# SYNTHESIS, STRUCTURE AND BIOACTIVITY OF PYRIMIDINE DERIVATIVES AND DEVELOPMENT OF SOME SYNTHETIC METHODS

**A thesis submitted in partial fulfillment of the requirements for award of the degree of Doctor of Philosophy**


By

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# Abstract

**Background**

Heterocycles in general and nitrogen heterocycles in particular constitute the lion's part of the classical divisions of organic chemistry and are of immense importance biologically, industrially, and indeed to the healthy functioning of any developed society. The majority of pharmaceuticals/ biologically significant/ agrochemicals/ commercially used (additives and modifiers in industries, as cosmetics, reprography, information storage, plastics etc.)/ available entities are heterocyclic compounds. They are available in living systems (plant as well as animal sources), viz. DNA-RNA bases, vitamins, enzymes, hormones etc. which play a crucial role in the biochemical processes.

Relevant to the proposed project are the nitrogen heterocycles, which normally form the basic skeleton of alkaloids. To be specific, this proposed project involves the works on pyrimidines – the RNA nucleobase, whose biochemical role is well known. This pyrimidine unit, annelated with a variety of other ring systems (pyridine, pyrimidine, purine, pyrazole etc.) is available in natural sources including nucleosides and nucleotides etc.

**Objective**

Feeling the ever-increasing vitality, demand and specially considering the bioactivity, heterocyclic chemistry now becomes an interdisciplinary subject of immense practical and theoretical importance bridging all branches of chemistry, biochemistry, material sciences etc. Furthermore, cycloaddition and annulation are central concerns of organic synthetic chemists to synthesize complex fused systems, given that the ring systems are a feature of important compounds, including many of pharmaceutical interest. Substances with a fused pyrimidine ring, such as purines, pteridines and riboflavin play a very important role in the biochemistry of the living cell. Many drug candidates have been modeled on these compounds, particularly for cancer and virus research. Development of clinically useful anticancer (5-fluorouracil) and antiviral drugs (AZT, DDC, DDI, BVDU) has renewed interest in the synthetic manipulation of uracils.

Keeping in mind the above significances of pyrimidine derivatives, we began our study with the following objectives:

1. The main objective is to search for novel compounds with pharmaceutical value.
2. To investigate and extend the application and scopes of hetero-Diels Alder methodology to the synthetically modified pyrimidine moiety.
3. To promote economical and environmentally friendly experimental procedures (green chemistry) by performing the reactions under microwave irradiation, in ionic liquids, in/on water and preferably in the absence of solvents.
4. To study the site- selectivity and regio-selectivity of the reactions.
5. To accomplish the synthesis of an array of heterocycles / fused heterocycles by new synthetic procedures.
6. Study and characterization of the prepared compounds by using NMR and IR spectroscopy, Mass spectrometry, Elemental analysis/HRMS and single crystal X-ray spectroscopy. Whenever required, help from other new techniques would be sought.
7. Finally, all the new compounds will be screened for possible bioactivity.

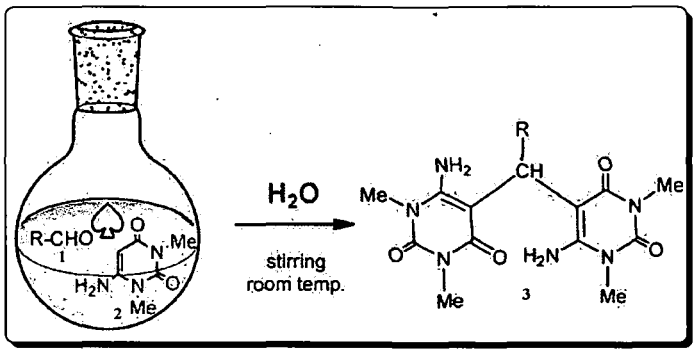
**Layout and contents of the thesis**

This thesis entitled "**Synthesis, Structure and Bioactivity of Pyrimidine Derivatives and Development of Some Synthetic Methods**" comprises of **six Chapters**. Each chapter is divided into sections namely **Introduction, Materials and Methods, Results and Discussion** (including **Bioactivity test**), **Conclusion, Experimental Section** and **References** respectively (excluding the **Chapters 1 & 6**).

The first chapter (i.e. **Chapter 1**), is a literature survey on pyrimidines. It includes a general description of pyrimidines and their derivatives (history, physical and chemical properties) and special emphasis has been given to the biological and medicinal applications of pyrimidines and their derivatives.

Author's own experimental work starts from **2nd Chapter**. This chapter describes a very simple, clean and green synthesis of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimethyluracil-5-yl)methanes (**3**) (or in short, bis-uracil) from 6-aminouracil (**2**) and aldehyde derivatives (**1**) (**Scheme 1**). The reaction was carried out at RT with simple stirring in water without using any outdriving forces like heat, dehydrating agent,

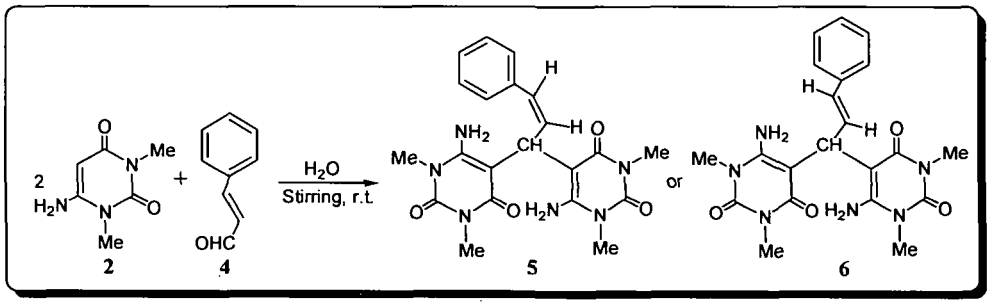
catalyst, surfactant or additive. The methodology is coupled with easy work-up and excellent yield. No chromatography is required. To note, if that strategy can be used for the synthesis of bioactive molecules, then chemists can feel the warmth of synthesis and then relax for not doing any harm to the environment while performing chemistry! The structures of the compounds were established using various spectroscopies and single crystal X-ray crystallography.




R= Ph, *p*-$OCH_3$Ph, *p*-ClPh, *p*-OHPh, *p*-$CH_3$Ph, *p*-$NO_2$Ph, *m*-$NO_2$Ph, H, $CH_3$, $CH_3$-$(CH_2)_3$, $CH_3$-$(CH_2)_2$,

**Scheme 1**

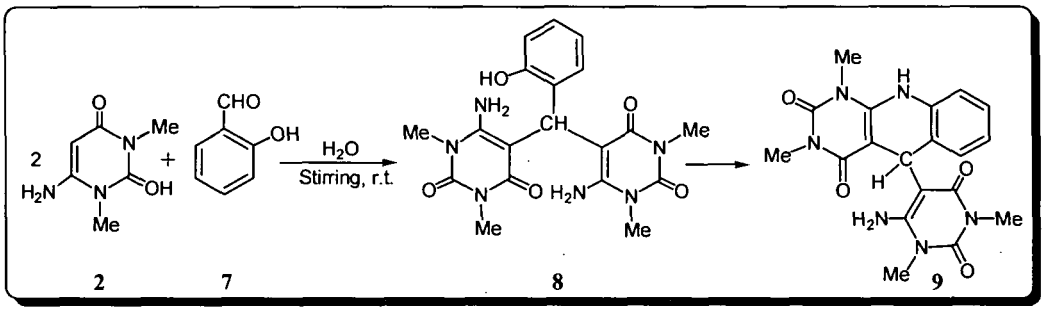
In the same way, when the reaction of (**2**) with cinnamaldehyde (**4**) was performed, formation of both *cis* / *trans* bis-uracil (**5/6**) (**Scheme 2**) adducts were observed. However, attempt to separate the *trans* or *cis* product by column or thin layer chromatographic technique failed. But upon recrystallisation, only the *trans* product (**6**) recrystallized out in the form of square size transparent crystals. Finally, the product




**Scheme 2**

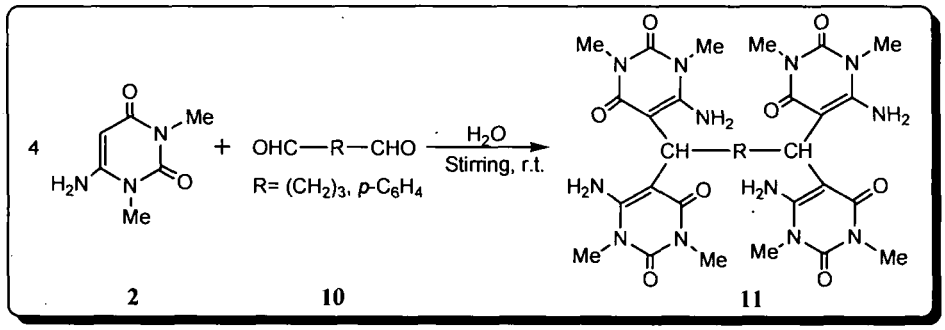
was confirmed by single crystal X-ray analysis.

Interestingly, when salicyldehyde (**7**) was reacted with (**2**), 5-(6-amino-1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxopyrimidin-5-yl)-1,3-dimethylpyrimido[4,5-*b*] - quinoline-2,4(1*H*,3*H*,5*H*,10*H*)-dione (**9**) formed and reaction proceeded via bis-uracil adduct (**8**) (**Scheme 3**). The structure of the compound was confirmed by single crystal X-ray analysis. NMR and IR spectroscopy, mass spectrometry and elemental analysis support the structure.




**Scheme 3**

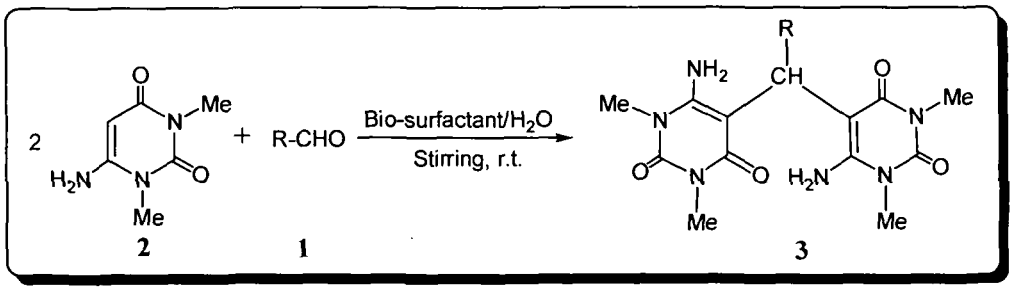
Following the same reaction scheme, tetrakis-uracil derivatives (**11**) were synthesized from the reaction between (**2**) and dialdehydes (**10**) (**Scheme 4**).




**Scheme 4**

In some of the cases the reaction required longer reaction time and also yields were somewhat low. So, to overcome this problem a green reaction methodology was

developed by using bio-surfactant (A **Green Surfactant**). By using **Green Surfactant** the products were obtained within short time (**Scheme 5**) with improved yields.




R= Ph, *p*-$OCH_3$Ph, *p*-ClPh, *p*-OHPh, *p*-$CH_3$Ph, *p*-$NO_2$Ph, *m*-$NO_2$Ph, H, $CH_3$, $CH_3$-$(CH_2)_3$, $CH_3$-$(CH_2)_2$,

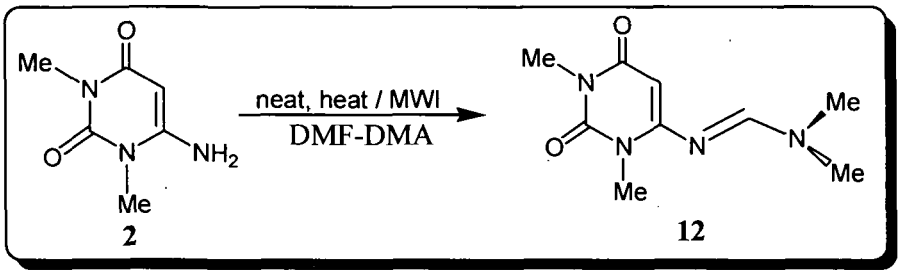
**Scheme 5**

The structures of the products were established using various spectroscopies and single crystal X-ray crystallography. ORTEP diagram for the compound 6,6'-diamino-1,1',3,3'-tetramethyl-5,5,-(benzylidene)-bis[pyrimidine-2,4(1*H*,3*H*)-dione] shows that both the amino groups and the two uracil rings are oriented oppositely and hence they exist in different magnetic environments.

Finally, all the synthesized compounds were evaluated for **Antioxidant, Antibacterial** and **Antifungal** activities. Free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) method was used for estimating the antioxidant activities. Among those compounds; aliphatic, nitro-substituted aromatic ring and furan moiety with bis-uracil ring showed very good antioxidant activities. We have also studied the antibacterial activities against seven non-pathogenic bacteria *K. pneumoniae*, *S. aureus*, *B. subtilis*, *E. coli*, *A. faecalis*, *P. aeruginosa*, *P. aeruginosa* and two antifungal strains *C. ablicans*, *F. oxysporium* respectively. These compounds were found to be equally active against both the gram (+) and gram (-) bacteria. All the tested compounds showed high to moderate antibacterial and poor antifungal activity.

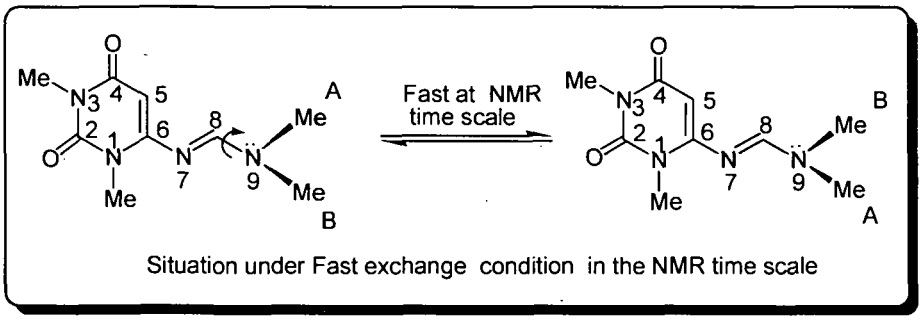
In the **3rd Chapter**, author describes the study of dynamic $^1$H NMR spectroscopy to determine the C-N rotational barrier of 6-[(dimethylamino)methylene]1,3-dimethylamino -uracil (**12**) in 1,1,2,2-tetrachloroethane-$d_2$. (**12**) was readily obtained by

the reaction of (**2**) with *N,N*-dimethylformamide dimethylacetal (DMF-DMA) under solvent less condition at refluxing temperature or under microwave irradiation (**Scheme 6**).




**Scheme 6**

At room temperature, in the $^1$H NMR spectrum, the two N9-methyl groups split into two sharp singlets with equal intensity indicating their magnetically nonequivalent character (**Scheme 7**). This non-equivalence is attributed to the hindered rotation of the dimethylamino moiety about the C8-N9 bond. A barrier is observable when exchange of the studied nuclei into different electronic environments is slow enough on the NMR timescale.




**Scheme 7**

In the limit of slow exchange (lower temperatures), distinct and sharp $^1$H resonances are observed for the two N9-$CH_3$ groups. However, as the temperature is increased, the exchange rate increases and the two sharp N9-$CH_3$ peaks broaden, come closer, and eventually coalesce at 89 °C (Coalescence temperature) (**Fig. 1**).

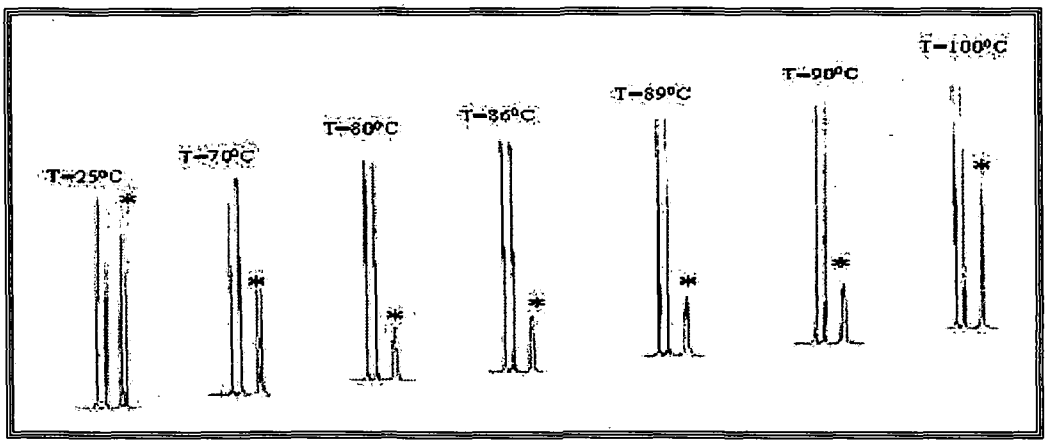
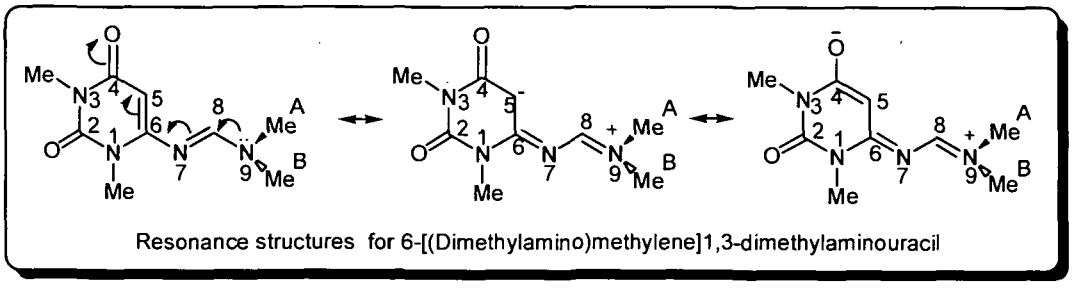



**Fig. 1**

This classical view is similar to the resonance observed in amide, where the lone pair of electron over nitrogen gets delocalised over the whole moiety and also resembles in push pull type enamine (N9-C8-N7-C6-C5-C4=O) with an extra double bond (C6-C5) in between. The combination of the very strong electron-donating ability of its enamine system and the strong electron attracting carbonyl group will polarize the C=N and C=C bonds to some extent, that will also contribute to the partial double bond character of N9-C8 and hence to the restricted rotational barrier (**Scheme 8**).

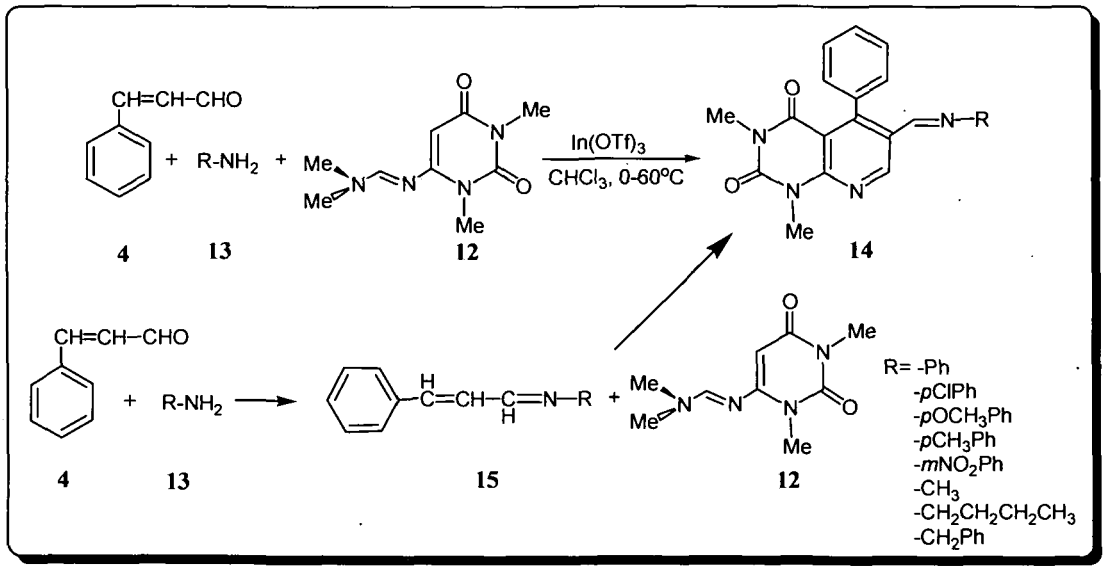



**Scheme 8**

The X-ray study of compound showed that a planar six-membered uracil ring attached to an exocyclic enamine side chain makes an angle of 174.3° with the plane of the uracil ring. The crystal structure also contains two water molecules which form O-H- - -O hydrogen bonds with the O atoms of the carbonyl group in the uracil moiety.

This experimental work was supported by density functional theory (DFT) studies. All the geometries were fully optimized at the B3LYP/6-31+G* level of DFT. Theoretical calculations show that the origin of this barrier lies in the formation of partial C-N double bond in the ground state. The computed barrier (19.8 kcal/mol) is in excellent agreement with the observed value (18.97 kcal/mol). Theoretical calculations show that the origin of the barrier lies in delocalization of the lone pair of electrons on the end nitrogen atom into the anti-bonding orbital of the adjacent C-N double bond in the ground state.

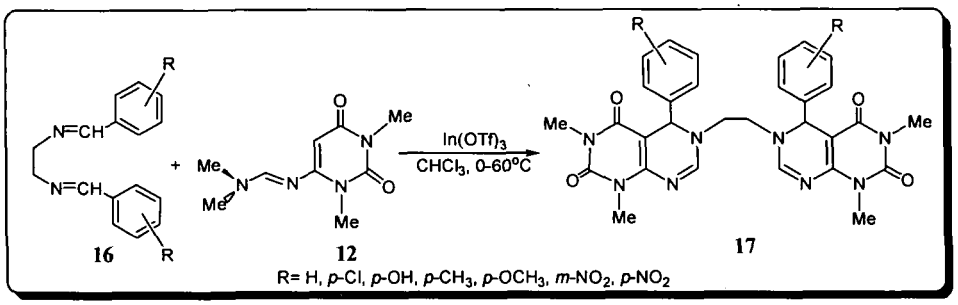
In the **4th Chapter**, author describes the synthesis of pyrido[2,3-*d*]pyrimidine-2,4-dione derivatives (**14**) by Multi Component Reaction (MCR) via Aza-Diels-Alder methodology (**Scheme 9**) in the presence of $In(OTf)_3$ as a catalyst.



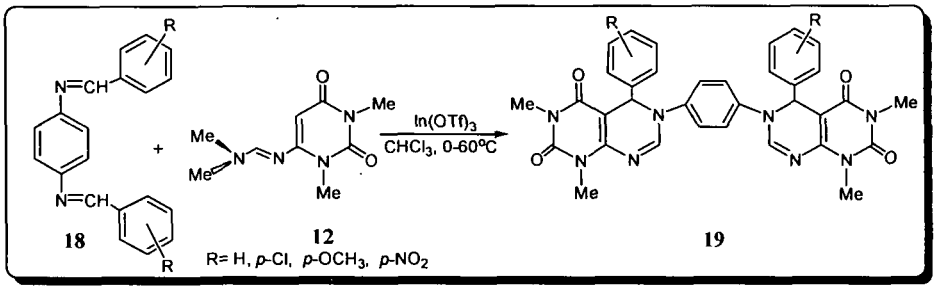

**Scheme 9**

Following the same reaction procedure we continued our generalization for the synthesis of some fused bis-pyrimido[4,5-*d*]pyrimidine derivatives (**17**), (**19**) and (**21**) by the reaction between bis-imines (**16**), (**18**) and (**20**) with (**12**) respectively (**Scheme 10, 11** and **12**). However, a three component strategy in schemes **10** and **11** failed.

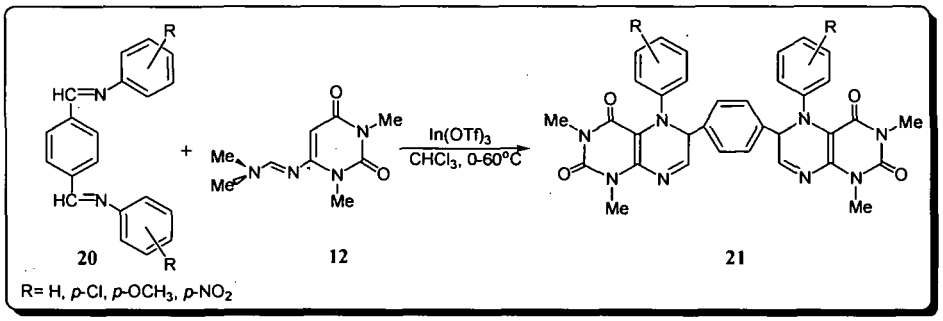
But, same products (**21**) were also isolated in one-pot three component reaction of terephthaldehyde (**22**), aromatic amine (**23**) and (**12**) by refluxing in toluene (**Scheme 13**) without using any catalyst.



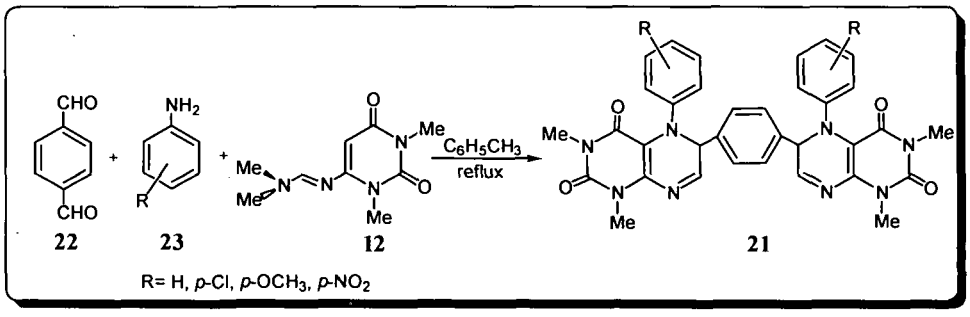

**Scheme 10**




**Scheme 11**




**Scheme 12**



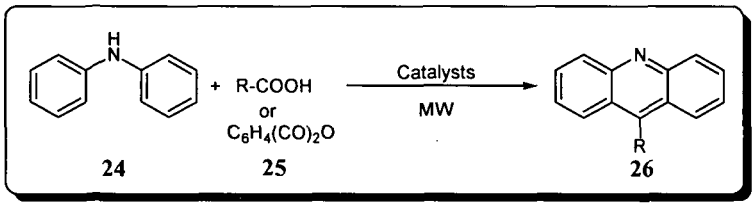

**Scheme 13**

All the synthesized compounds were confirmed by various spectroscopies and single crystal X-ray analysis.

Finally, all the synthesized compounds were evaluated for **Antibacterial, Antifungal, Cell viability test and Cytochrome P450** activities. For that, we have studied the antibacterial activities against two non-pathogenic bacteria *S. aureus*, & *E. coli* and two fungal strains *C. ablicans* and *F. oxysporium* respectively. Pyrido[2,3-*d*]pyrimidine-2,4-dione derivatives were found to be active against gram positive bacteria and inactive against gram negative bacteria and they also showed very good anti-bacterial activities. Cell-Viability test of pyrido[2,3-*d*] -pyrimidine-2,4-dione derivatives against animal cell line showed that with increasing the concentration of compound loading reduces the cell growth. Induction of cytochrome P450 by the methoxy-substituted-pyrido[2,3-*d*]pyrimidine derimatives exhibited maximum absorbance at wavelength 420 nm, which indicated the denaturation of cytochrome P450.

Bis-pyrimido[2,3-*d*]pyrimidine derivatives (**17**), (**19**) and (**21**) were found to have very good activities against gram negative bacteria, however, they remained inactive against gram positive bacteria.

The concept of '**Green**' has entered into every sphere of science and technology. In the **5th Chapter**, author describes green development of some synthetic methods by performing the reaction in absence of solvent under microwave irradiation and performing the reaction using ionic liquid.



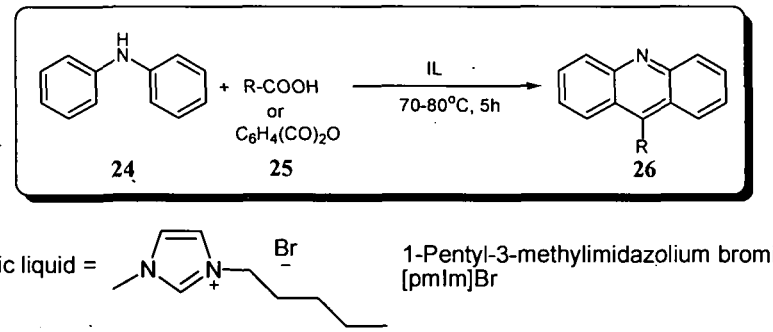

R= -$C_6H_5$, *p*-$ClC_6H_4$, *o*-$ClC_6H_4$, *p*-$NO_2C_6H_4$, *p*-$NH_2C_6H_4$, *p*-$OHC_6H_4$, -$(CH_2)_4$-COOH, -$(CH_2)_2$-COOH, -$CH_2$-COOH, *o*-$C_6H_4$COOH

Catalysts = *p*-TSA, Basic alumina, CAN, Zirconium oxychloride octahydrate, Potassium dichromate, Anhydrous aluminium chloride

**Scheme 14**

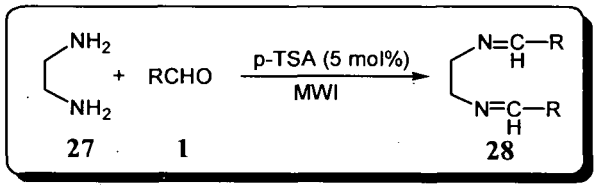
Bernthsen reaction, which has been carried out under microwave irradiation (MWI) in the presence of *p*-TSA (10 mol%) as catalyst in a solventless reaction to provide 9-substituted acridines (**26**) by the reaction between diphenylamine (**24**) and carboxylic acid moieties (**25**) (**Scheme 14**). This is a good development over existing methods.

We also studied the effect of ionic liquid in Bernthsen reaction. At room temperature, we got very low yield of the products (**26**), but upon applying heat we got very good yield of the products (**Scheme 15**). Out of several catalysts tested, *p*-toluenesulphonic acid (*p*-TSA) was found to be superior one.

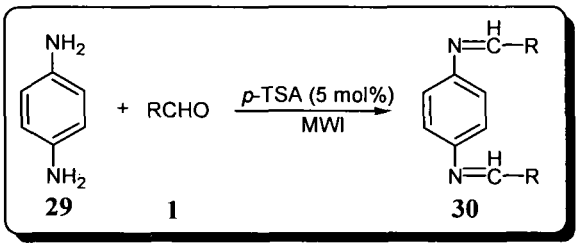



**Scheme 15**

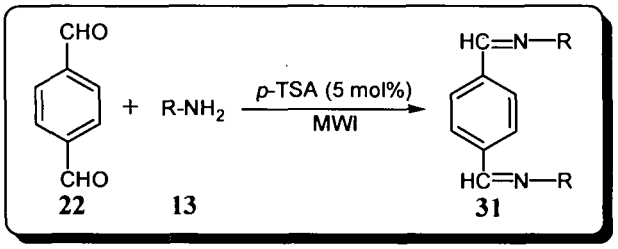
A short library of symmetrical bis-imines (**28**), (**30**) and (**31**) has been constructed efficiently from the reaction between diamines (**27** & **29**) (**Scheme 16** and **17**) and aldehydes (**1**) or dialdehydes (**22**) and amines (**13**) (**Scheme 18**) under MWI catalysed by *p*-TSA. The methodology is associated with shorter reaction time, good yields and simple workup.




**Scheme 16**




**Scheme 17**




**Scheme 18**

The last chapter of the thesis, i.e. the **6th Chapter** delineates the overall conclusion and future scopes of the works presented in the thesis.

## DECLARATION BY THE CANDIDATE

The thesis entitled "**Synthesis, Structure and Bioactivity of Pyrimidine Derivatives and Development of some Synthetic Methods**" is being submitted to the Tezpur University in partial fulfillment for the award of the degree of Doctor of Philosophy in Chemical Sciences is a record of bonafide research work accomplished by me under the supervision of Dr. A. J. Thakur, Assoc. Prof. Dept. of Chemical Sciences, Tezpur University.

All helps received from various sources have been duly acknowledged.

No part of this thesis has been submitted elsewhere for award of any other degree.

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## *CERTIFICATE OF THE SUPERVISOR*

This is to certify that the thesis entitled, ***Synthesis, Structure and Bioactivity of Pyrimidine Derivatives and Development of some Synthetic Methods"*** submitted to the School of Science and Technology, Tezpur University in part fulfillment for the award of the degree Doctor of Philosophy in Chemical Sciences (School of Science and Technology) is a record or research work carried out by Mr. Subrata Das under my supervision and guidance. He has complied with all the requirements as laid down in the regulations of Tezpur University for the award of Doctor of Philosophy in Chemical Sciences (School of Science and Technology) including course work.

All help received by him from various sources have been duly acknowledged.

No part of this thesis has been submitted elsewhere for award of any other degree.

Date: 31/10/11
Place: Tezpur


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## Certificate of the External Examiner and ODEC

This is to certify that the thesis entitled "**Synthesis, Structure and Bioactivity of Pyrimidine Derivatives and Development of some Synthetic Methods**" submitted by Mr. Subrata Das to Tezpur University in the Department of Chemical Sciences under the school of Science and Technology in partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy in Chemical Sciences has been examined by us on _____ and found to be satisfactory.

**Signature of:**

Principal Supervisor

External Examiner

Date: _____

# Acknowledgement

*I would like to express my deep sense of gratitude to my supervisor Dr. Ashim Jyoti Thakur, for his inspiring guidance and giving me the opportunity to work in his laboratory. It would have not been possible for me to bring out this thesis without his help, support, and constant encouragement throughout the research work.*

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*(Subrata Das)*

## Contents

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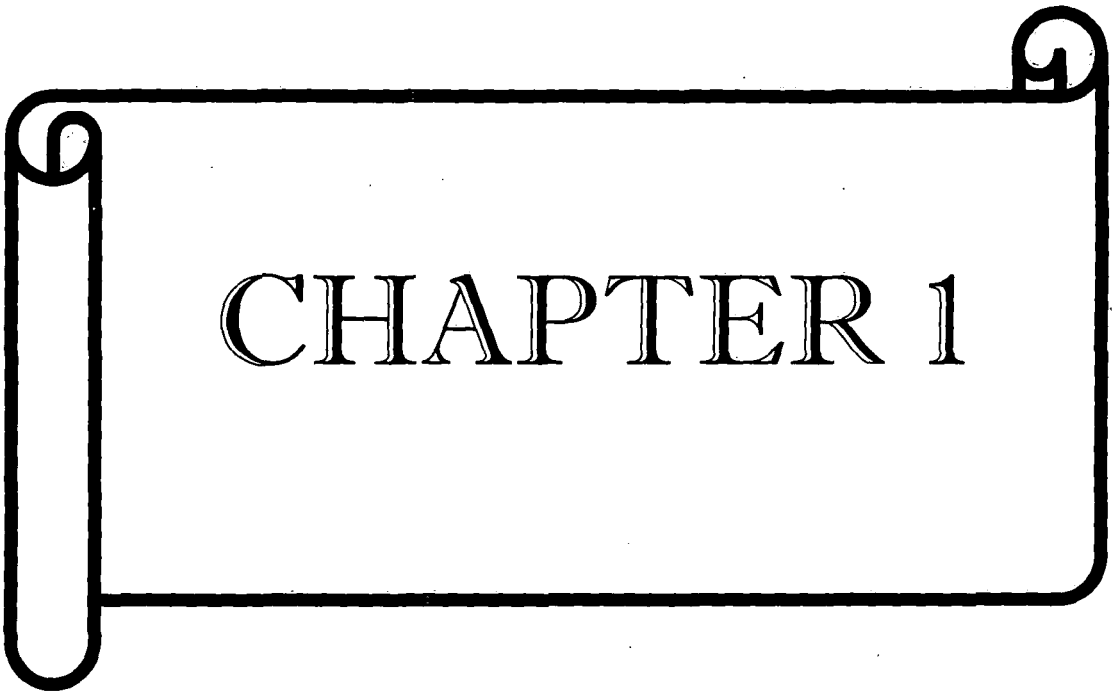
## Abbreviations used in the thesis

| | |
|---|---|
| RNA | Ribonucleic acid |
| DNA | Deoxyribonucleic acid |
| *t*RNA | Transfer ribonucleic acid |
| MIC | Minimun inhibitory concentration |
| SAR | Structure activity relationship |
| QSAR | Quantitative structure-activity relationship |
| ECM | Extracellular matrix |
| IMPDH | Inosine monophosphate dehydrogenase |
| HBV | Hepatitis B virus |
| HSV | Herpes simplex virus |
| HCV | Hepatitis C virus |
| HCMV | Human cytomegalovirus |
| TK | Thymidine kinase |
| HIV | Human immunodeficiency virus |
| $IC_{50}$ | Half maximal inhibitory concentration |
| $EC_{50}$ | Half maximal effective concentration |
| ABTS | 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) |
| DPPH | 1,1-Diphenyl-2-picrylhydrazyl |
| ASA | Acetylsalicylic acid |
| PK | Pharmacokinetic |
| PD | Parkinson's disease |
| CNS | Central nervous system |
| Caco2 | Colon carcinoma cells |
| TsC | 1-(*p*-Toluenesulfonyl)cytosine |
| BMsU | 5-Bromo-1-(methanesulfonyl)uracil |
| MCF-7 | Michigan cancer foundation - 7 |
| TP | Thymidine phosphorylase |
| $F_3$dThd | 2′-Deoxy-5-(trifluoromethyl)uridine |

| | |
|---|---|
| AIDS | Acquired immune deficiency syndrome |
| MAO-B | Monoamine oxidase B |
| CSC | (*E*)-8-(3-Chlorostyryl) caffeine |
| GTPγS | Guanosine gamma thio-phosphate |
| MSS | Musculoskeletal syndrome |
| MMP | Matrix metalloprotease |
| GnRH | Gonadotropin-releasing hormone |
| FSH | Follicle-stimulating hormone |
| LH | Luteinizing hormone |
| PDEs | Phospodiesterases |
| ERKs | Extracellular signal-regulated kinases |
| DMU | *N,N'*-Dimethyl urea |
| NMR | Nuclear magnetic resonance |
| FTIR | Fourier transform infrared spectroscopy |
| SDA | Sabouraud dextrose agar |
| MTT | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| HCl | Hydrochloric acid |
| HBr | Hydrobromic acid |
| HI | Hydroiodic acid |
| $H_2SO_4$ | Sulfuric acid |
| TEBAC | Triethylbenzyl ammonium chloride |
| TLC | Thin layer chromotography |
| ORTEP | Oak ridge thermal ellipsoid plot |
| *SA* | *Staphylococcus aureus* |
| *BS* | *Bacillus subtilis* |
| *EC* | *Escherichia coli* |
| *PA* | *Pseudomonas aeruginosa* |
| TMS | Tetramethylsilane |
| CHN | Carbon hydrogen nitrogen |
| m.p. | Melting point |
| DMF | Dimethyl formamide |

| | |
|---|---|
| DMSO | Dimethyl sulfoxide |
| DMAc | Dimethyl acetamide |
| CMC | Critical micelle concentration |
| *p*-TSA | *p*-Toluene sulphonic acid |
| AU | Adenine |
| AZT | Azidothymidine |
| DDI | Dideoxyinosine |
| DDC | Dideoxycytidine |
| BVDU | (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine |
| VT-DNMR | Variable temperature-dynamic nuclear magnetic resonance |
| DFT | Density functional theory |
| DMF-DMA | *N*,*N*-Dimethyl formamide dimethylacetal |
| GS | Ground state |
| TS | Transition state |
| IRC | Intrinsic reaction coordinate |
| HOMO | Highest occupied molecular orbital |
| LUMO | Lowest unoccupied molecular orbital |
| MCR | Multi-component reaction |
| DA | Diels-Alder |
| CYP450 | Cytochrome P450 |
| MWI | Microwave irradiation |
| MORE | Microwave organic reactions enhancement |
| DPA | Diphenylamine |
| CAN | Ceric ammonium nitrate |
| PEG | Polyethylene glycol |
| EDA | Ethylenediamine |
| *p*-PDA | *p*-Phenylenediamine |
| THF | Tetrahydrofuran |
| λ | Wave length |


CHAPTER 1

**Introduction:**

Pyrimidine (**1**) (**Fig. 1**) is one of the important nitrogen containing heterocyclic aromatic compounds, a family of diazine group, containing two nitrogen atoms at position 1 and 3 of the six-membered ring. Three isomeric diazines are theoretically possible- *o*-diazine or pyridazine (**2**), *m*-diazine or pyrimidine (**1**) and *p*-diazine or pyrazine (**3**) respectively (**Fig. 2**).[1]

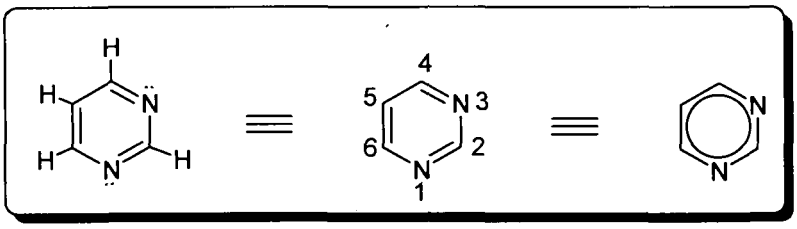



**Fig. 1:** The pyrimidine moiety

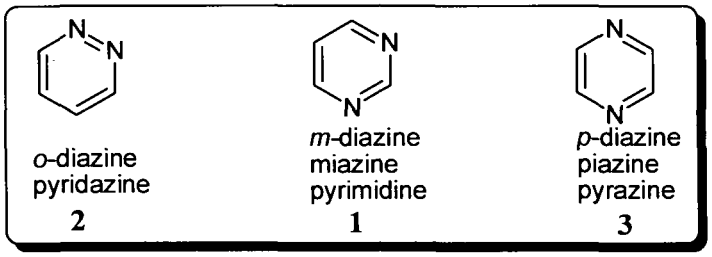
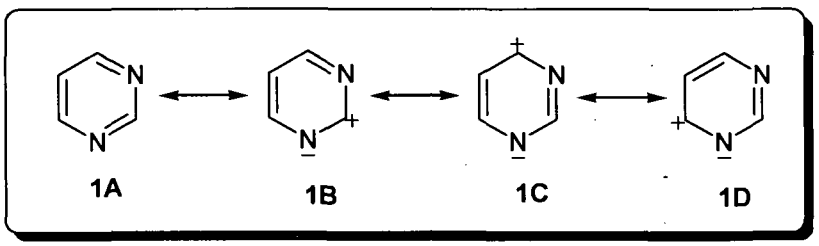


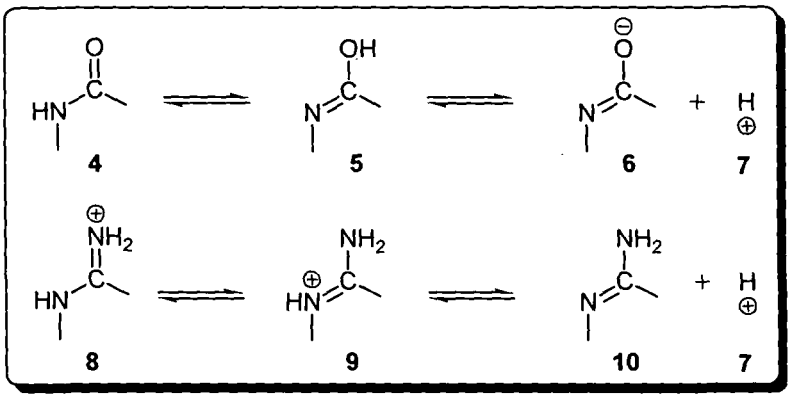

**Fig. 2:** Three possible isomeric diazines

Pyrimidine is neutral in solution, but forms salts with acid. Pyrimidine is probably a resonance hybrid of the following resonating structures (**A, B, C & D**) (**Scheme 1**).



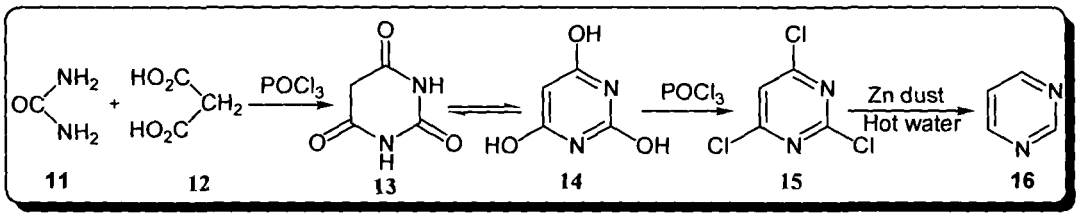

**Scheme 1:** Possible resonance structures of pyrimidine

Thus the ring is deactivated, and position 5 has the highest electron density. It can be therefore expected that attack by electrophilic reagents will be difficult, but attack by nucleophilic reagents (at positions 2, 4 and 6) will be facilitated. Chlorine atoms at 2, 4 or 6 are readily replaced by hydroxyl or amino group. When a hydroxyl or an amino group is present in pyrimidine nucleus, the compound no longer behaves entirely as an aromatic derivative. Because hydroxyl- or amino- substituents occur next to ring nitrogens, which shows keto-enol tautomerism (**Scheme 2**). On the other-hand, pyrimidine has many chemical properties common with pyridine. As the number of nitrogen atoms in the ring increases, the ring pi-electrons become less energetic and electrophilic aromatic substitution becomes more difficult and due to which pyrimidines are less basic.




**Scheme 2:** Keto-enol tautomerism of hydroxyl- or amino- substituent groups

Barbituric acid (**13**) is a very important pyrimidine derivative and it was originally prepared by condensing urea with malonic acid in the presence of phosphoryl chloride from which pyrimidine unit (**16**) was first prepared (**Scheme 3**).[2] Pyrimidine has drawn




**Scheme 3:** Synthetic route for pyrimidine (**16**)

attention such that everyday various simplest procedures have been developed for the synthesis of pyrimidine and their annelated derivatives.

Pyrimidine unit, annelated with a variety of ring systems are available in natural sources, like nucleoside and nucleotides etc. Three nucleobases found in nucleic acids, namely uracil (**17**), thymine (**18**) and cytosine (**19**) which are pyrimidine derivatives. Along with these little amounts of orotic acid (**20**) is also found in nucleic acids (**Fig. 3**).

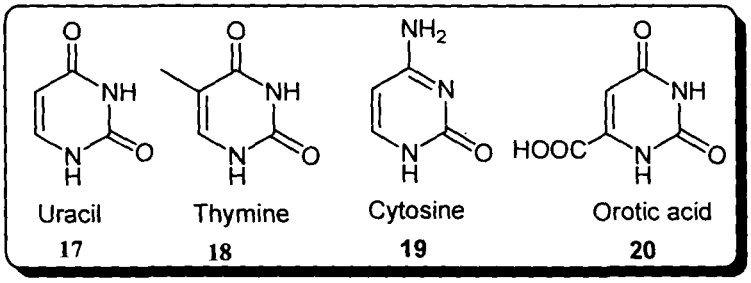



**Fig. 3:** Naturally occurring pyrimidine compounds

These pyrimidine nucleobases combine with purine nucleobases and five-carbon sugar to form a nucleotide molecule. The *N*-glycosidic link of pyrimidine nucleotides is in between C-1 of the ribose (or deoxyribose) and *N*-1 of pyrimidines. An invariant minor component of *t*RNA is pseudouridine, in which ribose is attached to C-5 of uracil. These 'pyrimidine nucleotides' can act as short-term carriers of chemical energy to help in hundreds of cellular reactions and well known metabolic intermediates.[3] Pyrimidine nucleotides play a key role in DNA and RNA synthesis, in the activation of sugar as a prerequisite for glycosylation of proteins and lipids, polysaccharide synthesis and detoxification. In DNA and RNA, these bases form hydrogen bonds with their complementary purines. Thus the purine bases adenine (A) and guanine (G) pair up with the pyrimidine bases, thymine (T), uracil (U) and cytosine (C) respectively (**Fig. 4**).[4] These are incorporated into the growing chain during RNA and/or DNA synthesis.

Pyrimidine nucleotides and purine nucleotides are used in large quantities in the biosynthesis of nucleic acids, so despite their complex structures they are synthesized *de novo* by most organisms. In higher animals, the biosynthetic pathways are present in many types of cell, although other cells depend on scavenger pathways which synthesize

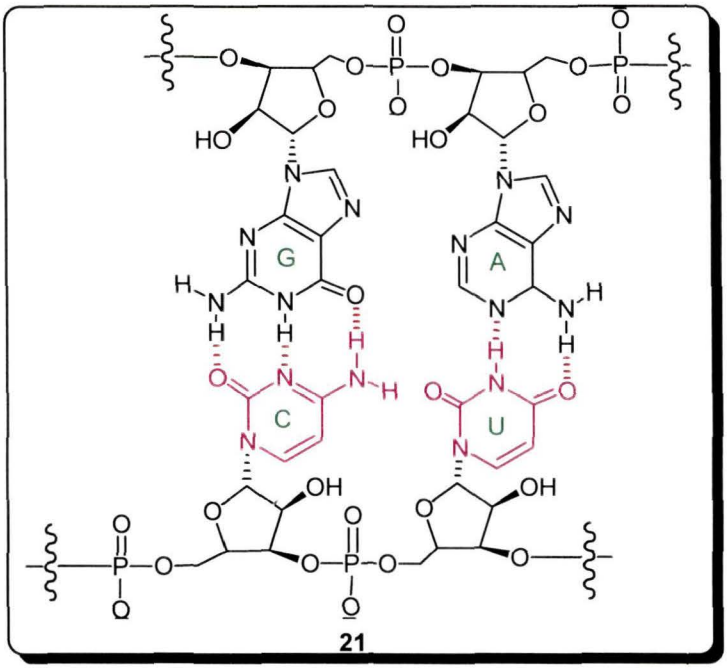



**Fig. 4:** Base pairing in RNA (Pyrimidine nucleobases in pink and hydrogen bonds in red)

nucleotides from purines and pyrimidines salvaged from the degradation of nucleic acids.[5]

The origin of the atoms in the pyrimidine ring is shown in the **Fig. 5**. The biosynthetic pathways appear to be basically similar in all organisms, although regulatory mechanisms differ.

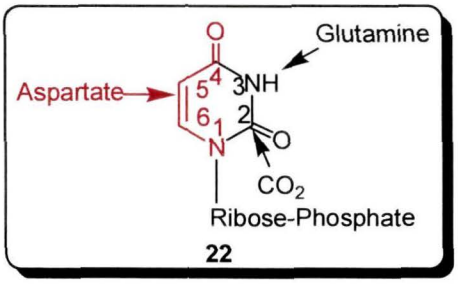
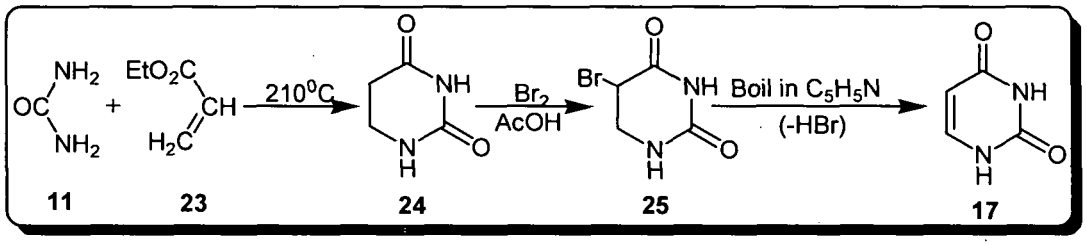



**Fig. 5:** Origin of atoms in the pyrimidine nucleotides

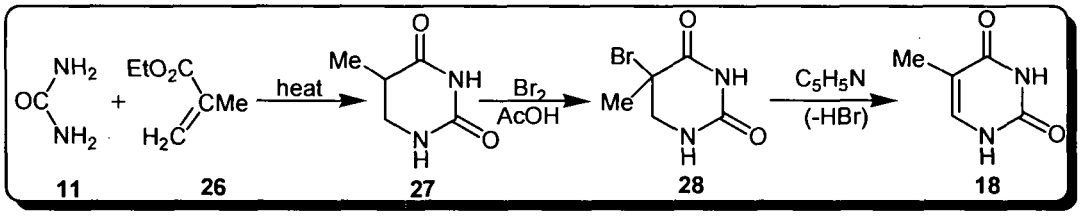
Thymine was the first pyrimidine to be purified from a natural source, had been isolated from calf thymus and beef spleen in 1893-94. Cytosine was discovered by Kossel

and Steudel in 1903.[6] They also discovered uracil in the same year.[7] In the laboratory, these nucleobases have been synthesized from reaction between urea/thiourea and ethyl acrylate/sodioformylacetic ester (**Scheme 4, 5 & 6**).

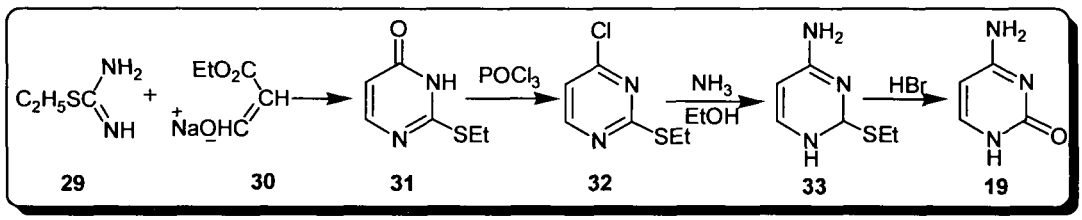
Not only pyrimidines are present in the DNA or RNA as a nucleobase, but also found in plant as well as animal and are available in vitamins, enzymes, hormones etc. (**Fig. 6**). More specifically, these nitrogen containing pyrimidine heterocyclic compounds are found normally as basic constituents of alkaloids. These natural products have given birth to several smaller units, which are highly important in present day's life quality as well as commerce. Most of the alkaloids and naturally available substances are build-up with pyrimidine core unit.




**Scheme 4:** Fischer and Roeder method for the synthesis of uracil (**17**)




**Scheme 5:** Fischer and Roeder method for the synthesis of thymine (**18**)




**Scheme 6:** Wheeler and Johnson method for the synthesis of cytosine (**19**)

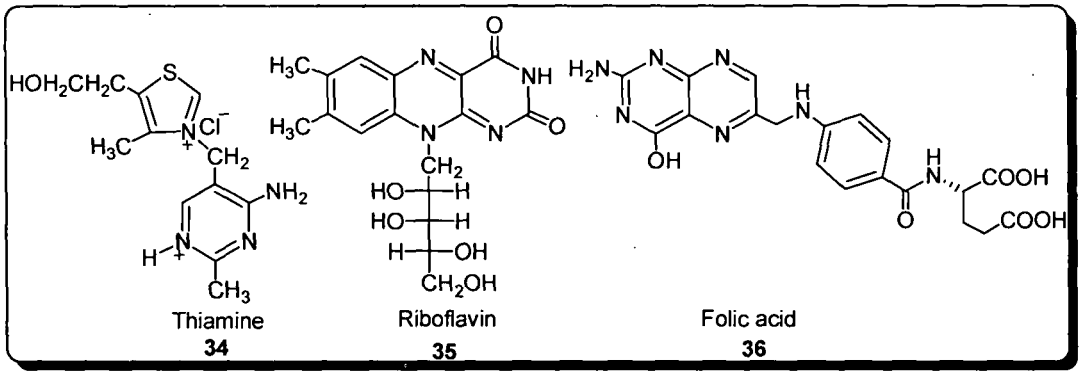
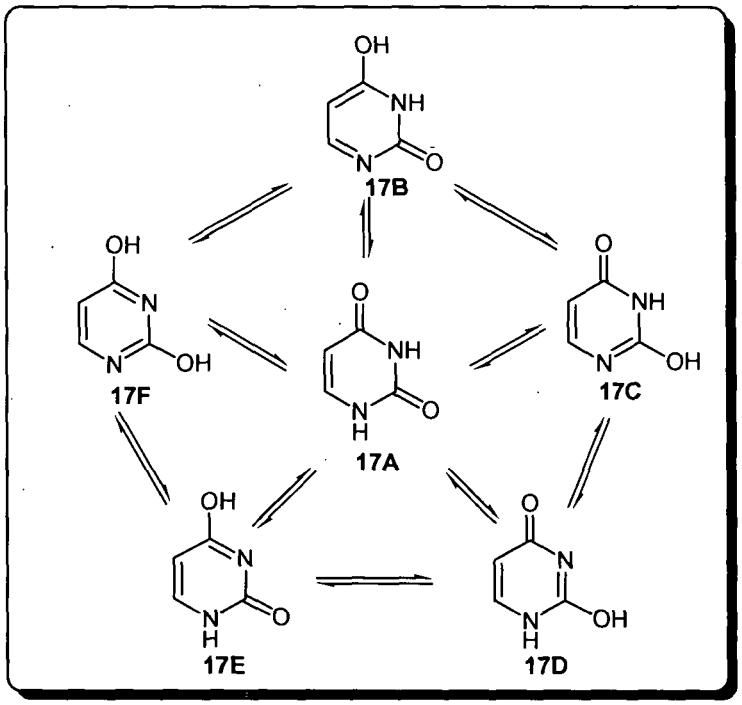



**Fig. 6:** Pyrimidine ring found in vitamins

Uracil, one of the nucleobases of pyrimidine family, which is found in RNA, comprises one of the major motifs in the formation of biopolymer RNA.[8] From the electronic structure and tautomerism of the uracil ring, it can be seen that six tautomeric forms (**17A, 17B, 17C, 17D, 17E & 17F**) of uracil are possible (**Scheme 7**). Among these six possible tautomers of uracil, the most stable form is the dicarbonyl (**17A**). The Gibbs free energy ($\Delta G_{298k}$) of this tautomer is lower than those of 2-Hydroxypyrimidine-4(3*H*)-




**Scheme 7**: Electronic structure and tautomerism of the uracil ring

one (**17C**) and 4-Hydroxypyrimidine-2(1*H*)-one (**17E**) of 46 and 52 kJ/mol, respectively.[9]

Pyrimidine (1,3-diazine) (**16**) and uracil (pyrimidine-2,4(1*H*,3*H*)-dione) (**17**) are π-deficient systems with similar distributions of their electron density (**Fig. 7**). In the uracil ring, however, this effect is substantially intensified by the interaction of ring π-electrons with oxygen atoms; thus C5 is the only carbon centre susceptible to attack by electrophiles.[10]

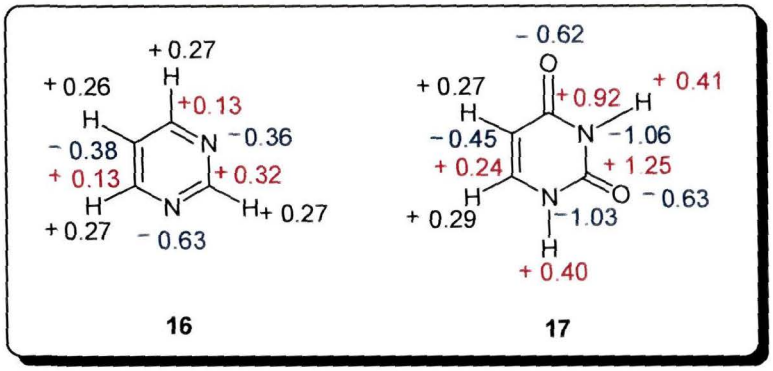



**Fig. 7:** Electron density distributions in pyrimidine (**16**) & uracil (**17**)

Uracil plays several roles in our life cycles and recently has challenged to solve the mechanism of its activity.[11] Uracil and its derivatives have drawn attention in recent times because they are chemotherapeutics with a unique broad spectrum of activities. For example simple 5-substituted uracil shows wide range of physiochemical properties and

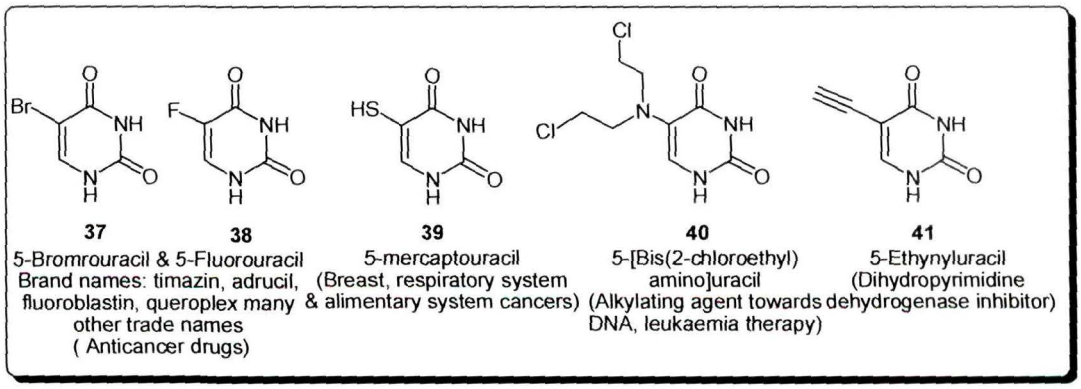



**Fig. 8:** Biological activities of 5-substituted uracil derivatives

biological activities (**Fig. 8**). Several uracil derivatives, including 5-bromouracil & 5-nitrouracil, can be incorporated into nucleic acids and thereby interfere with their transcription and translation processes.

Thus biological activities of uracil derivatives are closely concerned with the position of substituents on the ring.[12] 5-Fluorouracil[13] (**38**) and 5-mercaptouracil[14] (**39**) are used in the treatment of breast, respiratory system and alimentary system cancers as they inhibit the activity of the thymidylate synthase. Uramustine (5-[Bis(2-chloroethyl)amino]uracil, (**40**) is an alkylating agent towards DNA, and is used in leukaemia therapy.[15] 5-Ethynyluracil (**41**) as a dihydropyrimidine dehydrogenase inhibitor, impedes the biotransformation of 5-fluorouracil and consequently prolongs its therapeutic action.[16] *N*-substituted uracil and their derivatives also showed wide range biological activities (**Fig. 9**). Gemcatibane (**42**) is a popular cytostatic.[17a] Retrovir (**43**) an inhibitor of HIV reverse transcriptase was introduced to clinical use in 1987 as a great breakthrough in anti HIV therapy.[17b] 3-Phenacyl-*N*1-substituted uracil derivative (**44**) has hypnotic and sedative properties.[17c] 3-(3,5-Dimethylbenzyl)-1-cyanomethyl uracil (**45**) has been used as a anti-HIV agent.[17d]

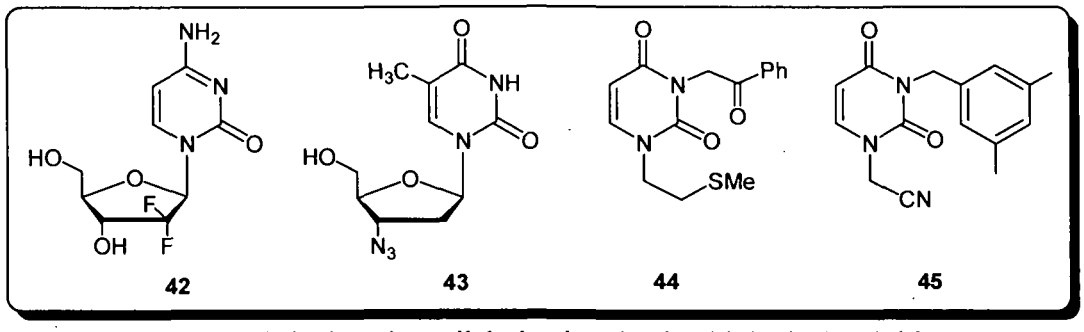



**Fig. 9:** *N*-Substituted uracil derivatives having biological activities

Structural exploitation and structural modification of uracil and their derivatives draw attention due to their wide biological activities. Papesch *et al* in 1951 and Blicke *et al* in 1954 first introduced the synthetic procedure for 1,3-dimethyl-6-aminouracil[18,19] by the reaction between *N,N'*-dimethylurea[20] and cyanoacetic acid. Synthesis of the 1,3-dimethyl-6-aminouracil was achieved using the Gould-Jacobs reaction[21] as the key step. Condensation of 6-amino-1,3-dibenzyluracil, and diethyl ethoxymethylenemalonate

under basic conditions, provided the aminomethylenemalonate, which was heated in Dowtherm (heat transfer fluid), resulted in intramolecular cyclization to produce the pyridopyrimidine ester (74%).

Below, an attempt has been made to highlight some of the important bioactivities associated with pyrimidine compounds from the recent literatures.

**Medicinal and biological significances of pyrimidine derivatives:**

**Antibiotic activity:**

30% or more hospitalized patients are treated with one or more courses of antibiotic therapy. Uracil derivatives have wide antibiotic activities.

A series of pyrimidine derivatives (**46-51**) were shown to be very active towards a variety of species of gram positive and gram negative bacteria in addition to some fungal plant pathogens (**Fig. 10**).[22]

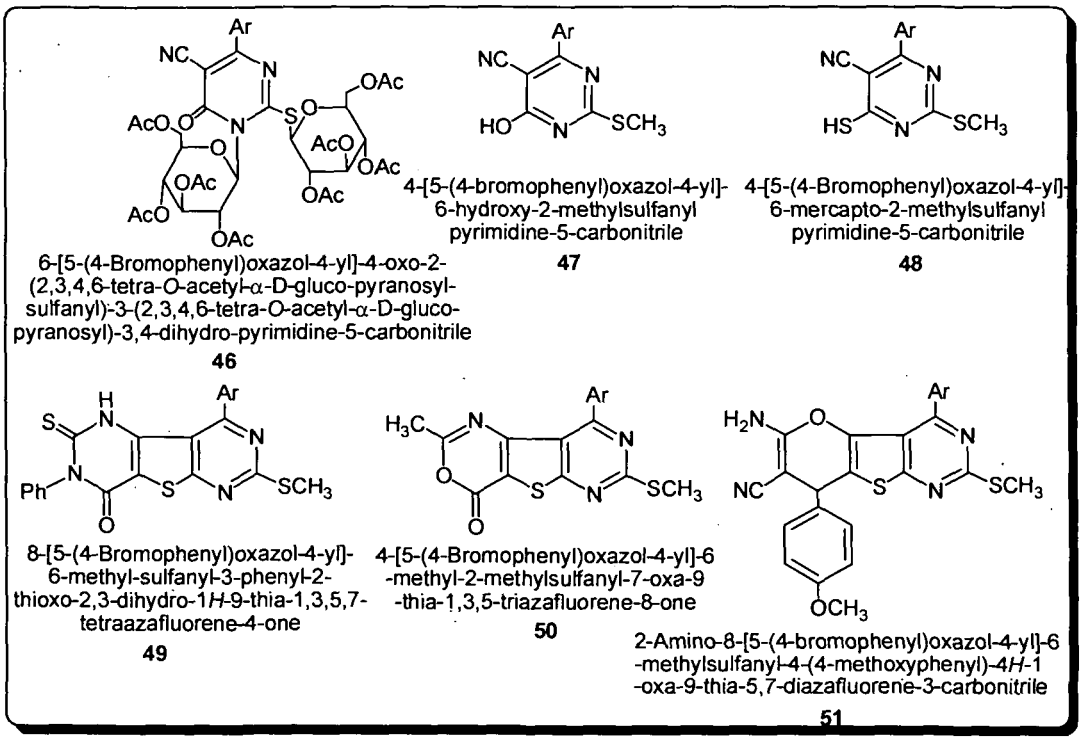



**Fig. 10:** Some pyrimidine derivatives having antibiotic activities

Some pyrimidine-2,5-diones[23] (**52**) showed potent antimicrobial activity against *E. coli* ATCC 13607, *P. diminuta* MTCC 3361, *S. aureus* ATCC 2943, *B. subtilis* ATCC 6633 and antifungal activity against *A. niger* ATCC 16404 and *C. albicans* ATCC 10231. The quantitative structure-activity relationship investigation showed that the antimicrobial activities of those derivatives against the test microorganisms were mainly governed by the molar refractivity, a polarizability parameter. Thus a proper substitution of the group with high polarizability at *N*-6 position probably improves the potency of these derivatives as antibacterial and antifungal agents (**Fig. 11**).

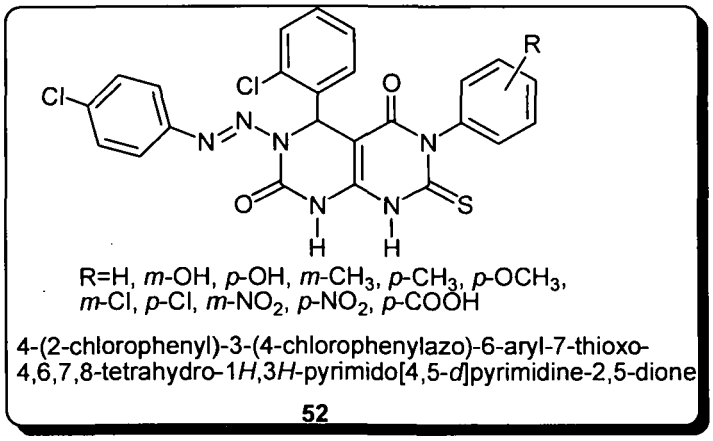



**Fig. 11:** Some pyrimidine derivatives having antibiotic activity

A series of structurally related 1*H*-pyrazolyl derivatives of thiazolo[4,5-*d*]pyrimidines[24] (**53-58**) have been evaluated for their *in vitro* antimicrobial activity against *E. coli* (ATCC 25922), *S. aureus* (ATCC 19433) and *C. albicans*. The minimal inhibitory concentrations (MICs, mg $mL^{-1}$) of the tested compounds revealed that most of the compounds exhibited promising antibacterial activities but they showed poor antifungal activity (**Fig. 12**).

The 2005 Nobel Prize in Physiology and Medicine was awarded to Barry Marshall and Robin Warren for the discovery of the bacterium *Helicobacter pylori* and for the determination that its colonization of the stomach mucosa which causes gastritis and peptic ulcers.[25] An association has been made between infection by the bacterium and a predisposition to gastric cancer. The diseases caused by *H. pylori* can be cured by

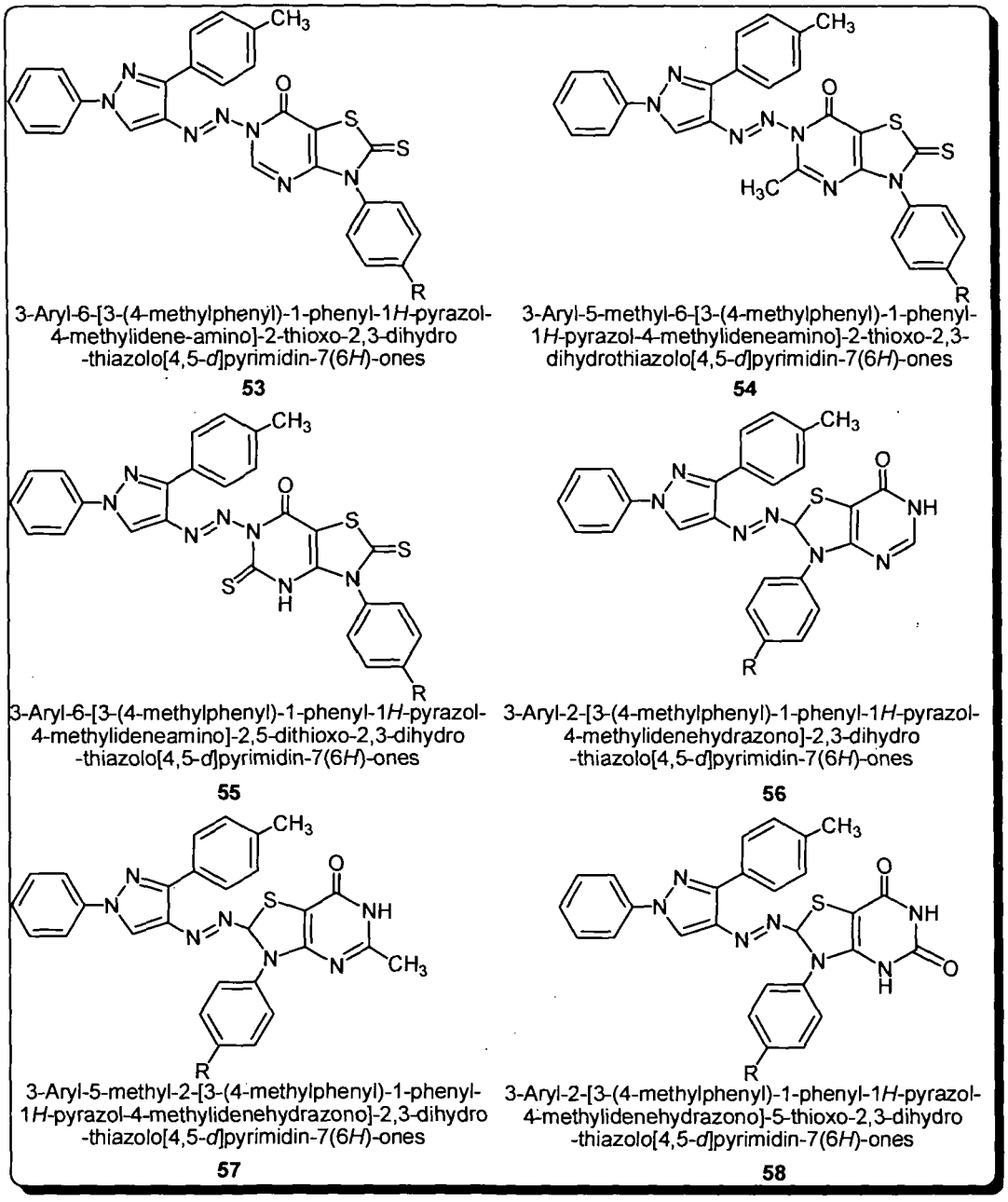



**Fig. 12:** Some pyrimidine derivatives having antibiotic activity

eradication of the pathogen with various antibacterial therapies.

Pyrazolopyrimidinedione[26] **(59) (Fig. 13)** hits as a selective, low micromolar inhibitor of *H. pylori* glutamate racemase (MurI). Variation of the substituents around the scaffold led to improved antibacterial activity. In order to achieve high bioavailability, a

novel pro-drug approach was implemented wherein a solubilizing sulfoxide moiety was oxidized *in vivo* to a sulfone.

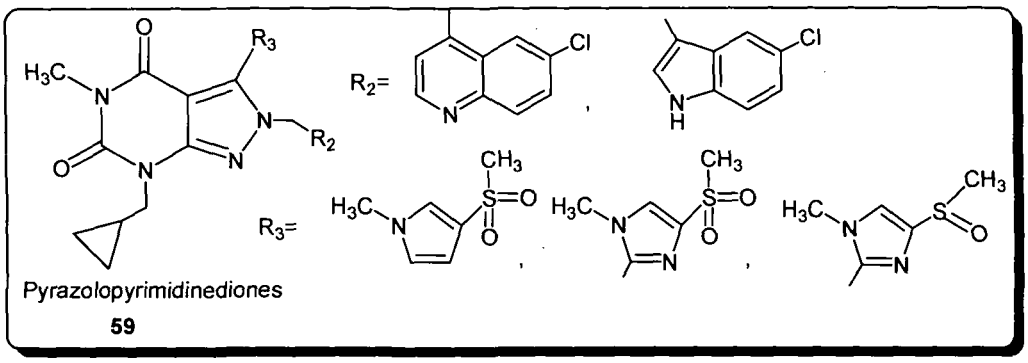



**Fig. 13:** Uracil derivatives as antibiotics

Cyclocondensation of $\alpha,\beta$-unsaturated ketones and 6-aminothiouracil followed by corresponding modification resulted a series of interesting pyrimidine derivatives (**60-63**) (**Fig. 14**).[27] The synthesized compounds were very effective *in vitro* antimicrobial

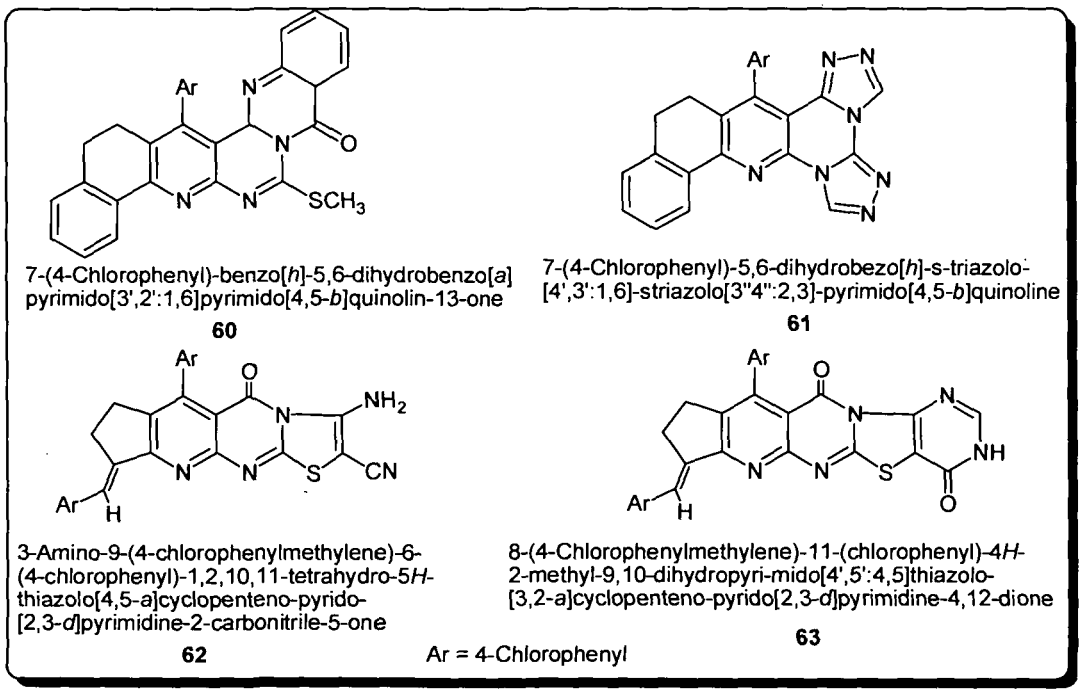
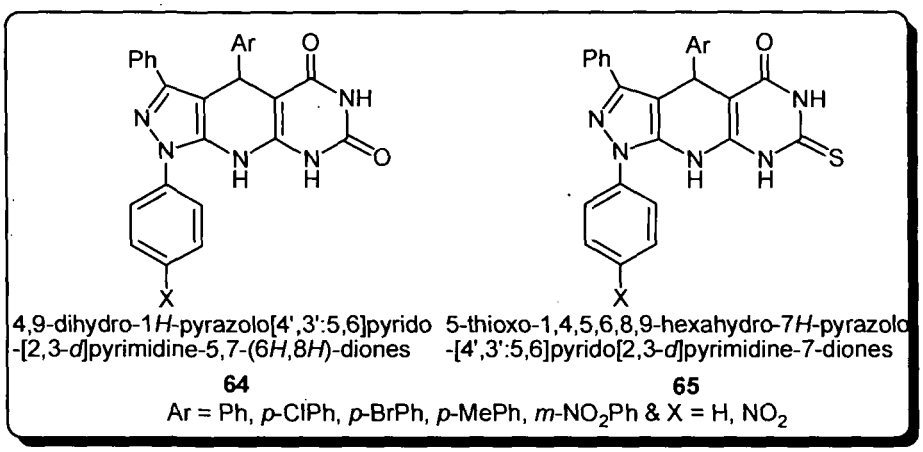



**Fig. 14:** Uracil derivatives having antibacterial activity

activities against four strains of bacteria species namely *S. aureus*, *B. subtilis*, *E. coli*, and *S. typhi* and two strains of fungi, *A. terreus* and *A. flavus* as revealed by the agar diffusion technique.

Similarly, several compounds of pyrimidine origin (**64,65**) were synthesized by condensation reaction of barbituric acids, 1*H*-pyrazol-5-amines and aldehydes under solvent-free conditions,[28] which were effective against the microorganisms *E. coli* ATCC 25922, *P. aeruginosa* ATCC 85327, *E. faecalis* ATCC 29737, *B. subtilis* ATCC 465, *B. pumilus* PTCC 1114, *M. luteus* PTCC 1110, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, and *S. mutans* PTCC 1601. Most of the compounds exhibited good to excellent antibacterial activity against all the tested strains. The introduction of -Cl and -Br at the aldehyde moiety, decrease the activity against *E. faecalis* and *S. aureu* (**Fig. 15**).




4,9-dihydro-1*H*-pyrazolo[4',3':5,6]pyrido -[2,3-*d*]pyrimidine-5,7-(6*H*,8*H*)-diones

**64**

5-thioxo-1,4,5,6,8,9-hexahydro-7*H*-pyrazolo -[4',3':5,6]pyrido[2,3-*d*]pyrimidine-7-diones

**65**

Ar = Ph, *p*-ClPh, *p*-BrPh, *p*-MePh, *m*-$NO_2$Ph & X = H, $NO_2$

**Fig. 15:** Uracil derivatives having antibacterial activity

New 1,3-diphenylpyrazoles bearing pyrimidine,[29] pyrimidinethione, thiazolo-pyrimidine, triazolopyrimidine, thio- and alkylthiotriazolopyrimidinone moieties (**66-69**) at the 4-position were synthesized by successive reaction of 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde with ethyl cyanoacetate and thiourea and synthesized compounds showed their antimicrobial activities against two strains of bacteria *B. cereus* and *E. coli* respectively, two strains of fungi *Botrytis* and *G. candidum* respectively and one strain of yeast *C. albicans* (**Fig. 16**).

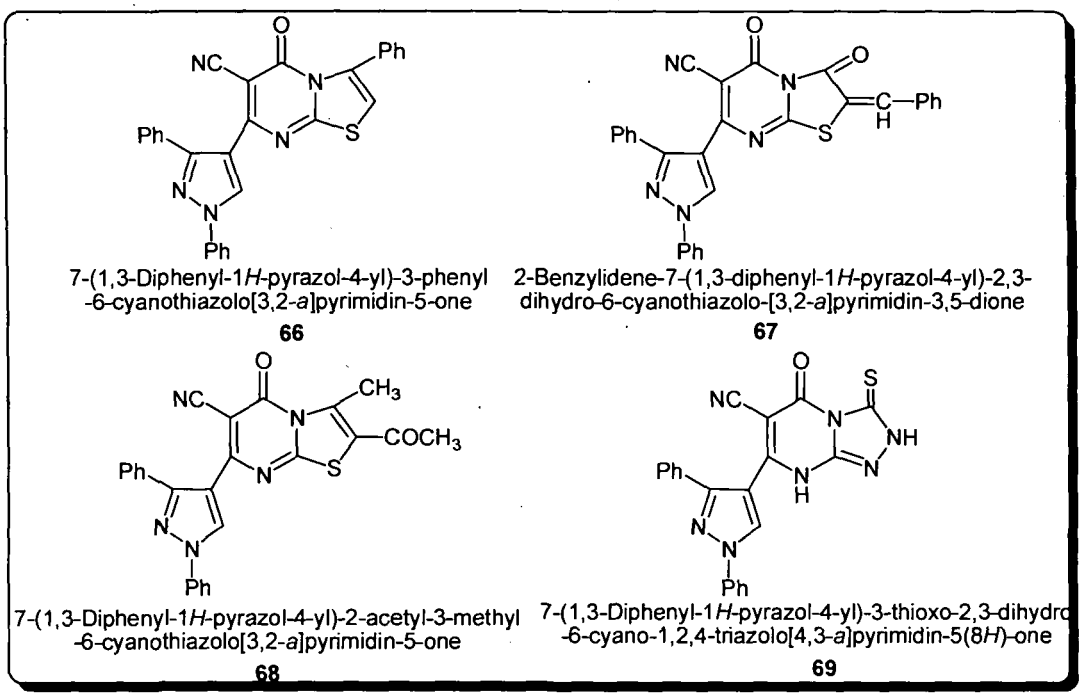



**Fig. 16:** Uracil derivatives having antibacterial activity

Pyrimidine derivatives[30] (**70,71**) with appended aryl, heteroaryl and alkylthio substituent at position 6 and also alkylthio substituent at position 2 were found to have *in vitro* activity against six pathogenic bacteria such as *M. tuberculosis* (*MT H37 Rv*), human isolates of *K. pneumoniae, P. aeruginosa, S. faecalis, S. aureus, E. coli* and antimycotic activity against *C. albicans (SKF), C. neoformans (CN-17), S. schenckii (SS-1), A. fumigatus (AF-27), T. mentagrophytes (A-280)* including virulent and non-virulent

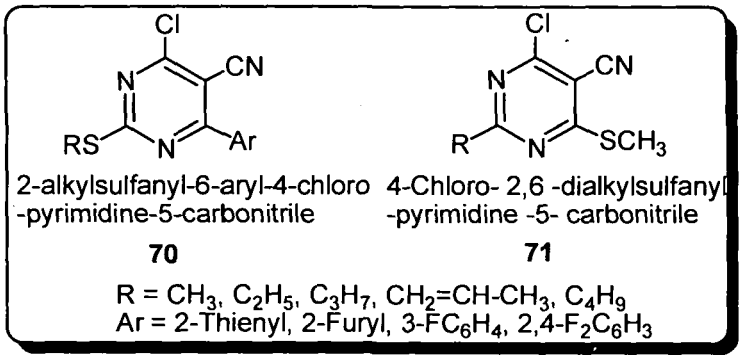



**Fig. 17:** Uracil derivatives showing antibacterial activity

strains of *M. tuberculosis*. Moreover, all the synthesized compounds have displayed potent *in vitro* antimicrobacterial activity (**Fig. 17**).

**Anticancer activity:**

A series of pyrimido[4,5-*c*]isoquinolinequinones, angucyclinoneaza-pyrido[2,3-*d*] - pyrimidine analogues (**72,73**) and corresponding hydroxyquinones[31] (**74**) (**Fig. 18**) exhibit moderate to high cytotoxic activity towards cancer cells, and therefore represent promising leads for the development of anticancer agents. Among the compounds, those with one and two pyridine moieties fused to the quinone system have shown the best effect. SARs established the main structural requirement for the activity of the new potential anticancer drugs.

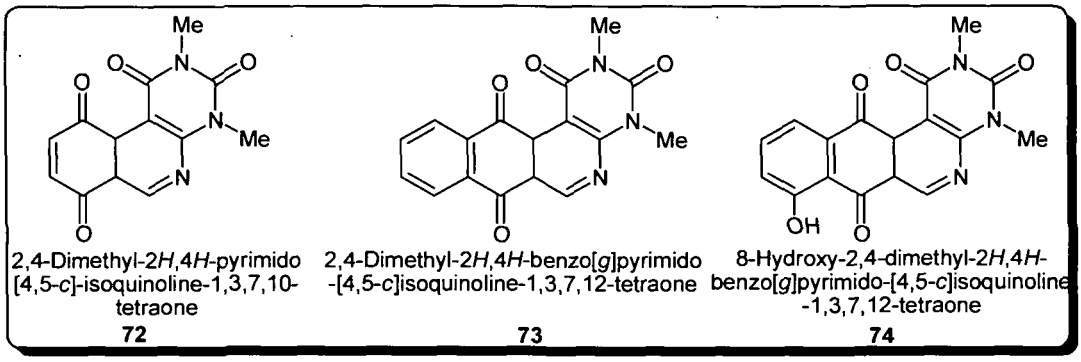



**Fig. 18:** Uracil derivatives as anticancer agents

Different derivatives of 5-[alkoxy-(4-nitro-phenyl)methyl]uracils with alkyl chain lengths C1-C12[32] were synthesized based on 5-[chloro-(4-nitro-phenyl)methyl]uracil and subsequent substitution of chlorine with appropriate alcohols. The resulting ethers (**75**) were tested for their cytotoxic activity *in vitro* against five cancer cell lines. The compounds were less active in lung resistance protein expressing cell lines, suggesting the involvement of this multidrug resistant protein in control of the biological activity. Cytotoxic substances induced rapid inhibition of DNA and modulation of RNA synthesis followed by induction of apoptosis. The data indicate that the biological activity of 5-[alkoxy-(4-nitro-phenyl)methyl]uracil depends on the alkyl chain length. Activity increases with chain lengths continuously in lines CEM and K562-Tax (**Fig. 19**).

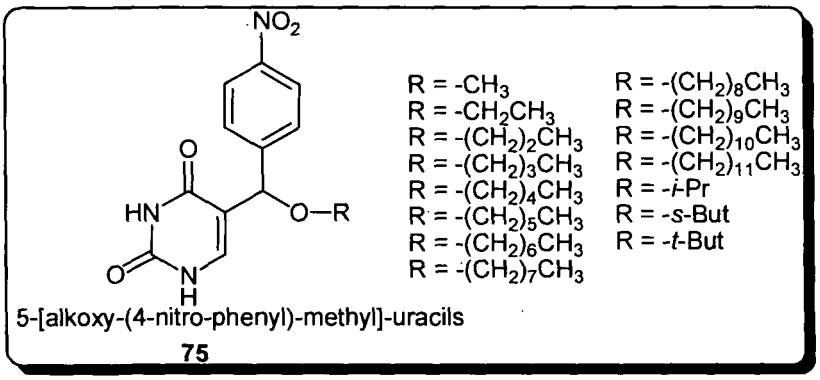



**Fig. 19:** Uracil derivatives as anticancer agents

**Antiviral activity:**

1,3-Dibenzyl-5-hydroxy-2,4-dioxo-1,2,3,4-tetrahydro-pyrido[2,3-*d*]pyrimidine-6-carboxylic acid[33] (**76**) (**Fig. 20**) have strong antiviral activity against dengue virus. This compound is also highly active against another virus of the genus, Flavivirus, the yellow fever virus. In addition, the compound exhibits antiviral activity, albeit low, against other RNA viruses. The mechanism of the antiviral activity is likely to be associated with inhibition of the enzyme, IMPDH and this is supported by the observation that addition of guanosine to the cell culture medium reverses the antiviral activity.

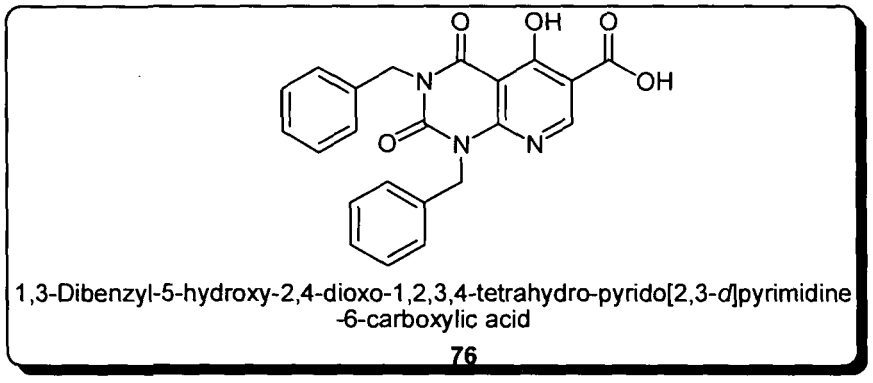



**Fig. 20:** Uracil derivative as antiviral agents

A number of sugar uracil-1-ylmethylhydrazones, *O*-acetylated derivatives of sugar uracil-1-ylmethylhydrazones and 4-acetyl-5-(*O*-acetylalditolyl)-2-(uracil-1-ylmethyl)-1,3,4-oxa-diazolines (**77,78**) (**Fig. 21**) were tested for antiviral activity against

Hepatitis-B virus.[34] Structure-activity correlation of the obtained results revealed that *O*-acetylated derivatives, followed by compounds in which the 1,3,4-oxadiazoline ring is attached to the *O*-acetylated sugar moiety, showed higher activity against HBV than the deprotected sugar hydrazones.

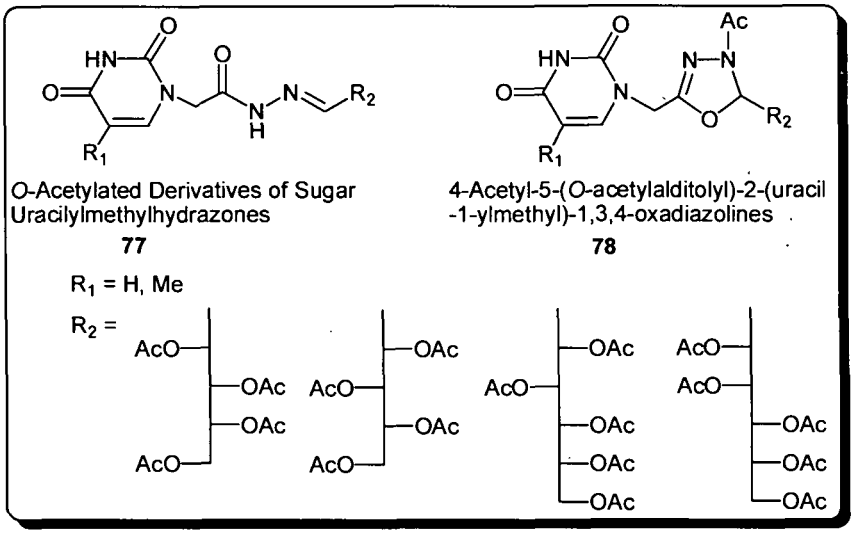



**Fig. 21:** Uracil derivatives having antiviral activity

Pyrimidine derivatives[35] (**79,80**) with a side-chain attached to the C-6 were subjected to *in vitro* phosphorylation tests, determination of their binding affinities to herpes simplex virus (HSV-1) thymidine kinase (TK) and catalytic turnover constants. 2,3-dihydroxypropyl and 2-fluoro-3-hydroxypropyl side-chains attached to C-6 of the pyrimidine moiety exhibited better binding affinity for HSV-1 TK and no cytotoxic effects in HSV-1 TK-transduced and non-transduced cell lines (**Fig. 22**).

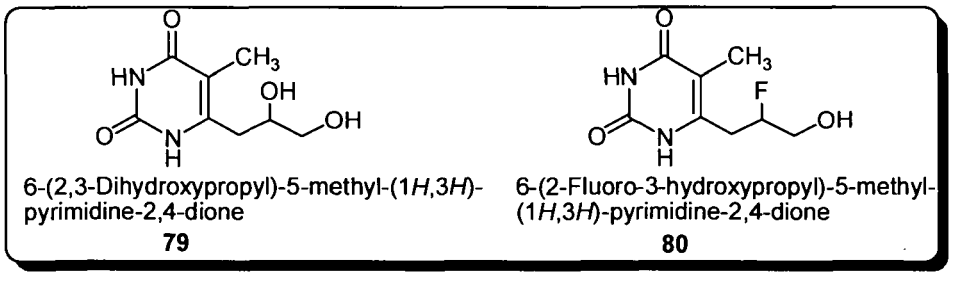



**Fig. 22:** Uracil derivatives as antiviral agents

Synthesis of a series of novel 2-alkoxy-3,4-dihydro-6-benzyl-4(3*H*)-pyrimidin-4-one analogues[36] (*S*-DABOs) and a novel dihydro-aryl/alkylsulfanyl-cyclohexylmethyl-oxopyrimidines (S-DACOs)[37] (**81-84**) were accomplished and evaluated as inhibitors of human immune deficiency virus type-1 (HIV-1). Key structural modifications included replacement of the 6-arylmethyl group by a 6-arylcarbonyl or 6-(*R*-cyanoarylmethyl) group. Most of the compounds showed only micromolar potency against HIV-1 in MT-4 cells *in vitro*, though two of them 2-benzoylmethylthio-6-[α-cyano-(1-naphthylmethyl)]-3,4-dihydro-5-methylpyrimidin-4(3*H*)-one & 6-(α-Cyanobenzyl)-3,4-dihydro-2-isopropylthio-5-methylpyrimidin-4(3*H*)-one were unusually potent ($IC_{50}$= 0.09 and 0.002 $\mu$M, respectively) and selective (SI = 1500 and 4600, respectively) (**Fig. 23**).

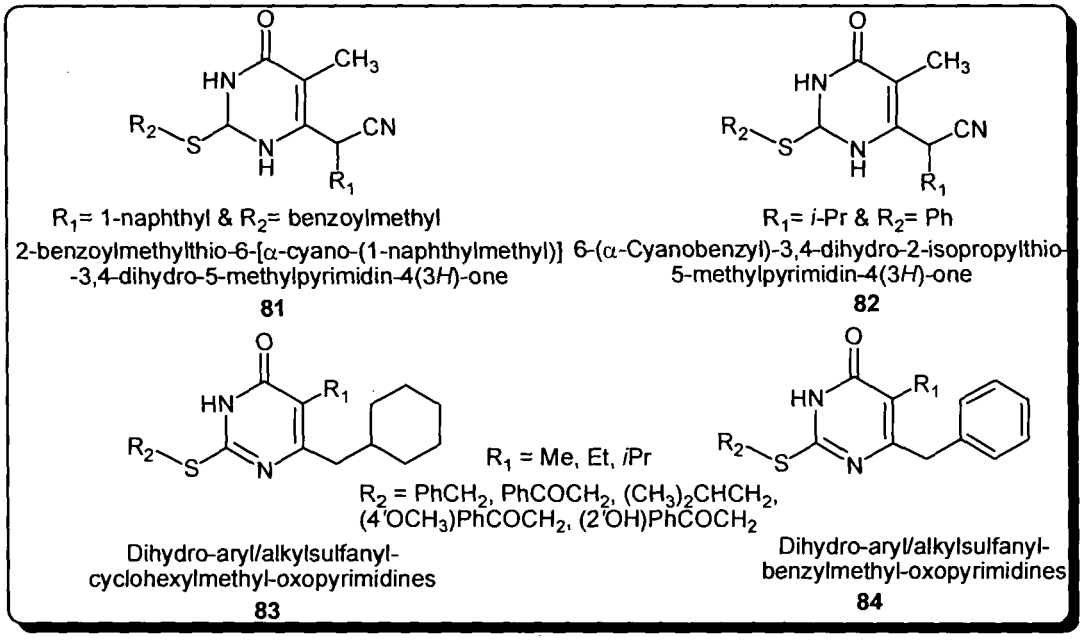



**Fig. 23:** Uracil derivatives showing anti-HIV activity

*N*-1-alkylated-5-aminoaryalkylsubstituted-6-methyluracil derivatives[38] (**85-87**) showed potent inhibitory activity against HIV-1 RT. The most active compounds showed activity in the low micromolar range with $IC_{50}$ values ($IC_{50}$ = 0.82-5.09 μM) comparable to that of nevirapine ($IC_{50}$ = 10.60 μM). Compounds showed 2- to 13-fold more activity than nevirapine by introducing the terminal 2,4,5-trichloroanilino, benzenemethanamine, and cyclohexylamino groups at pyrimidine C-5 (**Fig. 24**).

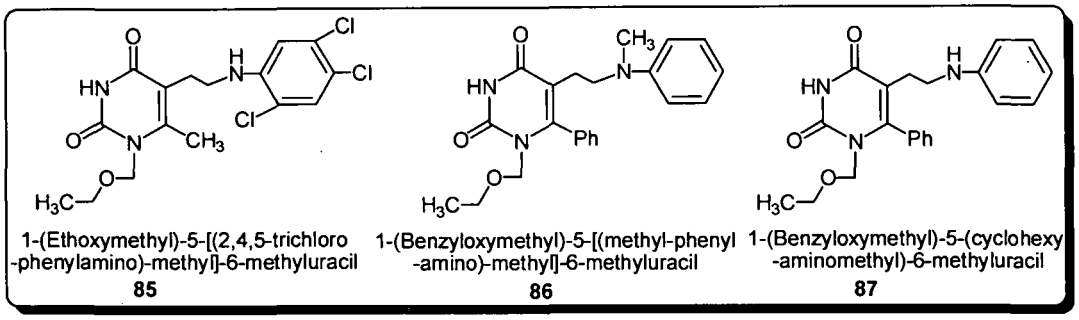



**Fig. 24:** Uracil derivatives having anti-HIV activity

**Anti-HCV activity and anti-HBV activity:**

A series of (2*R*,4*R*)- and (2*S*,4*S*)-nucleosides were tested against several viruses such as HIV-1 (MT-4 cells), HSV-1 (CCL81 cells), HSV-2 (CCL81 cells), HCMV (AD-169) and HBV (2.2.15 cells). None of the final nucleosides was found to be active against HIV-1, HSV-1 and HSV-2 up to 100 mg/mL, but many nucleosides exhibited weak to potent antiviral activities against HCMV and HBV. (2*R*,4*R*)-1-(2-Hydroxymethyl-3-methylenetetrahydrofuran-4-yl)-1*H*-pyrimidine-2,4-dione (**88**)[39] was the most active against HCMV (**Fig. 25**).

Another series of 2,3′-anhydro analogs of 5-substituted 1-(2-deoxy-β-D-lyxofuranosyl)uracils (**89**) and a related 1-(3-*O*-mesyl-2-deoxy-β-D-lyxofuranosyl) pyrimidine nucleoside analog (**90**) have been synthesized for evaluation as a new class of potential anti-HBV agents (**Fig. 25**). Few compounds demonstrated most potent anti-HBV activities against duck HBV (DHBV) and human HBV with $EC_{50}$ values in the range of 2.5-10 and 5-10 μg/mL, respectively, at non-toxic concentrations ($CC_{50}$ = >200

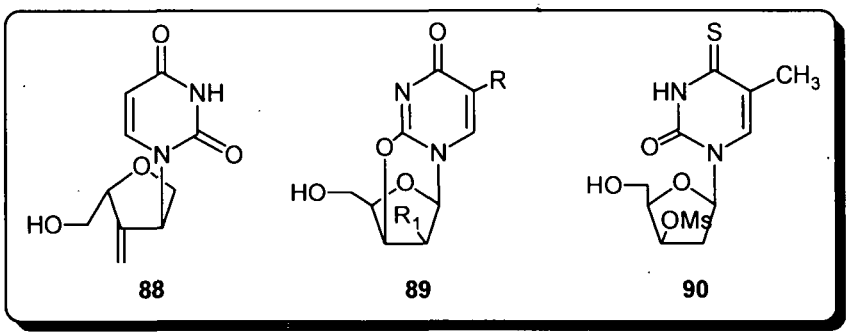



**Fig. 25:** Uracil derivatives as anti-HCV & anti-HBV agents

μg/mL).[40]

**Anti-oxidant activity:**

A series of pyrimido-quinolines, its amino derivatives and pyrimido-quinazolines[41] (**91**) were tested and evaluated for anti-oxidant, anti-inflammatory and analgesic activities. Out of them, 5,10-Dihydro-2-thioxo-pyrimido[4,5-*b*]quinolines showed the highest inhibitory anti-oxidant activity either using erythrocyte hemolysis or ABTS methods. Compounds of phenyl derivatives have proved to be more active than that of methoxy or chloro derivatives (**Fig. 26**).

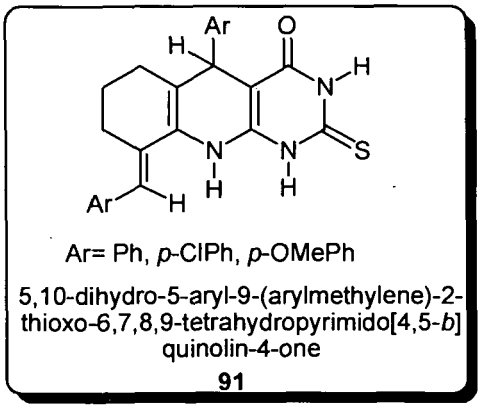



**Fig. 26:** Uracil derivatives having antioxidant activity

**Anti-inflammatory or analgesic activity:**

Various pyrido-pyrimidines[42] (**92-94**) (**Fig. 27**) were tested for analgesic activity and anti-inflammatory activity. Compound with morpholine substituent showed good activity; with the increased lipophilicity (*N*-methylpiperazine group), showed increased activity. Replacement of *N*-methylpiperazine group with piperidinyl group retains the activity. Interestingly these compounds showed one-third of ulcer index of the reference aspirin and diclofenac.

A series of 4,4,6-trimethyl-tetrahydropyrimidine-2-thione and 4,4,6-trimethyl-2-thioxo-3,4-dihydropyrimidine derivatives and their bis-pyrimidine derivatives[43] (**95-97**) were screened for anti-inflammatory and analgesic activities. Compounds 6-hydroxy-1-[2-(1*H*- imidazol-4-yl)ethyl]-4,4,6-trimethyl-tetrahydropyrimidine-2-thione and 1-3-

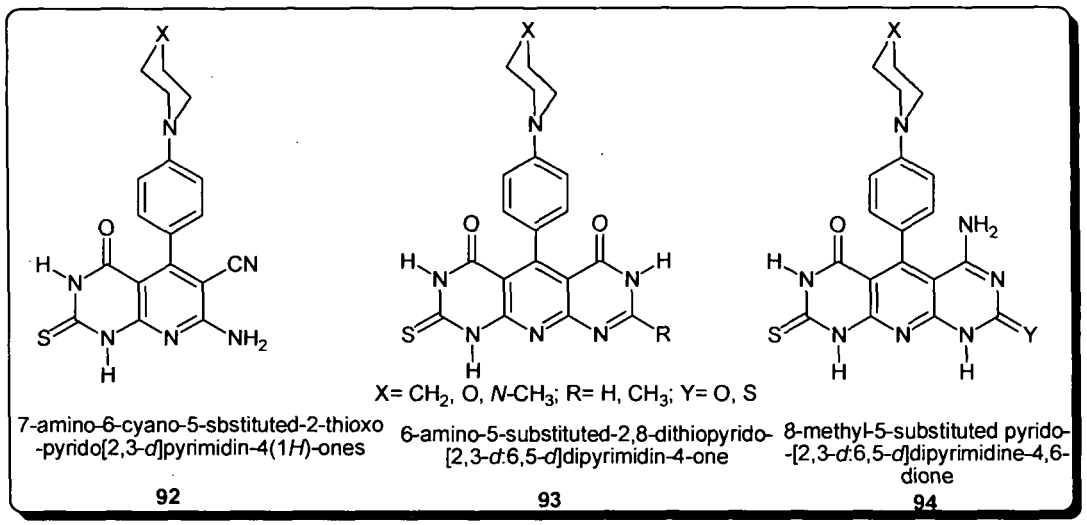



**Fig. 27:** Uracil derivatives having anti-inflammatory or analgesic activities

(6-hydroxy-4 -methyl-2-thioxo-tetrahydropyrimidine-1(2*H*)-ylpropyl)-pyrrolidin-2-one exhibited good anti-inflammatory and analgesic activities. Anti-inflammatory activity of 6-hydroxy-1-[2-(1*H*-imidazol-4-yl)ethyl]-4,4,6-trimethyl-tetrahydro-pyrimidine-2-thione is comparable while analgesic activity was found to be better than that of standard drug **(Fig. 28)**.

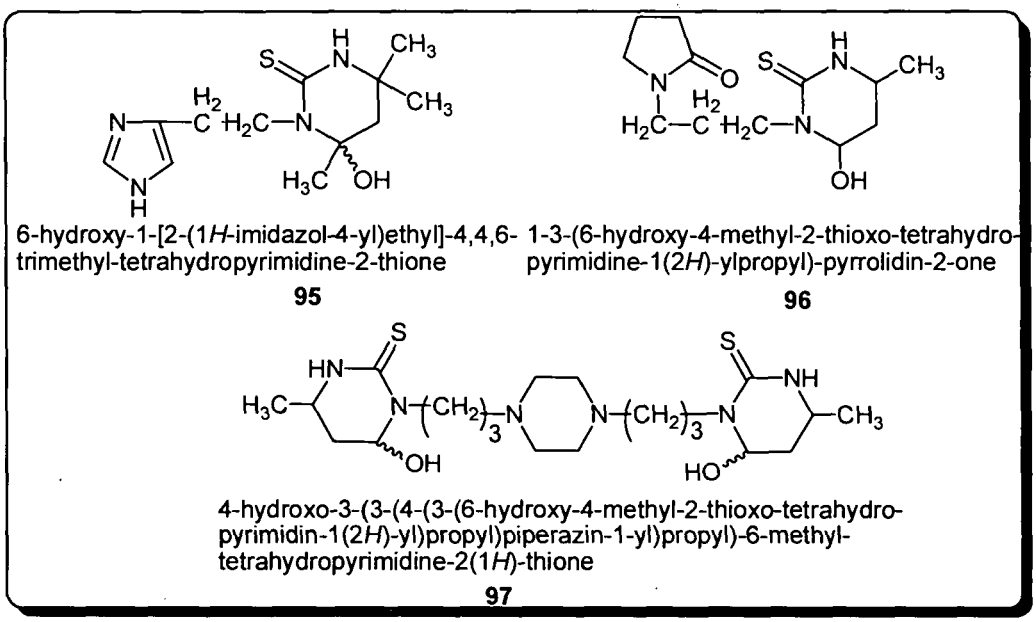



**Fig. 28:** Uracil compounds showing anti-inflammatory or analgesic activity

4-amino-5-cyano-2,6-diarylpyrimidines derivatives[44] and some new phthalimide derivatives (**98-101**) were evaluated against inflammation. Some of 4-amino-5-cyano-2,6-diaryl -pyrimidine derivatives with simple phenyl and -$OCH_3$ substituted phenyl exhibited better anti-inflammatory activity when compared with acetylsalicylic acid (ASA). Anti-inflammatory activity of phthalimide derivatives have been found to be twice more active than aspirin. Cytotoxical evaluations of compounds using neoplastic cells (NCI-$H_{292}$ and Hep-2) presented 41% of growth inhibition of neoplastic cells NCI-$H_{292}$ (**Fig. 29**).

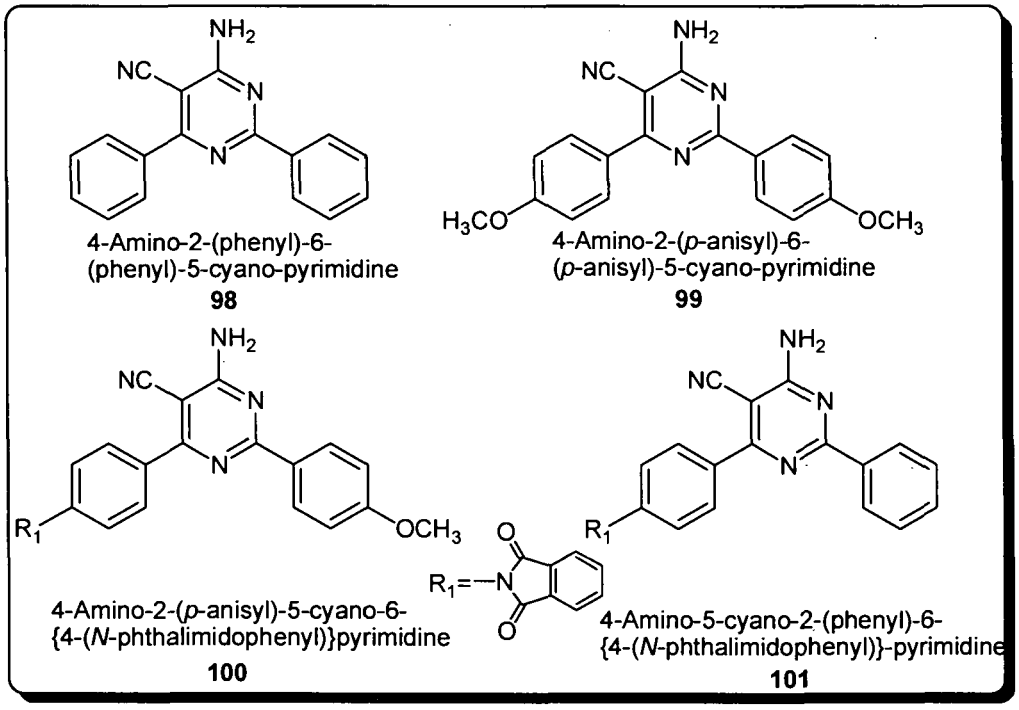



**Fig. 29:** Pyrimidine derivatives as anti-inflammatory agents

A series of [4,6-(substituted aryl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl]-acetic acid[45] derivatives (**102-104**) were screened for anti-inflammatory activity with standard drug diclofenac sodium. These activity data shows that presence of *p*-methoxy phenyl group at C-4 plays an important role to increase its activity and the presence of phenyl group at C-4 and C-6 reduces the anti-inflammatory activity but phenyl group at C-6 and *p*-chlorophenyl at C-4 increases the activity of compounds (**Fig. 30**).

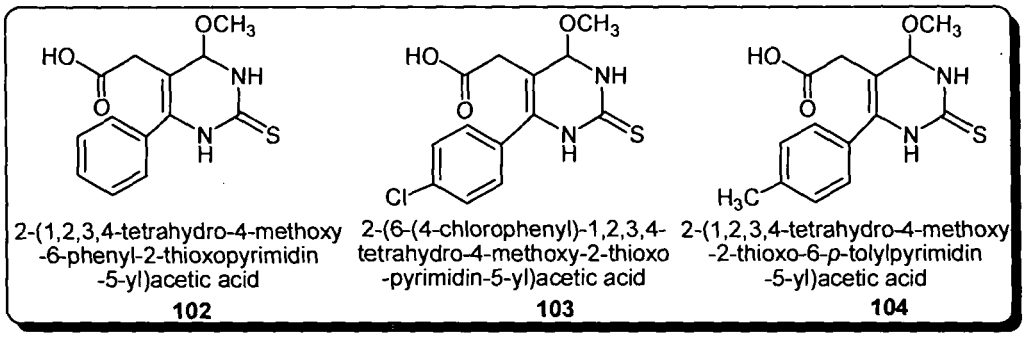



**Fig. 30:** Uracil derivatives showing anti- inflammatory activity

**Anti-histaminic activity (Histamine H receptor antagonists):**

Phenothiazine carboxylic acid derivatives[46] (**105-107**) having 6-amino-pyrimidine-2,4(1*H*,3*H*)-dione (**Fig. 31**) moiety via an appropriate linker, were found to have affinity towards human histamine $H_1$ receptor and Caco-2 cell permeability. Selected compounds were further evaluated for their oral antihistaminic activity in mice and bioavailability in rats. Finally, promising compounds were examined for their anti-inflammatory potential in mice OVA-induced biphasic cutaneous reaction model. Among the compounds tested, phenothiazineacetic acid compound showed both histamine $H_1$-receptor antagonistic activity and anti-inflammatory activity *in vivo* model.

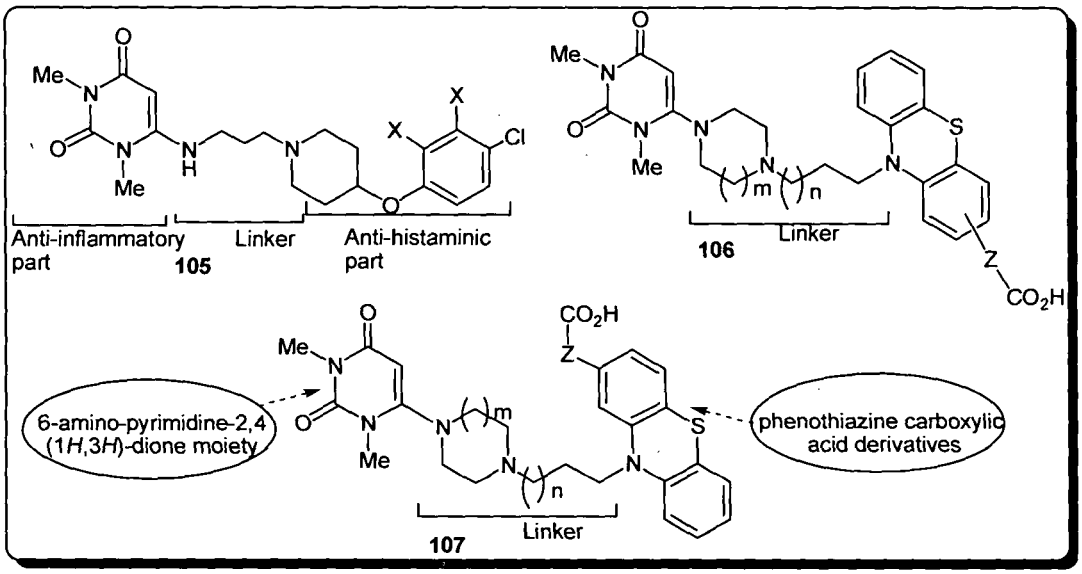



**Fig. 31:** Uracil derivatives as antihistaminic agents

6,7-Dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-2-ylamines **(108-111)** **(Fig. 32)** showed potent and selective *in vitro* histamine $H_4$ receptor antagonism across multiple species, good CNS penetration, improved PK properties compared to reference $H_4$ antagonists, functional $H_4$ antagonism in cellular and *in vivo* pharmacological assays, and *in vivo* anti-inflammatory and antinociceptive efficacy.[47] 4-((3*R*)-3-Aminopyrrolidin-1-yl)-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-2-ylamine, combined the best features of the series in a single molecule and is an excellent tool compound to probe $H_4$ pharmacology. It is a potent $H_4$ antagonist and has high selectivity for $H_4$, and combines good PK in rats and mice ($t_{1/2}$ of 2.6 and 1.6 h, oral bioavailability of 37% and 90%) with anti-inflammatory activity ($ED_{50}$= 37 $\mu$mol/kg, mouse) and efficiency in pain models (thermal hyperalgesia, $ED_{50}$= 72 $\mu$mol/kg, rat).

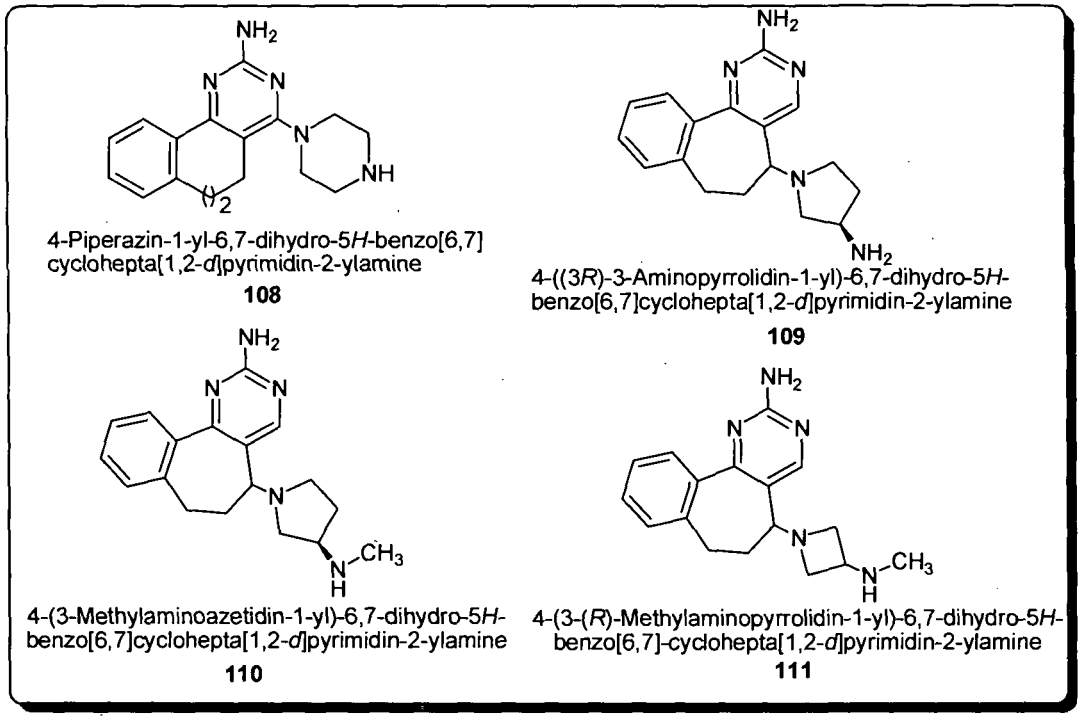
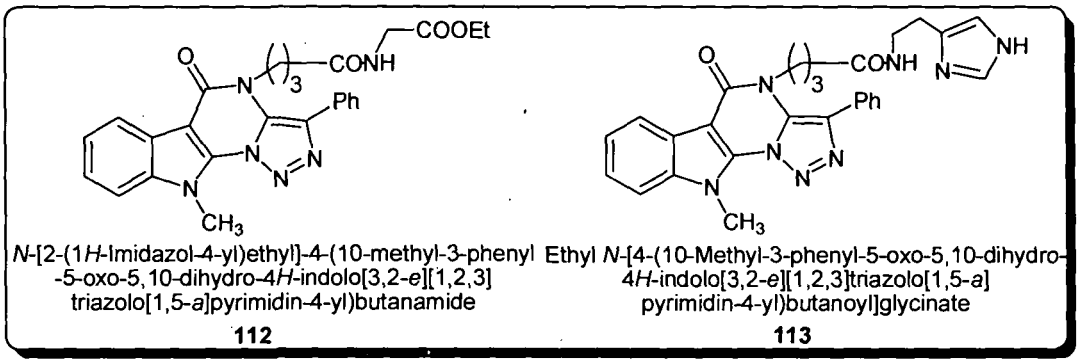



**Fig. 32:** Pyrimidine derivatives showing antihistaminic activity

**Antitumor activity:**

New derivatives of the (pyrrolo[3,4-*e*] and pyrrolo[2,3-*e*])[1,2,3]triazolo[1,5-*a*]pyrimidine and (indolo[2,3-*e*] and indolo[3,2-*e*])[1,2,3]triazolo[1,5-*a*]pyrimidine

(**112,113**),[48] were studied for the antitumor activity of three parameters for each cell line: $pGI_{50}$Value, pTGI value and $pLC_{50}$ value. The more active derivatives were shown to be *N*-[2-(1*H*-Imidazol-4-yl)ethyl]-4-(10-methyl-3-phenyl-5-oxo-5,10-dihydro-4*H*-indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-4-yl)butanamide and Ethyl *N*-[4-(10-Methyl-3-phenyl-5-oxo-5,10-dihydro-4*H*-indolo[3,2-*e*][1,2,3]-triazolo[1,5-*a*]pyrimidin-4-yl)butanoyl] - glycinate, endowed with significant anti-proliferative activity against the renal and CNS subpanels, respectively (**Fig. 33**).




*N*-[2-(1*H*-Imidazol-4-yl)ethyl]-4-(10-methyl-3-phenyl-5-oxo-5,10-dihydro-4*H*-indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-4-yl)butanamide

**112**

Ethyl *N*-[4-(10-Methyl-3-phenyl-5-oxo-5,10-dihydro-4*H*-indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-4-yl)butanoyl]glycinate

**113**

**Fig. 33:** Uracil derivatives having anti-tumour activity

Novel *N*-1-sulfonylpyrimidine derivatives (**114,115**) have a strong antiproliferative activity and an ability to induce apoptosis in treated tumor cells. Two *N*-1-sulfonylpyrimidine nucleobases[49] have showed catalytic activity of tumor cells' enzymes involved in DNA and RNA synthesis, and in *de novo* and salvage pyrimidine and purine syntheses. Investigations were performed *in vitro* on colon carcinoma cells (Caco2). The biosynthetic activity of the tumor cells enzymes was determined using sensitive radio-assays. Enzyme activity in treated cells was calculated relative to untreated control cells. Both of the investigated compounds, 1-(*p*-toluenesulfonyl)cytosine (TsC) and 5-bromo-1-(methanesulfonyl)uracil (BMsU) inhibited activities of specific enzymes involved in nucleic acid synthesis. BMsU strongly inhibited activities of DNA polymerase a (53%), thymidine kinase (68%), thymidilate synthase (43%), and ribonucleotide reductase (46%). *De novo* biosynthesis of pyrimidine and purine was reduced by 20%. TsC was able to inhibit RNA polymerase (37%), orotatephosphoribosyl transferase (39%), uridine kinase (44%), ribonucleotide

reductase (47%), and *de novo* purine synthesis (61%). Antitumor activity of TsC and BMsU are closely associated with their inhibitory activity on enzymes that play an important role in the metabolism of tumor cells (**Fig. 34**).

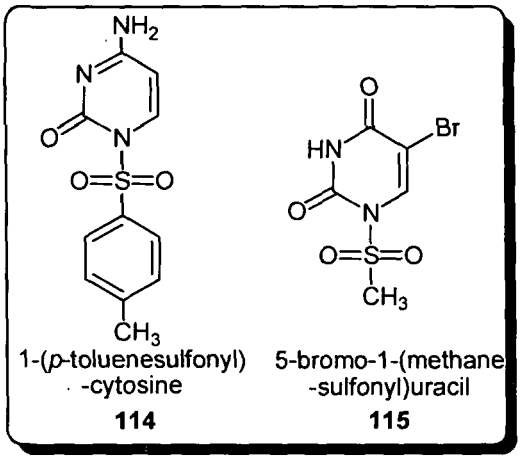



**Fig. 34:** Uracil derivatives having antiproliferative activity

The syntheses of the novel C-5 substituted pyrimidine derivatives (**116,117**)[50] of L-ascorbic acid containing free hydroxy groups at C-2′ or C-2′ and C-3′ positions of the lactone ring were evaluated for their cytostatic activity against malignant tumour cell lines: murine leukaemia (L1210), human T-lymphocytes (Molt4/C8 and CEM), cervical carcinoma (HeLa), breast carcinoma (MCF-7), pancreatic carcinoma (Mia-PaCa-2), laryngeal carcinoma (Hep-2), colon carcinoma (SW620) and human normal fibroblasts (WI38). Some compounds in the series showed the best inhibitory activities. Cytostatic activities of conjugates were also studied. Among the pyrimidine derivatives of L-ascorbic acid, 5-fluorouracil and 5-(trifluoromethyl)uracil derivatives of 2,3-dihydroxy-L-ascorbic acid showed rather marked cytostatic activities. Compound 5-fluorouracil derivatives had the highest inhibitory activity against murine leukaemia (L1210) cells ($IC_{50}$: 5.2 lM), while 5-(trifluoromethyl)uracil derivatives showed pronounced inhibitory activity against all human malignant cell lines ($IC_{50}$: 5.6–12.8 lM) except for human Tlymphocytes (**Fig. 35**).

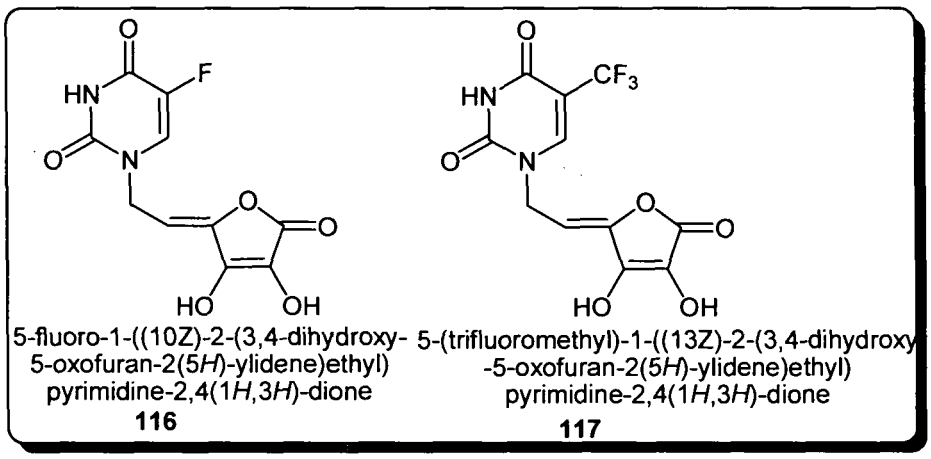



**Fig. 35:** Uracil derivatives describing cytostatic activity

5-Bromo-1-mesyluracil (**115**) (BMsU)[51] was used to elucidate the effects of BMsU on the biosynthetic activity of tumor cell enzymes involved in DNA, RNA and protein syntheses, and in *de novo* and salvage pyrimidine and purine synthesis. Investigations were performed *in vitro* on human cervix carcinoma cells (HeLa). BMsU displayed inhibitory effects on DNA and RNA synthesis in HeLa cells after 24 h of treatment. *De novo* biosynthesis of pyrimidine and purine was also affected. Antitumor activity of BMsU is closely associated with its inhibitory activity on the enzymes that play an important role in the metabolism of tumor cells. *In vivo* antitumor activity of BMsU was also investigated. Significant reduction in tumor growth time was achieved with BMsU administered at a dose of 50 mg/kg (**Fig. 36**).

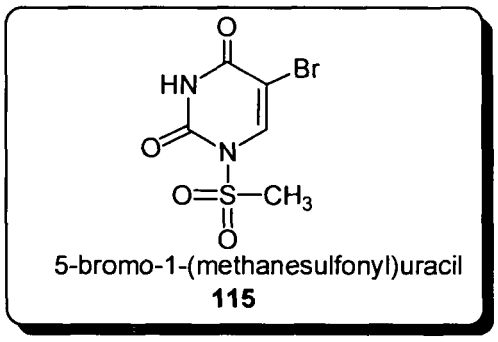



**Fig. 36:** Uracil derivative showing antitumor activitiy

A series of 6-methylene-bridged uracil derivatives (**118-123**)[52] have been used as inhibitors of human thymidine phosphorylase (TP). To enhance the *in vivo* antitumour activity of fluorinated pyrimidine 2'-deoxyribonucleosides such as 2'-deoxy-5-(trifluoromethyl) uridine ($F_3$dThd), a potent TP inhibitor preventing their degradation to an inactive compound, has become a target of medicinal chemistry. Introduction of an *N*-substituted aminomethyl side chain at the 6-position of 5-chlorouracil has improved water solubility and enhanced inhibitory activity compared with the known TP inhibitor, 6-amino-5-chlorouracil. 5-Bromo-6-(pyrrolidinylmethyl)uracil was reasonably well absorbed in mice after oral administration. Combining with $F_3$dThd, 5-bromo-6-(pyrrolidinyl -methyl)uracil exerted its TP inhibitory potency by increasing the maximum plasma concentrations of the former as evidenced in experiments with monkeys. Positive changes in pharmacokinetic profile were accompanied by the enhanced *in vivo* antitumor activity of this combination when compared to $F_3$dThd alone, in mice bearing human tumorxenografts (**Fig. 37**).

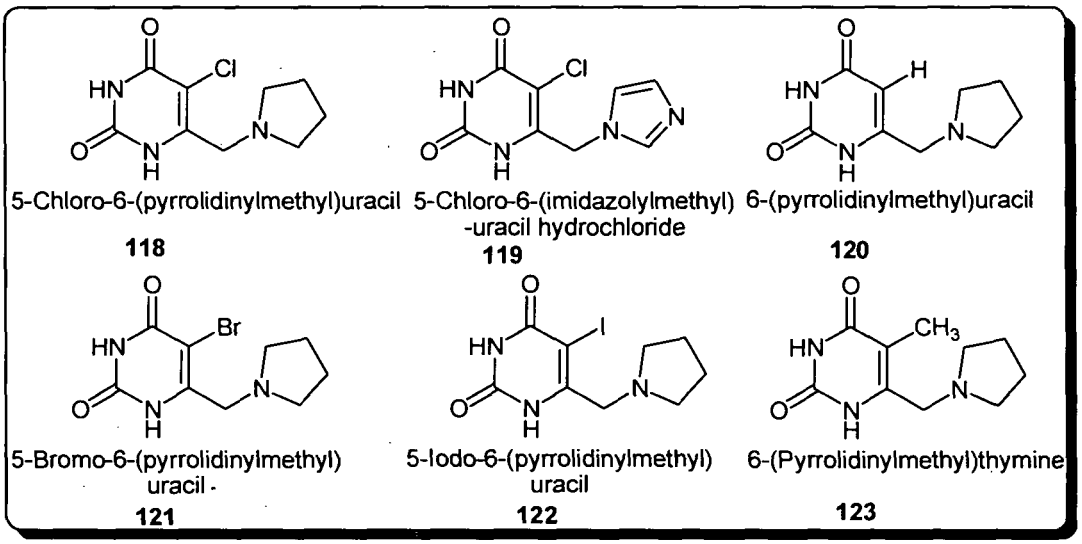



**Fig. 37:** Uracil derivatives describing antitumor activity

**Antitubercular activity:**

A series of 3,6-dimethyl-6-aryl-1,5,6,7-tetrahydro-8*H*-pyrazolo[3',4':4,5]thieno [2,3-*d*]pyrimidin-8-ones (**124**)[53] were screened for antitubercular activity against *M. tuberculosis* $H_{37}$ RV. Biological studies indicate that the presence of the 4-chloro phenyl

substituent in the 2- position of the pyrimidine ring of the pyrazolo[3′,4′:4,5]thieno[2,3-*d*]pyrimidin-8-ones gives useful biological activity (**Fig. 38**).

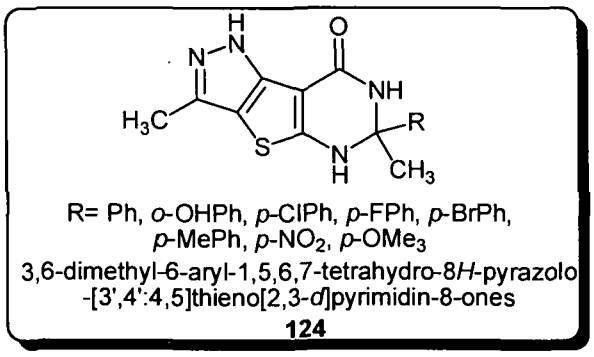



**Fig. 38:** Pyrimidine derivatives showing antitubercular activity

Several derivatives of $N^1$-(4-substituted-benzyl)-pyrimidines (**125**)[54] were synthesized as potential inhibitors of thymidine monophosphate kinase of *M. tuberculosis* (TMPKmt). Key SAR parameters included the chain length substitution in para position of the benzyl ring, the functional group terminating the alkyl chain, and the substituent on the C-5 pyrimidine ring. These molecules were assayed against both recombinant enzyme and mycobacteria cultures. The most potent compounds have $K_i$ values in the micromolar range and a $MIC_{50}$ of 50 μg/mL against *M. bovis* (**Fig. 39**).

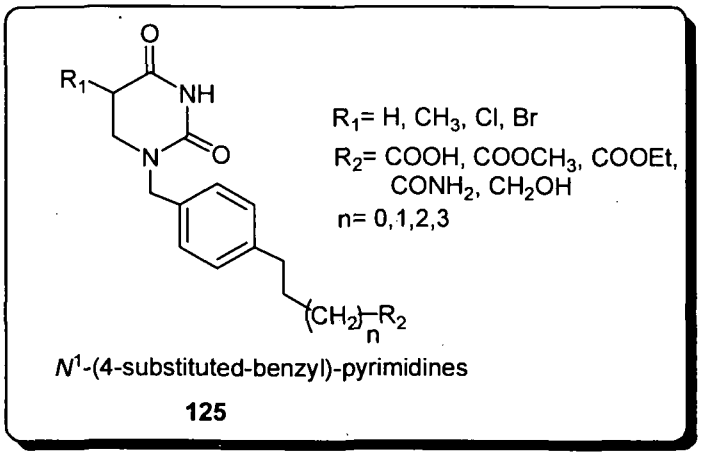



**Fig. 39:** Pyrimidine derivatives having antitubercular activity

*M. tuberculosis* and *M. avium* infections cause the two most important mycobacterioses, leading to increased mortality in patients with AIDS. Various 5-substituted 2′-deoxyuridines, uridines, 2′-*O*-methyluridine, 2′-ribofluoro-2′-deoxyuridines, 3′-substituted-2′,3′-dideoxyuridines, 2′,3′-dideoxyuridines, and 2′,3′-didehydro-2′,3′-dideoxyuridines derivatives (**126-129**)[55] were evaluated for their *in vitro* inhibitory activity against *M. bovis* and *M. avium*. 5-(C-1 Substituted)-2′-deoxyuridine derivatives emerged as potent inhibitors of *M. avium* ($MIC_{90}$ = 1-5 μg/mL range). The nature of C-5 substituents in the 2′-deoxyuridine series appeared to be a determinant of anti-mycobacterial activity. This new class of inhibitors could serve as useful compounds for anti-tuberculosis agents (**Fig. 40**).

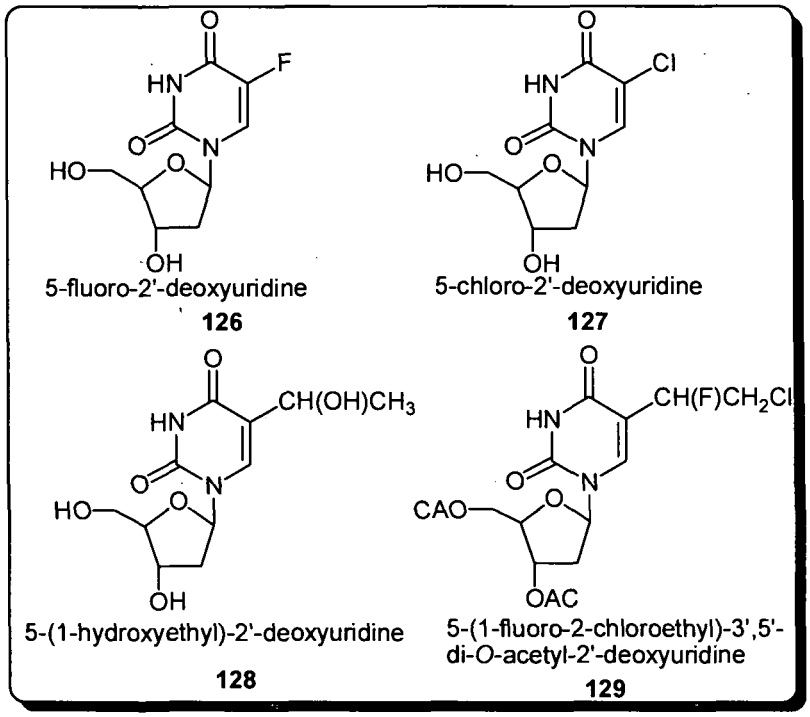



**Fig. 40:** Uracil derivatives having antitubercular activity

**Adenosine receptor antagonists:**

The 8-substituted-1,3-dimethylxanthines (**130-132**)[56] were evaluated in radio ligand binding studies at cloned human $A_1$ and $A_{2A}$ adenosine receptors. [$^3$H]DPCPX and [$^3$H]ZM-241385 were used as radio ligands for $A_1$ and $A_{2A}$ adenosine receptors,

respectively. The three series of xanthine derivatives such as disubstituted vanilloid based xanthine, isovanilloid based xanthine and monosubstituted xanthine derivatives exhibited varying degrees of affinity and selectivity towards $A_1$ and $A_{2A}$ receptor subtypes. The effects of varying the positions of 8-phenyl substituents on affinity and selectivity at $A_1$ and $A_{2A}$ adenosine receptors have been studied. Isovanilloid 1,3-dimethyl-8-[4-methoxy-3-(2-morpholin-4-ylethoxy)phenylxanthine displayed the highest affinity and selectivity towards $A_{2A}$ AR subtypes with $K_i$¼ 100 nM over $A_1$ receptors ($K_i$ > 100 mM). It has been observed that substitution pattern on 8-phenyl group greatly affects the affinity and selectivity at adenosine receptors, with $A_{2A}$ tolerating bulkier substituents than did $A_1$ receptors (**Fig. 41**).

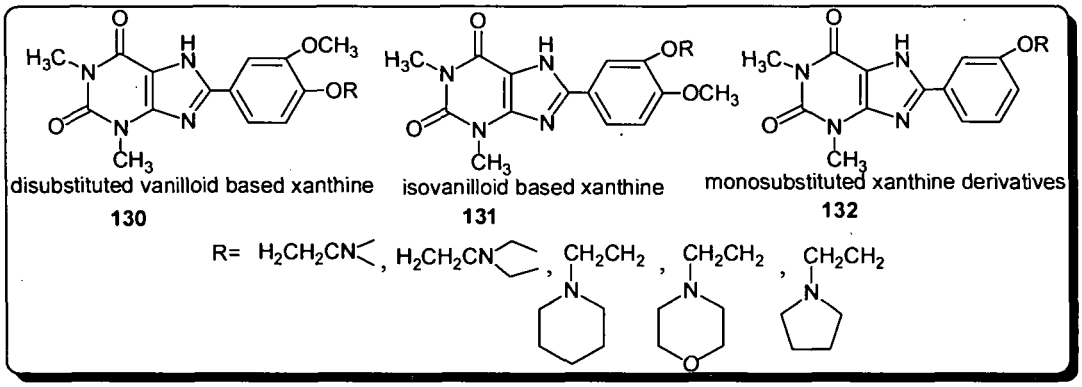



**Fig. 41:** Pyrimidine derivatives as adenosine receptor antagonists

New derivatives of 4-aryl-pyrido[1,2-*c*]pyrimidine containing the 3-(4-piperidyl)-1*H*-indole residue or its 5-methoxy derivative (**133-136**)[57] were characterized (i) *in vitro* by binding to 5-$HT_{1A}$ receptors and 5-HT transporter proteins in rat brain cortex membranes and (ii) *in vivo* in the mouse by induced hypothermia and forced swimming models for antagonist/agonist activity against the 5-$HT_{1A}$ auto receptors and postsynaptic 5-$HT_{1A}$ receptors, respectively. SAR evaluation indicated that the presence of the 3-(4-piperidyl)-1*H*-indole residue and ortho- or para-substituents with -F or -$CH_3$ groups in the aryl ring as well as an unsubstituted aryl in the 4-aryl-pyrido[1,2-*c*]pyrimidine moiety promoted low micromolar inhibition constants ($K_i$) values for both receptors. In contrast, the presence of a 5-methoxy-3-(4-piperidyl)-1*H*-indole residue as well as -Cl or -$OCH_3$ substituents at the para position markedly reduced the receptor affinity (**Fig. 42**).

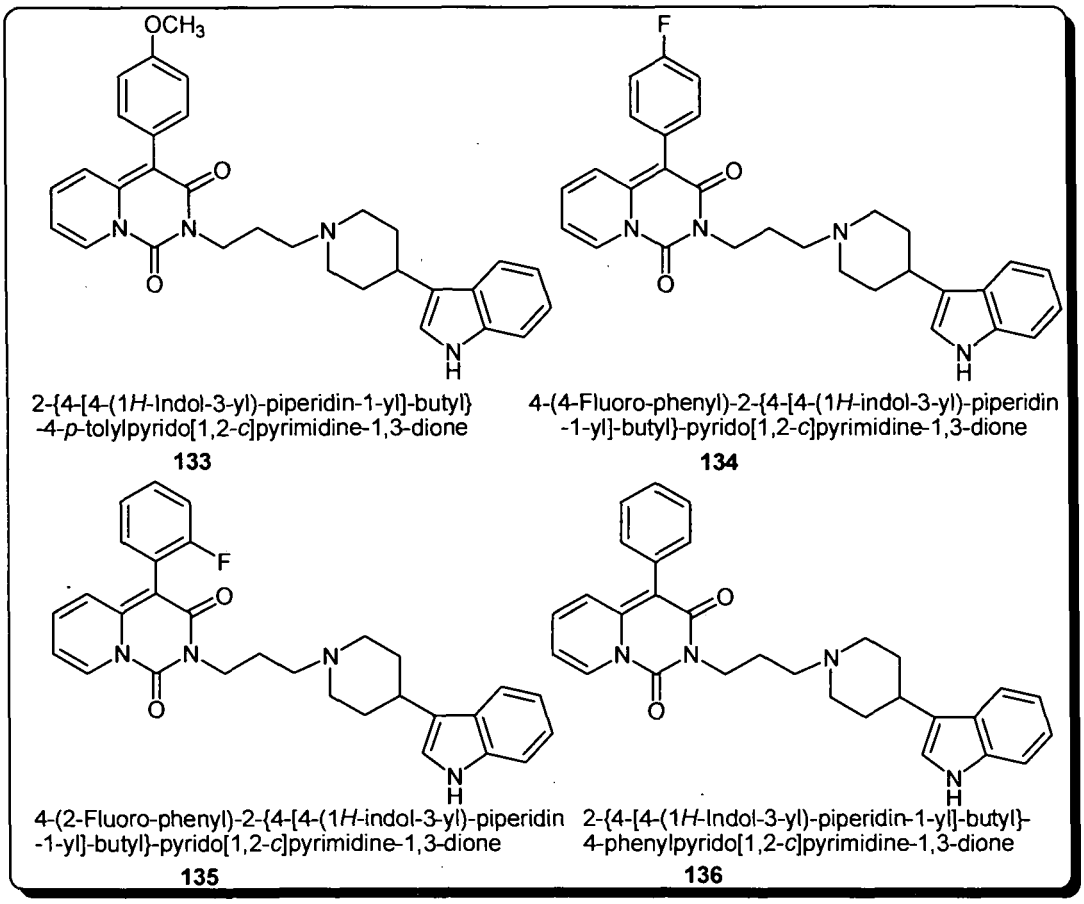



**Fig. 42:** Pyrimidine derivatives as adenosine receptor antagonists

A series of 5'-deoxy-5'-morpholine, piperidine, and pyrrolidine of pyrimidine nucleosides (**137-142**)[58] have been shown to have inhibitory action to RNase A. These compounds are moderate inhibitors of RNase A with mid-to-upper micromolar inhibition constants ($K_i$). Structure-activity relationship analysis has demonstrated that the compounds with the larger group in the 5′ position are more potent. Comparative structural analysis of these RNase A complexes with other similar RNase A-ligand complexes provides a structural explanation of their potency and suggests ways to improve their efficiency and selectivity. These inhibitors can be the starting point for the development of compounds that can be used as pharmaceuticals against pathologies associated with RNase A homologues such as human angiogenin, which is implicated in tumor induced neovascularization (**Fig. 43**).

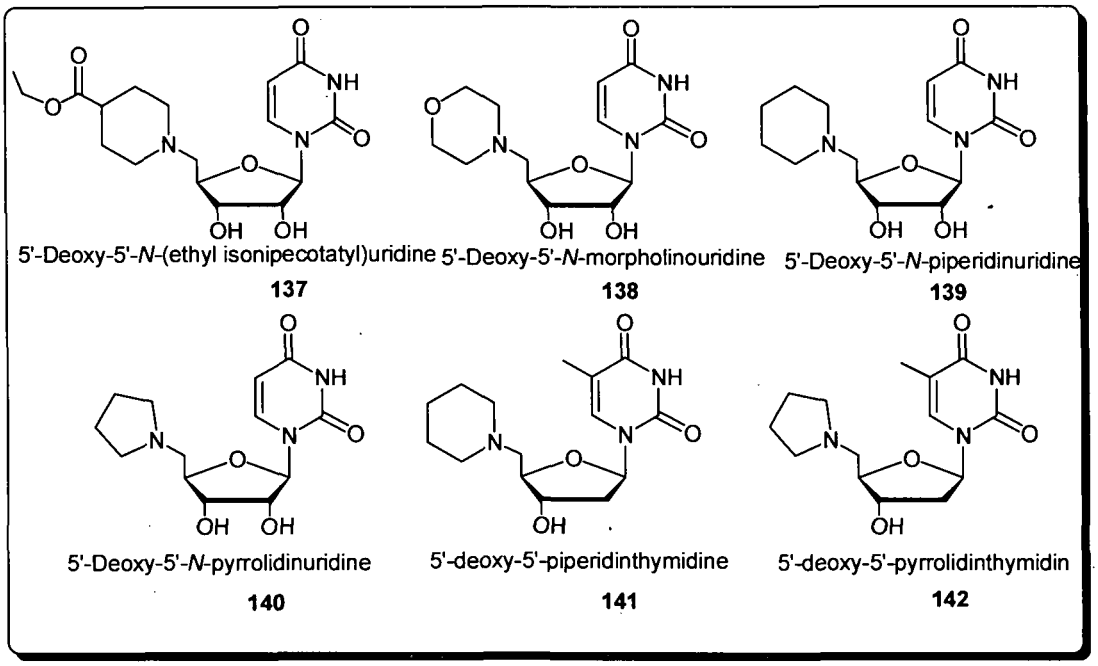



**Fig. 43:** Pyrimidine derivatives as adenosine receptor antagonists

A series of 1-benzyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-dione derivatives (**143** & **144**)[59] and a regioisomeric series of diaryl 2- or 4-amidopyrimidines (**145**)[60] are new, potent and selective $A_3$ adenosine receptor antagonists containing a xanthine core. On extension of SAR studies on related structures in which the effect of different kind of substitutions at the 1-, 3- and 8-positions has been evaluated in order to improve both the potency and the hydrophilicity of the originally synthesized molecules. The $A_3$ binding

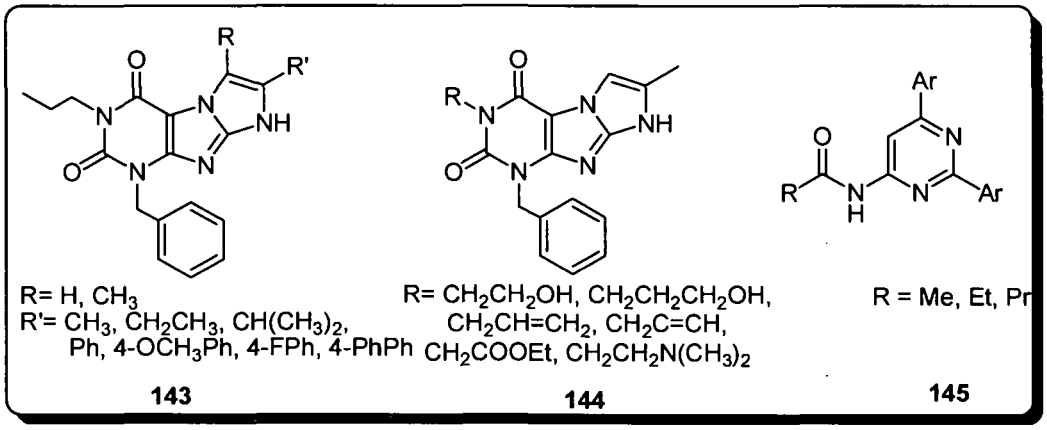



**Fig. 44:** Pyrimidine derivatives as adenosine receptor antagonists

disposition of these compounds was also investigated through docking and 3D-QSAR studies (**Fig. 44**).

A one-pot route to 8-substituted xanthenes (**146,147**)[61] has been developed from 5,6-diaminouracils and carbaldehydes. The process, promoted by (bromodimethyl)-sulfonium bromide, is mild and efficient and eliminates the need for external oxidants. Yields are good and the process is applicable to a range of substrates including a family of $A_{2A}$ adenosine receptor antagonists. Preparation of a new analog of the antagonist KW-6002 is presented, and in situ bromination of aryl substituted products demonstrated (**Fig. 45**).

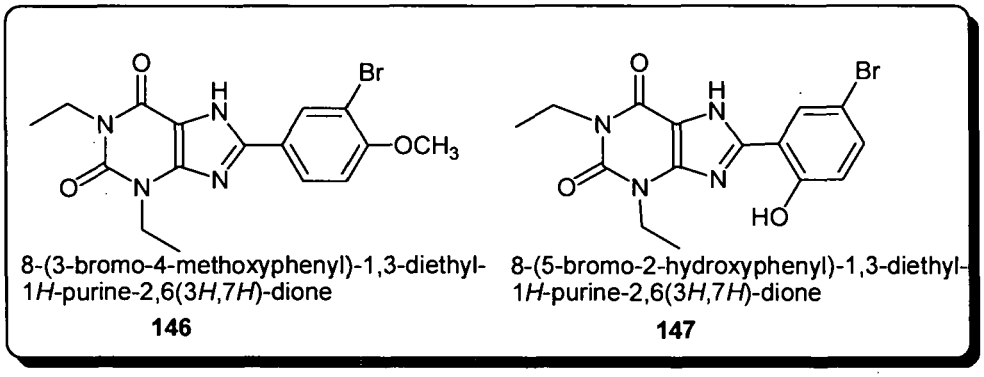



**Fig. 45:** Pyrimidine derivatives as adenosine receptor antagonist

The adenosine $A_{2A}$ receptor has emerged as an attractive target for the treatment of Parkinson's disease (PD). Evidence suggests that antagonists of the $A_{2A}$ receptor ($A_{2A}$ antagonists) may be neuroprotective and may help to alleviate the symptoms of PD. Several members of the (*E*)-8-styrylcaffeine class of $A_{2A}$ antagonists also are potent inhibitors of monoamine oxidase B (MAO-B). Since MAO-B inhibitors are known to possess anti-parkinsonian properties, dual-target-directed drugs that block both MAO-B and $A_{2A}$ receptors may have enhanced value in the management of PD. In an attempt to explore this concept further three additional classes of C-8 substituted caffeinyl analogues[62] were prepared. The 8-phenyl- (**148**) and 8-benzylcaffeinyl (**149**) analogues exhibited relatively weak MAO-B inhibition potencies while selected (*E,E*)-8-(4-phenylbutadien-1-yl)caffeinyl (**150**) analogues were found to be exceptionally potent reversible MAO-B inhibitors with enzyme-inhibitor dissociation constants ($K_i$ values)

ranging from 17 to 149 nM. Furthermore, these (*E,E*)-8-(4-phenylbutadien-1-yl)caffeines acted as potent $A_{2A}$ antagonists with $K_i$ values ranging from 59 to 153 nM. The (*E,E*)-8-(4-phenylbutadien-1-yl)caffeines are a promising candidate class of dual-acting compounds (**Fig. 46**).

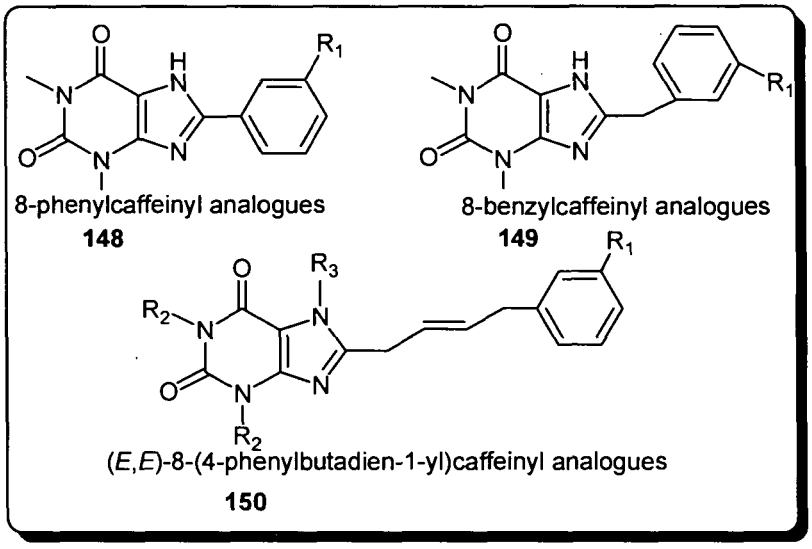



**Fig. 46:** Pyrimidine derivatives as adenosine receptor antagonist

D-mannosyl, D-galactosyl and D-glucosyl theophylline nucleosides (**151-152**)[63] (**Fig. 47**) are found to inhibit specific binding at $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ adenosine receptors.

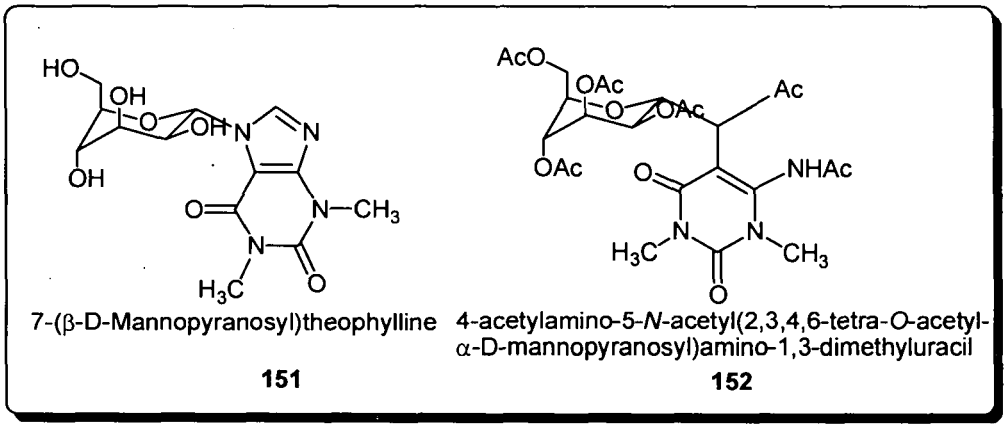



**Fig. 47:** Pyrimidine derivatives as adenosine receptor antagonist

A new series of 1,3-dipropyl-8-(1-phenylacetamide-1*H*-pyrazol-3-yl)-xanthine derivatives (**153**) [64] has been identified as potent $A_{2B}$ adenosine receptor antagonists. The products have been evaluated for their binding affinities for the human $A_{2B}$, $A_1$, $A_{2A}$, and $A_3$ adenosine receptors. *N*-(4-chloro-phenyl)-2-[3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-*H*-purin-8-yl)-5-methyl-pyrazol-1-yl] (**154**) showed a high affinity for the human $A_{2B}$ adenosine receptor $K_i$=7 nM and good selectivity ($A_1$, $A_{2A}$, $A_3/A_{2B}$> 140). The SAR also showed that the activity of the compounds depend on its substitution (**Fig. 48**).

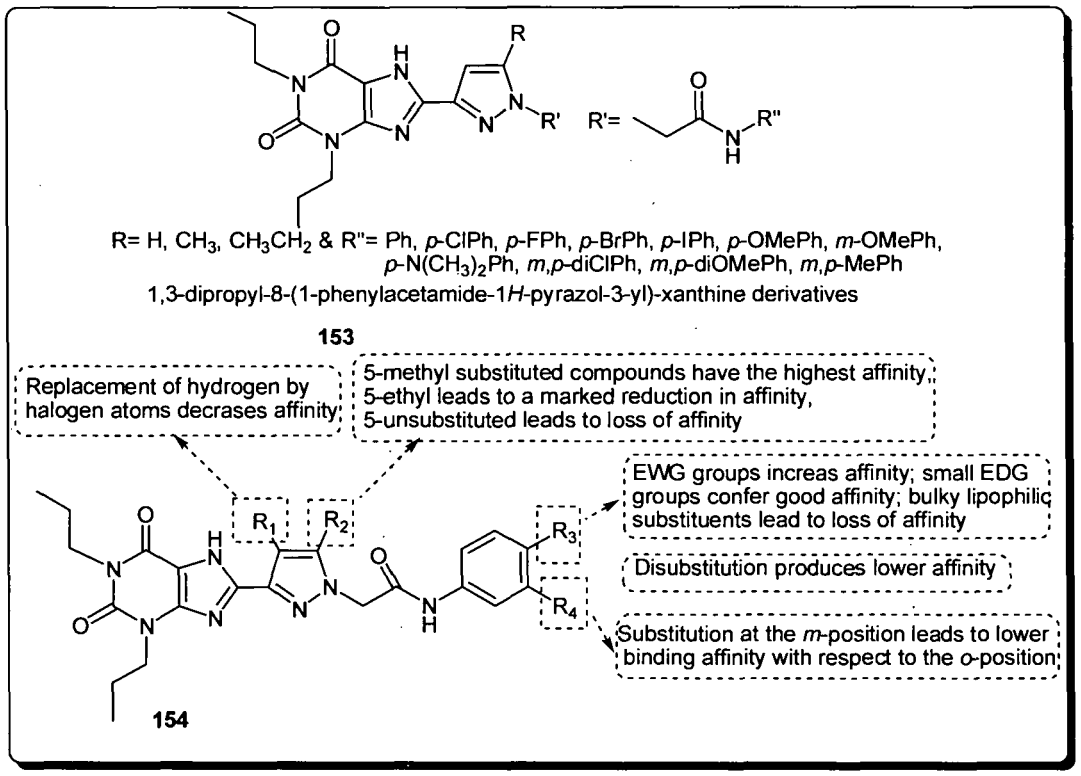



**Fig. 48:** Pyrimidine derivatives as adenosine receptor antagonist

*N*-benzyl pyrimido[2,1-*f*]purinedione derivatives (**155**)[65] were evaluated for their affinity to adenosine $A_1$ and $A_{2A}$ receptors, selected compounds were additionally investigated for affinity to the $A_3$ receptor subtype. The results of the radioligand binding assays to $A_1$ and $A_{2A}$ adenosine receptors showed that most of the 1,3-dimethyl-9-benzylpyrimidopurinediones exhibited selective affinity to $A_{2A}$ receptors at micromolar

or submicromolar concentrations. The best adenosine $A_1$ receptor ligand was *m*-chlorobenzyl derivative. SARs were discussed with the analysis of lipophilic and spatial properties of the investigated compounds (**Fig. 49**).

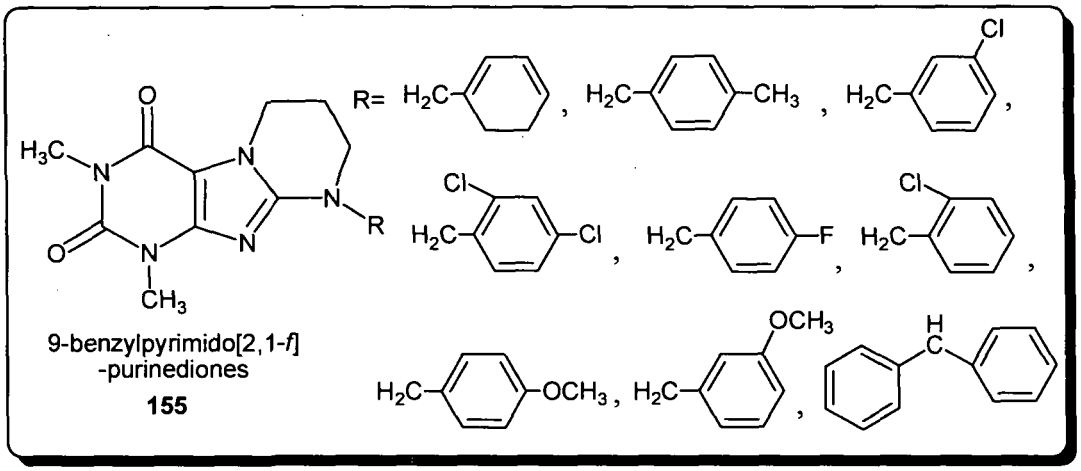



**Fig. 49:** Pyrimidine derivatives as adenosine receptor antagonist

The adenosine $A_{2A}$ receptor has emerged as a possible target for the treatment of PD. Evidence suggests that antagonism of the $A_{2A}$ receptor not only improves the symptoms of the disease but may also protect against the underlying degenerative processes. It has been reported that several known adenosine $A_{2A}$ receptor antagonists ($A_{2A}$ antagonists) also are moderate to very potent inhibitors of monoamine oxidase B (MAO-B). (*E*)-8-styrylcaffeinyl analogues (**156**) were found to be inhibitors of MAO-B. The most potent among these was (*E*)-8-(3-chlorostyryl) caffeine (CSC),[66] a compound frequently used when examining the *in vivo* pharmacological effects of $A_{2A}$ antagonists. Since MAO-B inhibitors are also thought to possess anti-parkinsonian properties, dual targeting drugs that block both MAO-B and $A_{2A}$ receptors may have enhanced therapeutic potential in the treatment of PD (**Fig. 50**).

Amino-substituted pyrido[2,3-*d*]pyrimidinediones (**157-160**)[67] have been found to bind to adenosine $A_1$ and $A_{2A}$ receptors in micromolar concentrations. The SARs of this class of compounds were provided with polar substituents, such as ethoxycarbonyl groups and basic amino functions, in order to improve their water-solubility. The most potent and selective compound of the present series was 6-carbethoxy-1,2,3,4-tetrahydro-

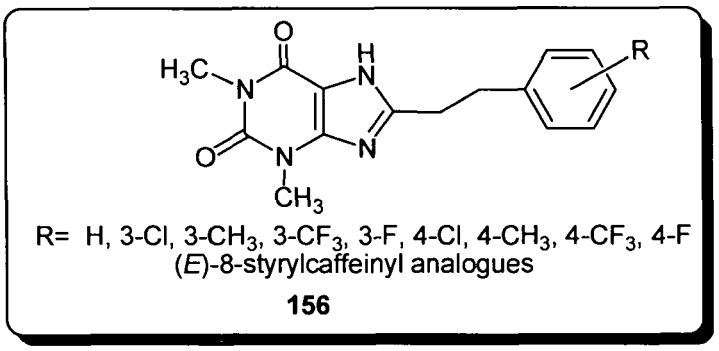



**Fig. 50:** Pyrimidine derivatives as adenosine receptor antagonists

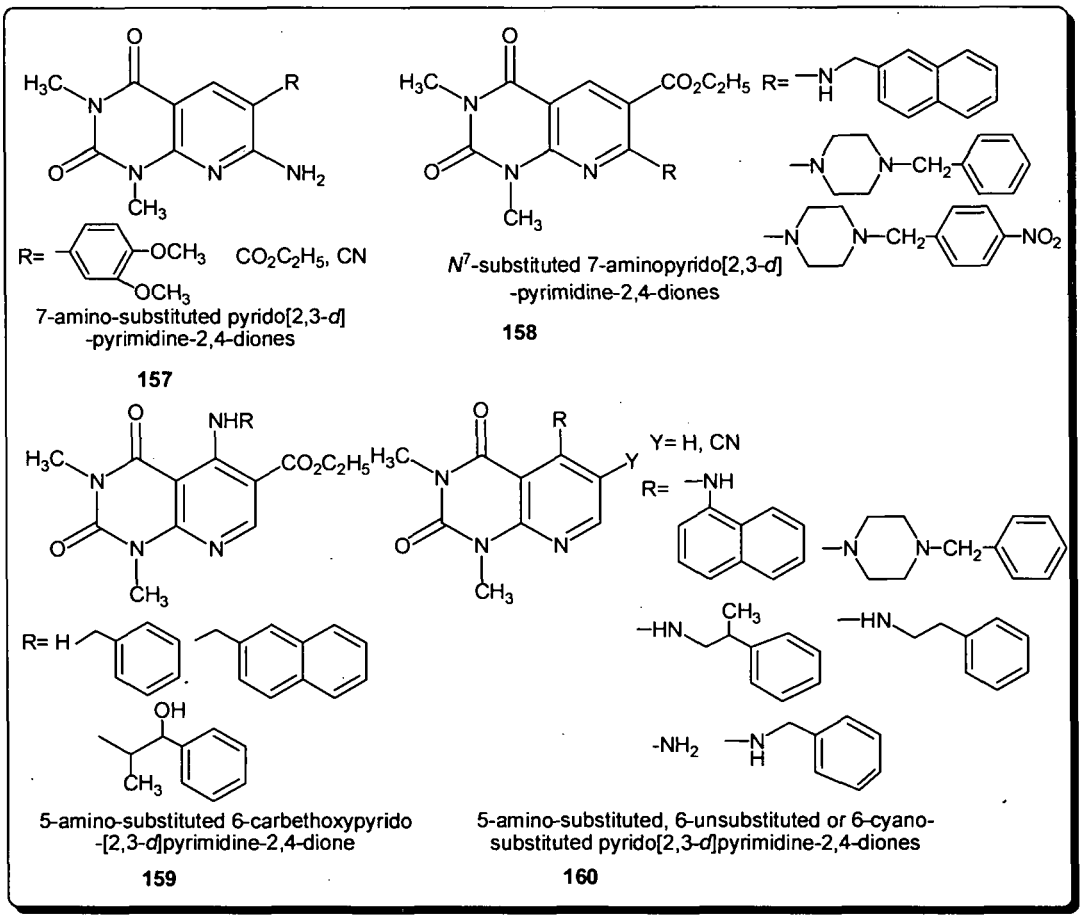



**Fig. 51:** Pyrimidine derivatives as adenosine receptor antagonists

1,3- dimethyl-5-(2-naphthylmethyl)-aminopyrido[2,3-*d*]pyrimidine-2,4-dione with a $K_i$ value of 5 nM at rat and 25 nM at human $A_1$ receptors. The compound was more than 60-

fold selective versus $A_3$ and more than 300-fold selective versus $A_{2A}$ receptors. It showed an over 300-fold improvement with respect to the lead compound. In GTPγS binding studies at membranes of Chinese hamster ovary cells recombinantly expressing the human adenosine $A_1$ receptor, behaved as an antagonist with inverse agonistic activity. A regioisomer of 6-carbethoxy-1,2,3,4-tetrahydro-1,3-dimethyl-7-(2-naphthylmethyl) - aminopyrido[2,3-*d*]pyrimidine-2,4-dione in which the 2-naphthylmethyl- amino substituent at position 5 was moved to the 7-position, was a relatively potent ($K_i$=226 nM) and selective (>20-fold) $A_3$ ligand. In the series of compounds lacking an electron-withdrawing ethoxycarbonyl or cyano substituent in the 6-position, compounds with high affinity for adenosine $A_{2A}$ receptors were identified, such as 1,2,3,4-tetrahydro-1,3-dimethyl-5-(1-naphthyl)aminopyrido[2,3-*d*]pyrimidine-2,4-dione ($K_i$ human $A_{2A}$ = 81.3 nM, $K_i$ human $A_1$ = 153 nM, and $K_i$ human $A_3$> 10,000 nM) (**Fig. 51**).

**MMP inhibitors:**

The most significant impediment to the development of an MMP (matrix metalloproteases) inhibitor for the treatment of any diseases musculoskeletal syndrome (MSS), a side effect clinically observed with non-selective MMP inhibitors. MSS is characterized by a stiffening of various joints and may derive from a lack of normal turnover of extra cellular matrix due to inappropriate inhibition of one or more non-targeted MMPs. Using SAR from two related series of pyrimidinetrione-based inhibitors,[68] compounds with potent MMP-13 inhibition and >100-fold selectivity against other MMPs have been identified. Despite high molecular weights and polar surface areas, the compounds are generally well absorbed and have excellent pharmacokinetic (PK) properties when dosed as sodium salts. In a rat fibrosis model, a compound from the series displayed no fibrosis at exposures many fold greater than its MMP-13 $IC_{50}$. The series of pyrimidine derivatives display good potency and promising selectivity. Two of these, the 4-aryloxazol-2-yl and 3-aryl-1,2,4-oxadiazol-5-yl systems (**161,162**), were investigated thoroughly with particular attention to the effects of substituents on the pendant aryl ring (**Fig. 52**).

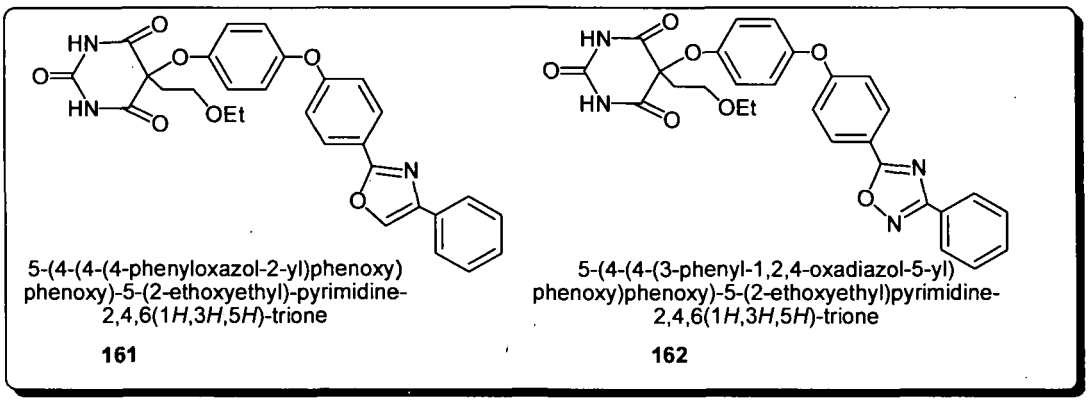



**Fig. 52:** Pyrimidine derivatives as MMP inhibitors

**Radio sensitizing activity:**

Two novel dual functional agents, 3[3-(2,4-dinitro-phenylamino)propyl]-5-fluoro -1*H*-pyrimidine-2,4-dione (**163**) and *N*-[3-(2,4-dinitro-phenylamino)propoxy]urea (**164**),[69] (**Fig. 53**) resulting from linkage of 2,4-dinitrophenylamine through three carbon atoms with 5-fluorouracil and hydroxyurea, respectively, were *in vitro* aerobic cytotoxicities in HT-29 cell line with and without radiation were determined. Compounds unlike their components were not cytotoxic but showed radiosensitizing activity.

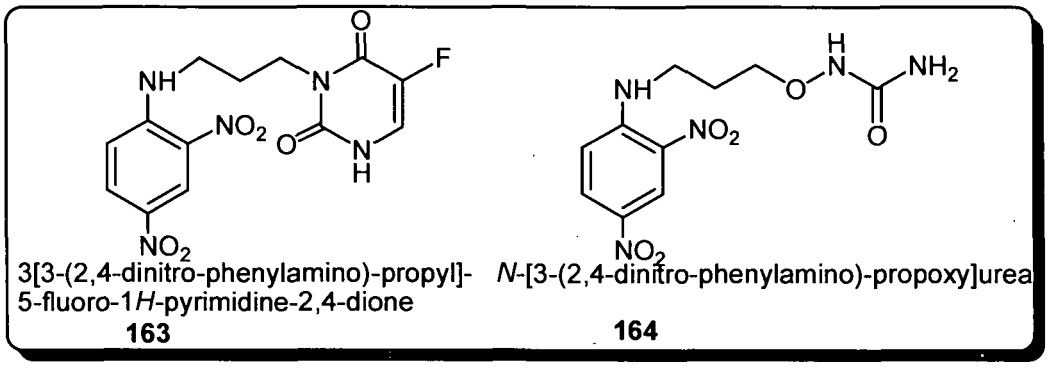
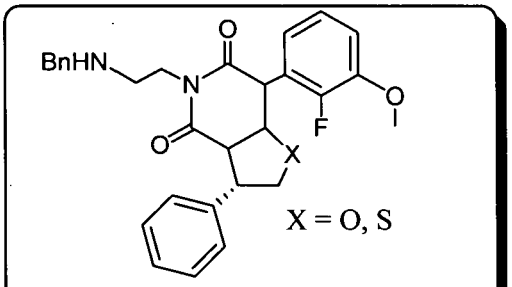



**Fig. 53:** Pyrimidine derivatives demonstrating radio sensitizing activity

**Gonadotropin-releasing hormone antagonist:**

Gonadotropin-releasing hormone (GnRH) is a decapeptide released from the hypothalamus. It stimulates the GnRH receptor in the pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn regulate

gonadal steroid hormone production. Down-regulation of the hypothalamic-pituitary-gonadal hormonal axis with peptidic GnRH agonists has been shown to alleviate disease conditions associated with endometriosis, uterine fibroids, breast, and prostate cancer. Treatment of various 2-methyl oxazolines or thiazolines with chlorocarbonylisocyanate gives the corresponding bicyclocoxazolino- or thiazolino[3,2-*c*]pyrimidin-5,7-dione derivatives[70] (**165**) in very good yield. This reaction has been applied to the rapid syntheses of human gonadotropin-releasing hormone (*h*GnRH) receptor antagonists for SAR study, resulting in (2-chloro, 3-methoxy)phenylthiazolino[3,2-*c*]pyrimidin-5,7-diones with binding affinity in the low nano molar range (4.5 nM) (**Fig. 54**).




(3*S*)-5-(2-(benzylamino)ethyl)-7-(2-fluoro-3-methoxyphenyl)-tetrahydro-3-phenylfuro [3,2-*c*]pyridine-4,6(2*H*,5*H*)-dione

**165**

**Fig. 54:** Pyrimidine derivatives as Gonadotropin-releasing hormone antagonist

**Antileishmanial agent:**

Leishmaniasis is caused by different species belonging to the genus *Leishmania*, a protozoan which is transmitted to humans by the bite of an insect vector, phlebotominesand fly. Infection by various strains of Leishmania causes a wide spectrum of disease in humans, with many different clinical manifestations, i.e., cutaneous, mucocutaneous and visceral. The visceral form of Leishmaniasis, commonly known as kala-azar, is caused by the parasite Leishmaniadonovani, which affects 61 out of the 88 countries worldwide. A series of dihydropyrido[2,3-*d*]pyrimidines (**166**)[71] have been screened for its *in vitro* antileishmanial activity profile in promastigote and amastigote models. Compounds have shown 83-100% inhibition against promastigotes and 79-100% inhibition against amastigotes at a concentration of 50 μg/mL (**Fig. 55**).

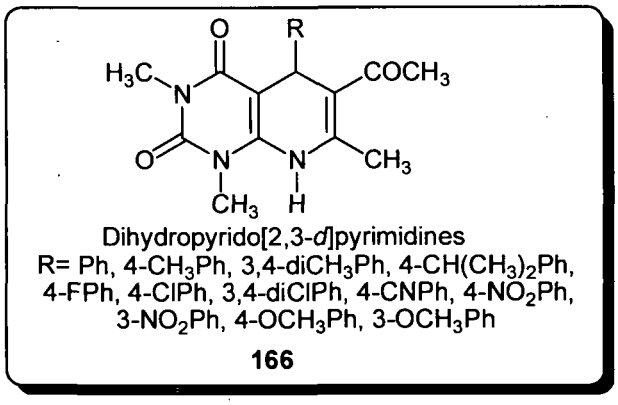



**Fig. 55:** Pyrimidine derivatives having antileishmanial activity

**PDE-4 inhibitors:**

The phospodiesterase (PDEs) family is particularly abundant in immune competent cells, where an increase of cAMP leads to the inhibition of the synthesis and release of pro-inplammatory mediators. Due to their crucial role in regulation in cell function, PDEs have become good clinical targets for the treatment of inplammation, asthma, erectile dysfuncion etc. A series of pyrido[2,3-*d*]pyrimidine-2,4-diones (**167-170**)[72] bearing substituents at C-3 and/or C-4 position on the pyridine ring were found to potent phosphodiesterase 4 (PDE 4) inhibitors (**Fig. 56**).

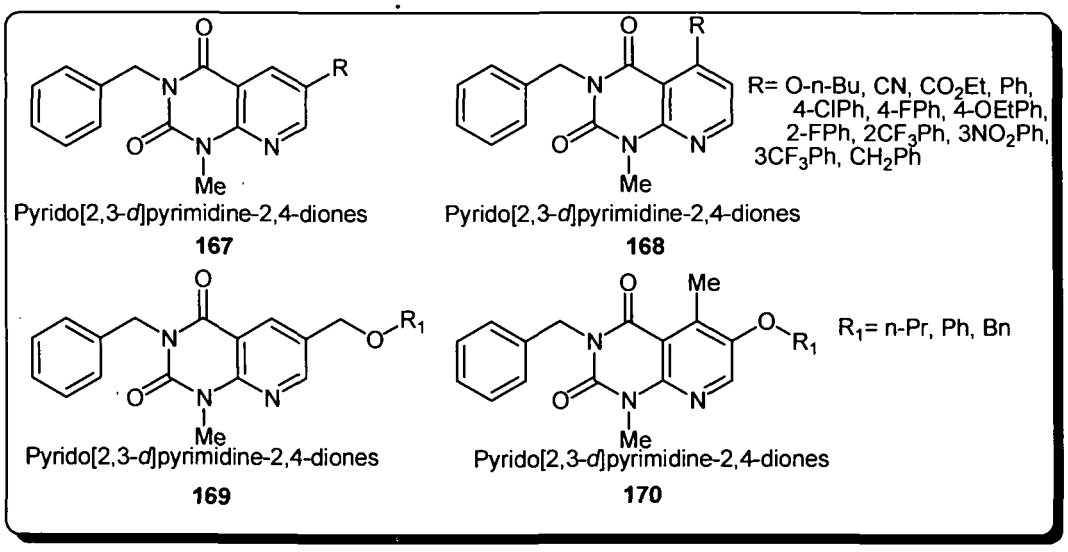



**Fig. 56:** Pyrimidine derivatives as PDE-4 inhibitors

**MEK inhibitors:**

The RAF–MEK–ERK pathway mediates proliferative and anti-apoptotic signaling from growth factors and oncogenic factors such as RAS and RAF in mutant phenotypes that promote tumor growth, progression, and metastasis. This pathway is inappropriately activated in 30% of all human cancers, resulting in the activation of ERK via phosphorylation. A novel series of 5-phenylamino-8-methylpyrido[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione (**171**) have shown MEK inhibitory effects which has been developed using structure-based drug design. Lead optimization of this series led to the discovery of TAK-733. This was advanced to Phase I clinical studies for cancer treatment[73] (**Fig. 57**).

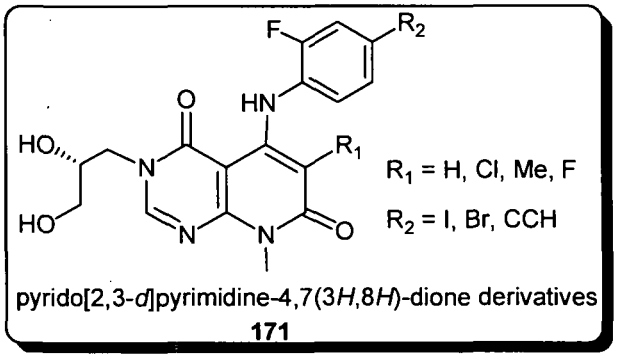



**Fig. 57:** Pyrimidine derivatives as MEK inhibitor

**References:**


1. a) Katritzky, A.R.; Rees, C.W. *Comprehensive Heterocyclic Chemistry*, Ed. Meth-Cohn, O. (Pergamon Press, Oxford, Part 1, pp143-199, 1984). b) Katritzky, A.R.; Rees, C.W.; Scriven, E.F.V. *Comprehensive Heterocyclic Chemistry II*, Ed. Boulton, A.J. (Pergamon Press, Oxford -New York -Tokyo, pp195-231, 1996).
2. a) Brown, D.J. In *The Chemistry of Heterocyclic Compounds, The Pyrimidines*, Ed. Taylor, E.C. (J. Wiley & Sons, New York -Chichester -Brisbane -Toronto -Singapore, 1994). b) Finar, I.L. *Organic Chemistry Volume 2: Stereochemistry and the Chemistry of Natural Products* (Pearson Publisher, 1956).
3. a) Lister, J.H. In *The Chemistry of Heterocyclic Compounds, Fused Pyrimidines Part II: Purines*, Ed. Brown, D.J. (Wiley-Interscience, New York -London -Sydney -Toronto, 1971). b) Brown, D.J. In *The Chemistry of Heterocyclic*

*Compounds, Fused Pyrimidines, Part III: Pteridines*, Ed. Taylor, E.C. (J. Wiley & Sons, New York-Chichester-Brisbane-Toronto-Singapore, 1988). c) Lecca, D.; Ceruti, S. Uracil nucleotides: from metabolic intermediates to neuroprotection and neuroinflammation, *Biochem. Pharmacol.* **75**, 1869-1881 (2008).

4. a) Armarego, W.L.F. In *The Chemistry of Heterocyclic Compounds, Fused Pyrimidines, Part I: Quinazolines*, Ed. Brown, D.J. (Interscience Publishers, New York -London -Sydney, 1967). b) Weissberger, A. Ed. In *The Pyrimidines*, Suppl. 1 of the *Chemistry of Heterocyclic Compounds*, (Wiley interscience, New York, 1970).
5. Berg, J.M.; Tymoczko, J.L; Stryer, L. Eds. *Biochemistry* (W.H. Freeman & Company, New York, 2002).
6. Kossel, A.; Steudel, H. Weitere untersuchungen über das cytosine, *Z. Physiol. Chem.* **38**, 49-59 (1903).
7. Kossel, A.; Steudel, H. Ueber das vorkommen des uracils im thierkörper, *Z. Physiol. Chem.* **37**, 245-247 (1903).
8. a) Fathalla, M. *et al* Base-pairing mediated non-covalent polymer, *Chem. Soc. Rev.* **38**, 1608-1620 (2009). b) Bradshaw, T.K.; Hutchinson, D.W. 5-Substituted pyrimidine nucleosides and nucleotides, *Chem. Soc. Rev.* **6**, 43-62 (1977). c) Sivakova, S.; Rowan, S.J. Nucleobases as supramolecular motifs, *Chem. Soc. Rev.* **34**, 9-21 (2005). d) Powner, M.W.; Gerland, B.; Sutherland, J.D. Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions, *Nature* **459**, 239-142 (2009). e) Pedersen, O.S.; Pedersen, E.B. Non-nucleoside reverse transcriptase inhibitors: The NNRTI boom, *Antiviral Chem. Chemother.* **10**, 285-314 (1999).
9. a) Gould, I.R.; Hillier, I.H. Accurate calculations of the oxo–hydroxy tautomers of uracil, *J. Chem. Soc., Perkin Trans. 2*, **2**, 329-333 (1990). b) Katritkzy, A.R.; Karelson, M. AM1 calculations of reaction field effects on the tautomeric equilibria of nucleic acid pyrimidine and purine bases and their 1-methyl analogs, *J. Am. Chem. Soc.* **113**, 1561-1566 (1991).
10. Shishkin, O.V.; Gorb, L.; Leszczynski, J. Modeling of the hydration shell of uracil and thymine, *Int. J. Mol. Sci.* **1**, 17-27 (2000).

11. a) Dinner, A.R.; Blackburn, G.M.; Karplus, M. Uracil-DNA glycosylase acts by substrate autocatalysis, *Nature* **413**, 752-755 (2001). b) Noia, J.D.; Neuberger, M.S. Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase, *Nature* **419**, 43-48 (2002). c) Katritzky, A.R.; Todadze, E. Heterocycles in the service of humankind, *Arkivoc* **ix**, 248-268 (2009). d) Masud, M.M. *et al* Synthesis of modified siRNA bearing C-5 polyamine-substituted pyrimidine nucleoside in their 3′-overhang regions and its RNAi activity, *Bioorg. Med. Chem. Lett.* **21**, 715-717 (2011).
12. Boncel, S.; Gondela, A.; Walczak, K. Michael-type addition as a convenient method for regioselective *N*-alkylation of ambident uracils, *Synthesis* **10**, 1573-1589 (2010).
13. Longley, D.B.; Harkin, D.P.; Johnston, P.G. 5-Fluorouracil: Mechanisms of action and clinical strategies, *Nat. Rev. Cancer* **3**, 330-338 (2003).
14. Safarjalani, O.N.A. *et al.* 5-(Phenylthio)acyclouridine: A powerful enhancer of oral uridine bioavailability: relevance to chemotherapy with 5-fluorouracil and other uridine rescue regimens, *Cancer Chemother. Phamacol.* **55**, 541-551 (2005).
15. Kennedy, B.J.; Torkelson, J.L.; Torlakovic, E. Uracil mustard revisited, *Cancer* **85**, 2265-2272 (1999).
16. Porter, D.J.; Chestunt, W.G.; Merril, B.M.; Spector, T. Mechanism-based inactivation of dihydropyrimidine dehydrogenase by 5-ethynyluracil, *J. Biol. Chem.* **267**, 5236-5242 (1992).
17. a) Hertel L.W. *et al.* Evaluation of the antitumor activity of gemcitabine (2′,2′-difluoro-2′-deoxycytidine), *Cancer Res.* **50**, 4417-4422 (1990). b) De Clercq, E. Antivirals and antiviral strategies, *Nature Rev. Microbiol.* **2**, 704-720 (2004). c) Maruyama, T.; Kozai, S.; Shimizu, T.; Watanabe, K.; Yamamoto, I. Synthesis and hypnotic-sedative activities of *N*-substituted uracil on mice, *Nucleic Acids Res. Suppl.* **3**, 25-26 (2003). d) Maruyama, T.; *et al* Synthesis and anti-HIV-1 and anti-HCMV activity of 1-substituted. 3-(3,5-dimethylbenzyl)uracil derivatives, *Chem. Pharm. Bull.* **54**, 325-333 (2006). e) Wang K. *et al* Synthesis and antitumor

activity of novel ribonucleosides with C-5 OH replaced by a diaminopyrimidinyl group, *Bioorg. Med. Chem. Lett.* **21**, 916-919 (2011).

18. Papesch, V.; Schroeder, E.F. Synthesis of 1-mono and 1,3-disubstituted 6-aminouracils. Diuretic activity, *J. Org. Chem.* **16**, 1879-1890 (1951).
19. Blicke, F.F.; Godt, H.C. Reactions of 1,3-dimethyl-5,6-diaminouracil, *J. Am. Chem. Soc.* **76**, 2798-2800 (1954).
20. Das, S. *N,N'*-Dimethyl Urea (DMU), *Synlett spotlight* **7**, 1138-1139 (2010).
21. a) Anderson, G.L. Regioselective synthesis of pyrido[2,3-*d*]pyrimidines. *J. Heterocycl. Chem.* **22**, 1469-1470 (1985). b) Heber, D.; Ravens, U.; Shulze, T. Synthesis and positive inotropic activity of several 5-aminopyrido[2,3-*d*]pyrimidines. V: Compounds with positive inotropic activity, *Pharmazie* **48**, 509-513 (1993).
22. Aly, A.A. Synthesis and pharmacological activity of annelated pyrimidine derivatives, *Phosphorus, Sulfur, and Silicon* **181**, 1285-1298 (2006).
23. Sharma, P.; Rane, N.; Gurram, V.K. Synthesis and QSAR studies of pyrimido[4,5-*d*]pyrimidine-2,5-dione derivatives as potential antimicrobial agents, *Bioorg. Med. Chem. Lett.* **14**, 4185-4190 (2004).
24. Bekhit, A.A.; Fahmy, H.T.Y.; Rostom, S.A.F.; Baraka, A.M. Design and synthesis of some substituted 1*H*-pyrazolyl-thiazolo[4,5-*d*]pyrimidines as anti-inflammatory-antimicrobial agents, *Eur. J. Med. Chem.* **38**, 27-36 (2003).
25. Mégraud, F. A humble bacterium sweeps this year's Nobel Prize, *Cell* **123**, 975-976 (2005).
26. Basarab, G.S.; Hill, P.J.; Rastagar, A.; Webborn, P.J.H. Design of helicobacter pylori glutamate racemase inhibitors as selective antibacterial agents: A novel pro-drug approach to increase exposure, *Bioorg. Med. Chem. Lett.* **18**, 4716-4722 (2008).
27. El-Gazzar, A.B.A.; Aly, A.S.; Zaki, M.E.A.; Hafez, H.N. Synthesis and preliminary antimicrobial activity of new pyrimido[4,5-*b*]quinoline and pyrido[2,3-*d*]pyrimidine, *Phosphorus, Sulfur and Silicon* **183**, 2119-2138 (2008).

28. Bazgir, A.; Khanaposhtani, M.M.; Soorki, A.A. One-pot synthesis and antibacterial activities of pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-dione derivatives, *Bioorg. Med. Chem. Lett.* **18**, 5800-5803 (2008).
29. El-Emary, T.I.; Abdel-Mohsen, S.A. Synthesis and antimicrobial activity of some new 1,3-diphenylpyrazoles bearing pyrimidine, pyrimidinethione, thiazolopyrimidine, triazolopyrimidine, thio- and alkylthiotriazolopyrimidinone moieties at the 4-position, *Phosphorus, Sulfur, and Silicon* **181**, 2459-2474 (2006).
30. Agarwal, N. *et al* Chloropyrimidines as a new class of antimicrobial agents, *Bioorg. Med. Chem.* **10**, 869-874 (2002).
31. Valderrama, J.A. *et al* Studies on quinones. Part 44: Novel angucyclinone *N*-heterocyclic analogues endowed with antitumoral activity, *Bioorg. Med. Chem.* **16**, 10172-10181 (2008).
32. Spáčilová, L. *et al* Synthesis and cytotoxic activity of various 5-[alkoxy-(4-nitro-phenyl)methyl]uracils in their racemic form, *Bioorg. Med. Chem. Lett.* **17**, 6647-6650 (2007).
33. Nair, V. *et al* A heterocyclic molecule with significant activity against dengue virus, *Bioorg. Med. Chem. Lett.* **19**, 1425-1427 (2009).
34. Ali, O.M.; Amer, H.H.; Abdel-Rahman, A.A.-H. Synthesis and antiviral evaluation of sugar uracil-1-ylmethylhydrazones and their oxadiazoline derivatives, *Synthesis* **18**, 2823-2828 (2007).
35. Raić-Malić, S. *et al* Synthesis, $^{18}$F-radiolabelling and biological evaluations of C-6 alkylated pyrimidine nucleoside analogues, *Nucleosides, Nucleotides & Nucleic Acids* **23**, 1707-1721 (2004).
36. Ji, L. *et al* Synthesis and anti-HIV-1 activity evaluation of 5-alkyl-2-alkylthio-6-(arylcarbonyl or α-cyanoarylmethyl)-3,4-dihydropyrimidin-4(3*H*)-ones as novel non-nucleoside HIV-1 reverse transcriptase inhibitors, *J. Med. Chem.* **50**, 1778-1786 (2007).
37. He, Y.P. *et al* Synthesis and biological evaluation of novel dihydro-aryl/alkylsulfanyl-cyclohexylmethyl-oxopyrimidines (S-DACOs) as high active anti-HIV agents, *Bioorg. Med. Chem. Lett.* **21**, 694-697 (2011).

38. Lu, X. *et al* The design and synthesis of *N*-1-alkylated-5-aminoarylalkylsubstituted-6-methyluracils as potential non-nucleoside HIV-1 RT inhibitors, *Bioorg. Med. Chem.* **15**, 7399-7407 (2007).
39. Yoo, S.J. *et al* Synthesis of novel (2*R*,4*R*)- and (2*S*,4*S*)-iso dideoxynucleosides with exocyclic methylene as potential antiviral agents, *Bioorg. Med. Chem.* **10**, 215-226 (2002).
40. Srivastav, N.C. *et al* Antiviral activity of 2,3′-anhydro and related pyrimidine nucleosides against hepatitis B virus, *Bioorg. Med. Chem. Lett.* **20**, 6790-6793 (2010).
41. El-Gazzar, A.B.A. *et al* Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines as anti-oxidant, anti-inflammatory and analgesic activities, *Eur. J. Med. Chem.* **44**, 609-624 (2009).
42. El-Gazzar, A.B.A.; Hafez, H.N. Synthesis of 4-substituted pyrido[2,3-*d*]pyrimidin-4(1*H*)-one as analgesic and anti-inflammatory agents, *Bioorg. Med. Chem. Lett.* **19**, 3392-3397 (2009).
43. Sondhi, S.M. *et al* One pot synthesis of pyrimidine and bispyrimidine derivatives and their evaluation for anti-inflammatory and analgesic activities, *Bioorg. Med. Chem.* **15**, 3334-3344 (2007).
44. Falcão, E.P.S. *et al* Synthesis and antiinflammatory activity of 4-amino-2-aryl-5-cyano-6-{3- and 4-(*N*-phthalimidophenyl)}pyrimidines, *Eur. J. Med. Chem.* **41**, 276-282 (2006).
45. Bahekar, S.S.; Shinde, D.B. Synthesis and anti-inflammatory activity of some [4,6-(4-substituted aryl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl]-acetic acid derivatives, *Bioorg. Med. Chem. Lett.* **14**, 1733-1736 (2004).
46. Kubota, K. *et al* Synthesis and structure-activity relationships of phenothiazine carboxylic acids having pyrimidine-dione as novel histamine H (1) antagonists, *Bioorg. Med. Chem. Lett.* **19**, 2766-2771 (2009).
47. Cowart, M.D. *et al* Rotationally constrained 2,4-diamino-5,6-disubstituted pyrimidines: a new class of histamine $H_4$ receptor antagonists with improved drug likeness and *in vivo* efficacy in pain and inflammation models, *J. Med. Chem.* **51**, 6547-6557 (2008).

48. A. Lauria, C. Patella, G. Dattolo, A. M. Almerico, Design and synthesis of 4-substituted indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine derivatives with antitumor activity, *J. Med. Chem.* **51**, 2037-2046 (2008).
49. Glavaš-Obrovac, L. *et al* Metabolic effects of novel *N*-1-sulfonylpyrimidine derivatives on human colon carcinoma cells, *Il Farmaco* **60**, 479-483 (2005).
50. Gazivoda, T. *et al* The novel pyrimidine and purine derivatives of L-ascorbic acid: synthesis, one- and two-dimensional $^{1}H$ and $^{13}C$ NMR study, cytostatic and antiviral evaluation, *Bioorg. Med. Chem.* **13**, 131-139 (2005).
51. Glavas-Obrovac, L. *et al* Synthesis and antitumor activity of 5-bromo-1-mesyluracil, *Nucleosides, Nucleotides and Nucleic Acids* **24**, 557-569 (2005).
52. Yano, S. *et al* Synthesis and evaluation of 6-methylene-bridged uracil derivatives. Part 1: Discovery of novel orally active inhibitors of human thymidine phosphorylase, *Bioorg. Med. Chem.* **12**, 3431-3441 (2004).
53. Vaghasiya, S.J.; Dodiya, D.K.; Trivedi, A.R.; Shah, V.H. Synthesis and biological screening of some novel pyrazolo[3′,4′:4,5]thieno[2,3-*d*]pyrimidin-8-ones via Gewald reaction, *Arkivoc* **xii**, 1-8 (2008).
54. Gasse, C. *et al* Substituted benzyl-pyrimidines targeting thymidine monophosphate kinase of *Mycobacterium tuberculosis*: synthesis and *in vitro* anti-mycobacterial activity, *Bioorg. Med. Chem.* **16**, 6075-6085 (2008).
55. Johar, M.; Manning, T.; Kunimoto, D.Y.; Kumar, R. Synthesis and *in vitro* anti-mycobacterial activity of 5-substituted pyrimidine nucleosides, *Bioorg. Med. Chem.* **13**, 6663-6671 (2005).
56. Bansal, R. *et al* Synthesis of a series of 8-(substituted-phenyl)xanthines and a study on the effects of substitution pattern of phenyl substituents on affinity for adenosine A(1) and A(2A) receptors, *Eur. J. Med. Chem.* **44**, 2122-2127 (2009).
57. Herold, F. *et al* Novel 4-aryl-pyrido[1,2-*c*]pyrimidines with dual SSRI and 5-$HT_{1A}$ activity, Part 1, *Eur. J. Med. Chem.* **44**, 1710-1717 (2009).
58. Samanta, A. *et al* Morpholino, piperidino, and pyrrolidino derivatives of pyrimidine nucleosides as inhibitors of ribonuclease A: Synthesis, biochemical, and crystallographic evaluation, *J. Med. Chem.* **52**, 932-942 (2009).

59. Baraldi, P.G. *et al* Structure–activity relationship studies of a new series of imidazo[2,1-*f*]purinones as potent and selective $A_3$ adenosine receptor antagonists, *Bioorg. Med. Chem.* **16**, 10281-10294 (2008).

60. Yaziji, V. *et al* Pyrimidine derivatives as potent and selective $A_3$ adenosine receptor antagonists, *J. Med. Chem.* **54**, 457-471 (2011).

61. Dong, M.; Sitkovsky, M.; Kallmerten, A.E.; Jones, G.B. Synthesis of 8-substituted xanthines via 5,6-diaminouracils: An efficient route to $A_{2A}$ adenosine receptor antagonists, *Tetrahedron Lett.* **49**, 4633-4635 (2008).

62. Pretorius, J. *et al* Dual inhibition of monoamine oxidase B and antagonism of the adenosine $A_{2A}$ receptor by (*E*,*E*)-8-(4-phenylbutadien-1-yl)caffeine analogues, *Bioorg. Med. Chem.* **16**, 8676-8684 (2008).

63. Rico-Gomez, R.M. *et al* Synthesis of new mannosyl, galactosyl and glucosyl theophylline nucleosides with potential activity as antagonists of adenosine receptors. DEMA-induced cyclization of glycosylideneiminouracils, *Carbohydrate Res.* **343**, 855-864 (2008).

64. Tabrizi, M.A. *et al* 1,3-Dipropyl-8-(1-phenylacetamide-1*H*-pyrazol-3-yl)-xanthine derivatives as highly potent and selective human $A_{2B}$ adenosine receptor antagonists, *Bioorg. Med. Chem.* **16**, 2419-2430 (2008).

65. Drabczynska, A. *et al* N9-Benzyl-substituted 1,3-dimethyl- and 1,3-dipropyl-pyrimido[2,1-*f*]purinediones: Synthesis and structure–activity relationships at adenosine $A_1$ and $A_{2A}$ receptors, *Bioorg. Med. Chem.* **15**, 5003-5017 (2007).

66. Vlok, N. *et al* Inhibition of monoamine oxidase B by analogues of the adenosine $A_{2A}$ receptor antagonist (*E*)-8-(3-chlorostyryl)caffeine (CSC), *Bioorg. Med. Chem.* **14**, 3512-3521 (2006).

67. Bulicz, J. *et al* Synthesis and pharmacology of pyrido[2,3-*d*]pyrimidinediones bearing polar substituents as adenosine receptor antagonists, *Bioorg. Med. Chem.* **14**, 2837-2849 (2006).

68. Reiter, L. A. *et al* Potent, selective pyrimidinetrione-based inhibitors of MMP-13, *Bioorg. Med. Chem. Lett.* **16**, 5822-5826 (2006).

69. Khalaj, A. *et al* Synthesis, aerobic cytotoxicity, and radiosensitizing activity of novel 2,4-dinitrophenylamine tethered 5-fluorouracil and hydroxyurea, *Bioorg. Med. Chem. Lett.* **16**, 6034-6038 (2006).

70. Pontillo, J.; Chen, C. Efficient synthesis of bicyclic oxazolino- and thiazolino[3,2-*c*]pyrimidine-5,7-diones and its application to the synthesis of GnRH antagonist, *Bioorg. Med. Chem. Lett.* **15**, 1407-1411 (2005).

71. Agarwal, A. *et al* Dihydropyrido[2,3-*d*]pyrimidines as a new class of antileishmanial agents, *Bioorg. Med. Chem.* **13**, 6678-6684 (2005).

72. Nam, G. *et al* Syntheses and evaluation of pyrido[2,3-*d*]pyrimidine-2,4-diones as PDE 4 inhibitors, *Bioorg. Med. Chem. Lett.* **11**, 611-614 (2001).

73. Dong, Q. *et al* Discovery of TAK-733, a potent and selective MEK allosteric site inhibitor for the treatment of cancer, *Bioorg. Med. Chem. Lett.* **21**, 1315-1319 (2011).

# CHAPTER 2

# Section A

## A clean, highly efficient and one-pot green synthesis of aryl/alkyl/ heteroaryl-substituted bis(6-amino-1,3-dimethyluracil-5-yl)methanes in water

**Introduction:**

The general belief is that life started from organic compounds in an aqueous environment. Szent-Gyorgyi rightly dubbed water as the "matrix of life"[1] and it is regarded as a universal solvent. Mimicking nature and bearing in mind environmentally friendly protocols, the use of water as a remarkable solvent with a difference in organic synthesis has received considerable attention in recent times.[2] The reason behind this is that water is the solvent of choice from not only an ecological but also from an economic point of view. Water is cheap, safe, natural, non-flammable, abundant, available and environmentally benign, that is, it is a 'green' solvent[3] associated with easy reaction work-up. Compared with common organic solvents, the unique and unusual physical (low viscosity, high specific heat, high surface tension, high dielectric constant, large cohesive energy density etc.) and chemical properties (ability to form hydrogen bonds, its amphoteric nature etc.) of liquid water help to increase the reactivity and selectivity of chemical reactions.[4] Moreover, hydrophobic interactions are also responsible for its reactivity. In spite of these potential advantages, water has not found use as a common organic solvent because most organic compounds are insoluble in water according to the assumption "*corpora non agunt nisi solute*" (substances do not interact unless dissolved). Regardless of this, however, there are many examples of aqueous media reactions,[5] water used as a co-solvent or on-water[6] reactions performed without dissolving the organic compounds (coined as the "on-water-effect"). Efforts have been made to dissolve organic compounds in water by adding additives like surfactants[7] and applying heat.[8] Rideout and Breslow's seminal observation that Diels–Alder reactions could be greatly accelerated by using a water suspension rather than solution instead of organic solvents established the importance of water in organic synthesis.[9] Since then, this area of research has

burgeoned. On the other hand, organic solvents contribute the lion's part to the pollution problem in practical chemistry,[10] both in the laboratory as well as in industry. Hence, the search for efficient synthetic methodologies for organic reactions without the use of organic solvents is an important challenge in reducing the amount of waste generated.[11] An ideal organic reaction demands that it would proceed neat, that is, with no solvent, or in an environmentally benign solvent such as water.

Uracil, a nucleobase of the pyrimidine family, is one of the major motifs present in the biopolymer RNA[12] and it plays several crucial roles in our life cycle.[13] The versatility of the uracil scaffold and its derivatives, as exhibited by their wide range of biological activities, has brought chemists[14] and biologists together.[15] Several patents also report promising pharmaceutical agents of uracil origin for the treatment of cancer and viral diseases.[16] To name but a few, uracil derivatives are also useful as bronchodilators and anticancer agents,[17] antiallergic agents,[18] antiviral agents,[19] antihypertensive agents[20] and adenosine receptor antagonists.[21] In addition, 5-substituted uracils and their nucleosides are widely used in the chemotherapy of cancer.[22] Pyrimidine derivatives have also been used in coordination chemistry.[23] In view of the biological significance of pyrimidine compounds, we have been focussing on the design and synthesis of such pyrimidine derivatives.[24]

**Materials and Methods:**

Melting points were determined with a Büchi 504 apparatus. IR spectra were recorded as KBr pallets with a Nicolet (Impact 410) FT-IR spectrophotometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded with a JNM ECS 400 MHz NMR spectrophotometer (JEOL) using tetramethylsilane (TMS) as the internal standard. X-ray intensity data were collected with a Bruker SMART APEX CCD area-detector diffractometer with Mo-$K\alpha$ radiation ($\lambda$ = 0.71073 Å). The structures were solved by SHELX97 and refined by full-matrix least-squares on $F^2$ (SHELX97).[42] Reactions were monitored by thin-layer chromatography using aluminium sheets with silica gel $60F_{254}$ (Merck). Elemental analyses were carried out with a Perkin–Elmer CHN analyser (2400 series II). Mass spectra were recorded with a Waters Q-TOF Premier and Aquity UPLC spectrometer. All the chemicals were used as received.

**Compounds:**

All the tested compounds were synthesized by our general reaction procedure. The structure was confirmed on the basis of IR, $^{1}H$ and $^{13}C$ NMR spectroscopy, mass spectrometry, elemental analyses and single crystal X-ray analysis.

**Chemicals:**

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Hi-Media (NE) and Gallic acid was purchased from Sigma-Aldrich (Mumbai, India) for the experiment. All the other chemicals used including the solvents, were of analytical grade. Bacterial strains were kindly provided by Prof. B. K. Konwar, Department of Molecular Biology & Biotechnology, Tezpur University, Assam, India and fungal strains were supplied by Department of Plant Pathology, Assam Agriculture University, Jorhat, Assam, India.

**Preparation of standards for bioactivity test:**

Each sample was dissolved in absolute ethanol (analytical grade) and stock solutions of 1mg/ml were prepared for the experiment.

Concentrations of 25mg/mL, 50mg/mL, 100mg/mL, 150mg/mL, 200mg/mL, 250mg/mL and 500g/mL of uracil derivatives in the sterilized DMSO were prepared. Sterilized DMSO without the test compound as negative control, another with gentamicin (1mg/mL) as positive control for the bacteria and for fungi clotrimazole (1mg/mL) were used to serve as positive control.

**Antioxidant assay procedure:**

Free radical scavenging activity of the extracts was measured using the method of Brand-Williams *et al*[25] with some modification. A 0.1mM solution of DPPH (1,1-diphenyl-2-picryl-hydrazyl) in ethanol was prepared and 3 ml of this solution was added to 1 ml of the extract in various concentrations (0.1-1.0 mg/ml). Gallic acid was used as standard antioxidant in same concentrations. The decrease in absorbance at 517 nm was measured after 30 min. Free radical scavenging activity was expressed as the percentage of DPPH decrease and percentage of scavenging activity was calculated from

$$\% \text{ scavenging activity} = (A_{\text{control}} - A_{\text{compound}})/ A_{\text{control}} \times 100$$

The assays were carried out in triplicate and the results were expressed as mean values ± standard deviation. The extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph of scavenging effect percentage against the extract concentration. The inhibition curves were prepared and $IC_{50}$ values were obtained.

**Antibacterial and antifungal assay procedure:**

The agar well diffusion technique was used in the present investigation, following the procedure described by Boakye-Yiadom,[26] Banso and Adeyemo,[27] and Radhika *et al.*[28] Five (5) wells, 8mm each were made on solidified nutrient agar and Sabouraud Dextrose Agar (SDA) media plates, respectively with the help of a sterile cork borer. 200$\mu$l of the log phase culture of the test microbes which includes *Staphylococcus aureus* (ATCC 11632), *Bacillus subtilis* (ATCC 11774), *E. coli* (ATCC 9637), *Pseudomonas aeruginosa* (MTCC 7812), *Pseudomonas aeruginosa* (MTCC 7814), *Pseudomonas aeruginosa* (MTCC 7815) and *Pseudomonas aeruginosa* (MTCC 7816) were seeded on the surface of the nutrient agar medium while *Candida albicans*, *Aspergillus niger*, *Colletotricum cappci* (MTCC 227), *Rhizoctonia solani*, *Fusarium oxysporium* were seeded on the SDA medium, using swab stick. The cut agar discs were removed with the aid of sterile forceps. Concentrations of 25mg/mL, 50mg/mL, 100mg/mL, 150mg/mL, 200mg/mL, 250mg/mL and 500mg/mL of the compound in the sterilized DMSO were separately introduced into separate wells. Two (2) control holes were set up, one filled with sterilized DMSO without the test compound as negative control, another with gentamicin as positive control for the bacteria and for fungi, clotrimazole was used to serve as positive control. The plates were incubated at 37 °C for 24 h and 15 days at 27 °C respectively for the bacterial and fungal cultures. The observed zones of inhibition were measured using transparent metric ruler.

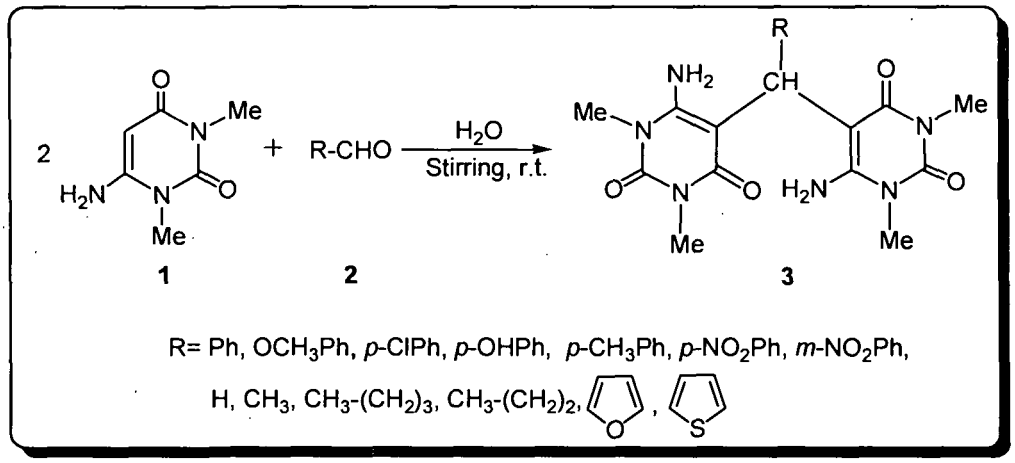
**MIC assay procedure:**

Bacteria culture maintained in nutrient ager slants at 4-5 °C were transferred to 10 ml of nutrient broth and incubated overnight at 37 °C. A pure culture was prepared by transferring 1 ml of the above culture to 9 ml of nutrient borth and incubated for 24h. Ampicillin (1 mg/ml) was used as positive control. Minimal inhibitory concentration

(MIC) of the test compounds were determined by borth dilution method. The different concentrations of test compounds were preparing by diluting it in pure sterilized nutrient borth. The MIC was recorded as the lowest dilatation of the tested sample inhibiting the visible growth of the test microorganism after 24 h for the bacterial cultures respectively on the 96 well micro titer plates.

**Results and Discussion:**

Thus, bearing in mind the importance of water and the uracil moiety together, herein, we report a clean, highly efficient and one-pot green method for the synthesis of bisuracil compounds (**3**) by the reaction of 6-amino-1,3-dimethyluracil (**1**; at the 5-position) and aldehydes (**2**) in water at room temperature without using any catalyst, surfactant, additive, dehydrating agent, heat or organic solvent (**Scheme 1**).



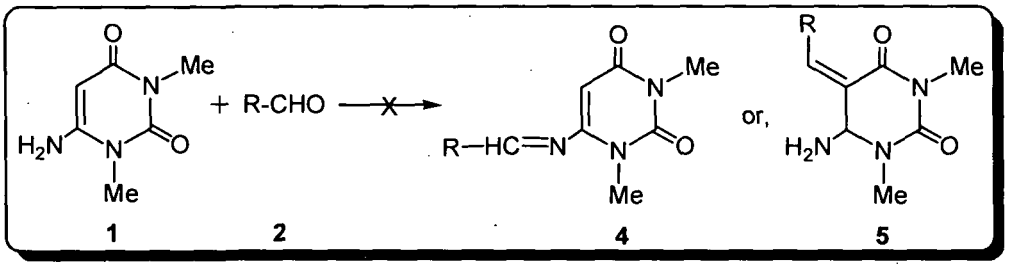

**Scheme 1:** Synthesis of bisuracil derivatives (**3**)

In 1986, Lam and Pridgen first reported the reaction of unsubstituted uracil with aromatic aldehydes in aqueous mineral acids (e.g., conc. HCl, HBr, HI or $H_2SO_4$) at reflux.[29] Depending on the nature of substituents on the aldehydes, they obtained a mixture of (hydroxymethyl)uracil and bis-uracil adducts. The condensation reaction of substituted 6-aminouracils and aromatic aldehydes in ethanol at reflux or under microwave irradiation wetted by DMSO afforded bis-uracil compounds.[30] However, in both the cases, the reaction failed with aliphatic and heteroaromatic aldehydes. Another

report presented the synthesis of bis-uracil compounds from aromatic aldehydes only in methanol and acetic acid.[31] Hlavác and co-workers in 2007 further explored the reaction of unsubstituted uracil with 4-nitrobenzaldehyde and obtained 5-[chloro(4-nitrophenyl)methyl]uracil at reflux in aqueous mineral acids and continued the generalization for the synthesis of 5-[alkoxy(4-nitrophenyl)methyl]uracils from 5-[chloro(4-nitrophenyl)methyl]uracil. They also studied the anticancer activity of the synthesized compounds.[32] Bazgir and co-workers in 2007 reported the formation of fused pyrimidine derivatives via bis-uracil as intermediate in a three-component reaction of 6-amino-1,3-dimethyluracil, aldehydes and urea catalysed by acetic acid under microwave irradiation.[33] Again, this method is limited to aromatic aldehydes only. An interesting report from Shi *et al*[34] showed that the three-component reaction of aromatic aldehyde, 6-aminopyrimidine-2,4-dione and meldrum's acid in water in the presence of triethylbenzylammonium chloride (TEBAC) at 90 °C for 18-32 h led to the formation of 5-benzylidenepyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione and 5,5′-(arylmethylene)bis[6-amino - pyrimidine-2,4(1*H*,3*H*)-dione]. However, none of these reported methods are general covering all types of aldehydes: aliphatic, aromatic and heterocyclic. Moreover, the use of mineral acid, acetic acid, additives, microwave irradiation or reflux conditions in common organic solvents are necessary for the synthesis of such substituted or unsubstituted bis-uracil adducts. Thus, a general and "green" method is required. Our method eliminates all of the above mentioned drawbacks with added advantage.

Bis-uracil and their analogues have also been isolated from marine sea hare *Dolabella auricularia.*[35] Semenov and co-workers have been actively working on the synthesis of *N*-substituted bis-uracil analogues and the bioactivities associated with the synthesized compounds.[36]

In an attempt to synthesize imines (**4**) from 6-amino-1,3-dimethyluracil (**1**) and benzaldehyde (**2**, R = Ph) (**Scheme 2**) we tested several reaction conditions, both conventional and non-conventional, using a catalyst or in the absence of catalyst and in the presence or absence of solvent. However, all the attempts went in vain and we observed the following interesting results.




**Scheme 2**: Synthesis of imine (**4**) or alkene (**5**)

As a model reaction, when we simply stirred 6-amino-1,3-dimethyluracil (**1**) and benzaldehyde (**2a**; R = Ph) in water at room temperature, we ended up with the formation of 5,5′-phenylmethylenebis(1,3-dimethyl-6-aminopyrimidine-2,4-dione) (**3a**) within 1 h in 95% yield (**Scheme 1**). The reaction was monitored by TLC. Interestingly, the reaction was very clean providing only one product, that is, (**3a**). We did not observe the formation of product (**4**) nor the other possible product (**5**) (**Scheme 2**). As the reaction progressed, we observed that the product started to precipitate from the aqueous solution and simple filtration afforded the product. The structure was confirmed by IR, $^{1}H$ and $^{13}C$ NMR spectroscopy, mass spectrometry and elemental analyses. The $^{1}H$ NMR peaks at $\delta$ = 3.27 (3 H), 3.34 (3 H) and 3.44 (6 H) ppm are due to the four *N*-methyl groups, the peak at $\delta$ = 5.79 ppm is due to the >CH proton, the broad singlet peaks at $\delta$ = 6.48 and 6.96 ppm are due to the two $-NH_2$ protons and the peaks at $\delta$ = 7.14–7.25 ppms are due to five aromatic protons. The two $-NH_2$ groups were confirmed by shaking with $D_2O$, i.e. the $-NH_2$ peak disappeared from the $^{1}H$ NMR spectrum upon shaking with $D_2O$. The structure was further confirmed by single crystal X-ray analysis. Suitable crystals were obtained by slow evaporation from ethanol solution.

Encouraged by this result, to study the scope and limitations of the reaction further, we extended the reaction to other differently substituted aromatic, aliphatic and heterocyclic aldehydes and ketones (entries **2a–p**) under the same optimized reaction conditions. The results are summarized in **Table 1** (entries **a–p**). All the aldehydes (entries **a–m**, **Table 1**) reacted with equal ease within short times to furnish the bis-uracils (**3a–m**) in good-to-excellent yields (75–99%) and with no side-products.

As illustrated in **Table 1** (entries **a–m**), it is evident that aromatic, aliphatic and heterocyclic aldehydes are equally effective for the synthesis of bis-uracil (**3**). We also

obtained very good yields (>90%) with aliphatic aldehydes (entries **j–m, Table 1**). With paraformaldehyde (entry **j, Table 1**), 6-amino-1,3-dimethyluracil (**1**) afforded the bis-uracil adduct (**3j**), 6,6′-diamino-1,1′,3,3′-tetramethyl-5,5′-methylene-bis[pyrimidine-2,4(1*H*,3*H*)-dione] (91% yield). However, under both acidic and basic conditions, unsubstituted uracil reacted with paraformaldehyde to produce 5-hydroxymethyluracil, as reported by Kong *et al.*[37] This is in sharp contrast to that report in which the reaction with aliphatic aldehydes was not mentioned. We performed the reaction with another three aliphatic aldehydes (entries **k–m, Table 1**) with different carbon chain lengths and all the products were confirmed by NMR ($^1$H and $^{13}$C) and FTIR spectroscopy, mass spectrometry, elemental analyses and single crystal X-ray analysis. ORTEP diagrams for

**Table 1**: Synthesis of bisuracil derivatives (**3**)

| Entry | Carbonyl compounds **2** | Time/h | m.p. (°C) | Yield (%)[a] |
|---|---|---|---|---|
| a | $C_6H_5CHO$ | 1 | 296-299 | 95 |
| b | *p*-$OMeC_6H_4CHO$ | 3 | 273-275 | 91 |
| c | *p*-$ClC_6H_4CHO$ | ¼ | 268-270 | 99 |
| d | *p*-$OHC_6H_4CHO$ | 7 | 245-248 | 75 |
| e | *p*-$MeC_6H_4CHO$ | ¼ | 276-279 | 99 |
| f | *p*-$NO_2C_6H_4CHO$ | 10 | 228-229 | 73 |
| g | *m*-$NO_2C_6H_4CHO$ | 5 | 224-225 | 82 |
| h | 2-furaldehyde | 1 | 246-250 | 99 |
| i | Thiophene-2-carbaldehyde | 6 | 309-312 | 87 |
| j | Paraformaldehyde | 1 | 328-334 | 91 |
| k | $CH_3CHO$ | 3 | 253-256 | 92 |
| l | $CH_3(CH_2)_2CHO$ | 3 | 232-233 | 93 |
| m | $CH_3(CH_2)_3CHO$ | 3 | 159-162 | 90 |
| n | $(CH_3)_2CO$ | 48 | -- | --[b] |
| o | $CH_3COPh$ | 48 | -- | --[b] |
| p | PhCOPh | 48 | -- | --[b] |

[a] Isolated yield [b] No product found

compounds (**3a**), (**3c**) and (**3j**) are shown in **Fig. 1**, **2** and **3**, and crystallographic data for the same are given in **Tables 2**, **3** and **4** respectively. From these ORTEP diagrams, it is clear that both the amino groups and the two uracil rings are oriented oppositely and hence they exist in different magnetic environments, which are manifested in their NMR spectra. Our methodology is also equally effective for heterocyclic compounds. 2-Furaldehyde (entry **h, Table 1**) and thiophene-2-carbaldehyde (entry **i, Table 1**) provided the desired products (**3h**) and (**3i**) in 99 and 87% yields, respectively. 2-Furaldehyde reacted within 1 h, but thiophene-2-carbaldehyde required a longer reaction time (6 h). No other products were detected. The structures of the products were confirmed by NMR (both $^{1}H$ and $^{13}C$) and FT-IR spectroscopy, mass spectrometry, elemental analysis and single-crystal X-ray analysis. The ORTEP diagram of product (**3h**) is shown in **Fig. 4** and detail crystallographic data for the same are given in **Table 5**.

Interestingly, on the other hand, in the case of *para*-hydroxy-substituted benzaldehyde (entry **e, Table 1**), low yields (73%) of the product was obtained probably due to the formation of intermolecular hydrogen-bonding with water.

Realizing the established difference in reactivity of aldehydes and ketones, we also studied the reaction of 6-amino-1,3-dimethyluracil (**1**) with ketones (**Scheme 1**, **Table 1**, entries **n–p**). However, the reaction failed to proceed even after a long time (24 h) with each ketone, which shows the chemoselectivity of the reaction, and all the starting materials were recovered as such. Further, this chemoselectivity was also established in a competitive experiment involving benzaldehyde (entry **2a**; 0.5 mmol, **Table 1**), benzophenone (entry **2p**; 0.5 mmol, **Table 1**) and uracil (**1**) (2.0 mmol). Benzaldehyde reacted as described in **Scheme 1** providing the desried product (**3a**), whereas benzophenone did not react at all. Unreacted benzophenone (**2p**) and uracil (**1**) were recovered along with the desired product (**3a**).

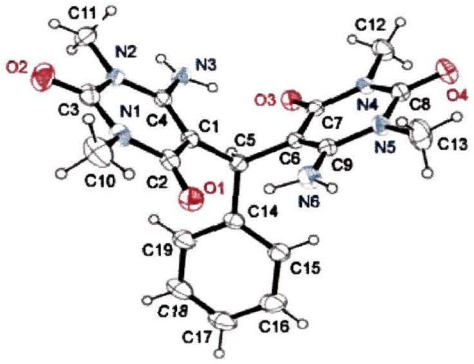



**Fig. 1:** ORTEP diagram of compound **(3a)**

**Table 2:** Detail crystallographic data of **(3a)**

| | |
|---|---|
| Formula | $C_{39}H_{22}N_6O_4$ |
| M | 398.43 |
| Crystal system | Monoclinic |
| Temperature/K | 296 (2) |
| Space group | *P21/n* |
| *a*/ Å | 12.3254(3) |
| *b*/ Å | 8.1844(2) |
| *c*/ Å | 19.5575(5) |
| α (°) | 90 |
| β (°) | 105.746(2) |
| γ (°) | 90 |
| *V*/ $Å^3$ | 1898.85(8) |
| *Z* | 4 |
| *Dc*/ $mg \cdot m^{-3}$ | 1.394 |
| Reflns. collected | 26001 |
| Reflns. unique | 4534 |
| R(*int*) | 0.0386 |
| Index ranges | -16<=h<=16, -9<=k<=10, -25<=l<=25 |
| Refinement method | Full-matrix, least squares on $F^2$ |
| $wR_2$ 0.1227 | $R_1$ 0.0412 *GoF* 1.091 |

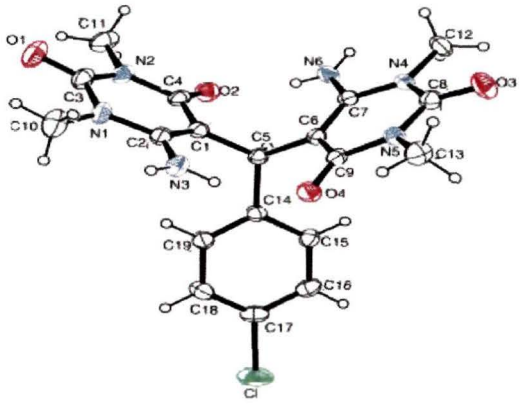



**Fig. 2:** ORTEP diagram of compound **(3c)**

**Table 3**: Detail crystallographic data of **(3c)**

| | |
|---|---|
| Formula | $C_{19}H_{21}N_6O_4Cl$ |
| M | 432.87 |
| Crystal system | Orthorhombic |
| Temperature/K | 296(2) |
| Space group | *P212121* |
| *a*/ Å | 11.5365(3) |
| *b*/ Å | 14.5935(4) |
| *c*/ Å | 23.2102(6) |
| α (°) | 90 |
| β (°) | 90 |
| γ (°) | 90 |
| *V*/ $Å^3$ | 3907.62(18) |
| *Z* | 8 |
| *Dc*/ $mg \cdot m^{-3}$ | 1.472 |
| Reflns. collected | 53787 |
| Reflns. unique | 9742 |
| R(*int*) | 0.0379 |
| Index ranges | -15<=h<=15, -19<=k<=19, -30<=h<=30 |
| Refinement method | Full-matrix, least squares on $F^2$ |

$wR_2$ 0.1449 $R_1$ 0.0558 *GoF* 1.049


**Fig. 3:** ORTEP diagram of compound **(3j)**

**Table 4**: Detail crystallographic data of **(3j)**

| | |
|---|---|
| Formula | $C_{13}H_{18}N_6O_4$ |
| M | 322.33 |
| Crystal system | Monoclinic |
| Temperature/K | 273(2) |
| Space group | *P21/c* |
| *a*/ Å | 14.2901(10) |
| *b*/ Å | 12.0192(8) |
| *c*/ Å | 8.2592(6) |
| α (°) | 90 |
| β (°) | 93.7040(10) |
| γ (°) | 90 |
| *V*/ $Å^3$ | 1415.60(17) |
| *Z* | 4 |
| *Dc*/ $mg \cdot m^{-3}$ | 1.512 |
| Reflns. collected | 6993 |
| Reflns. unique | 2477 |
| R(*int*) | 0.0175 |
| Index ranges | -14<=h<=16, -11<=k<=14, -9<=h<=9 |
| Refinement method | Full-matrix, least squares on $F^2$ |

$wR_2$ 0.1054 $R_1$ 0.0363 *GoF* 1.047

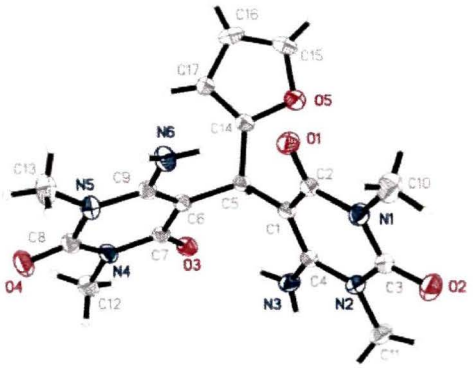


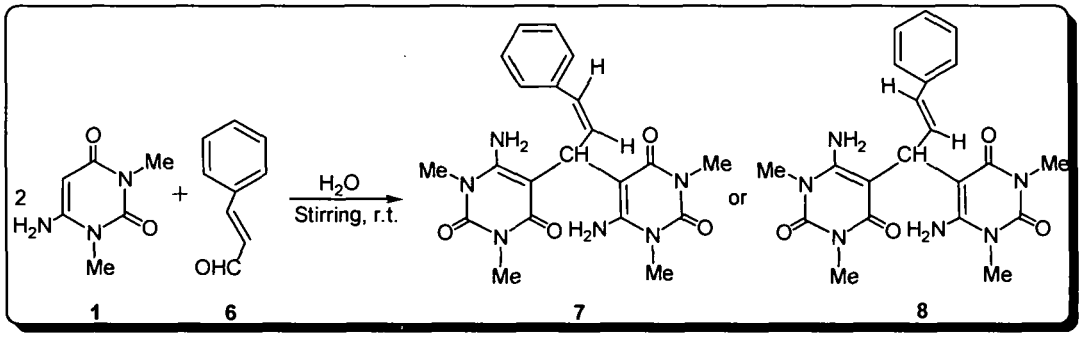

**Fig. 4:** ORTEP diagram of compound **(3h)**

**Table 5:** Detail crystallographic data of **(3h)**

| | |
|---|---|
| Formula | $C_{17}H_{20}N_6O_5$ |
| M | 388.39 |
| Crystal system | Monoclinic |
| Temperature/K | 273(2) |
| Space group | *P21/c* |
| *a*/ Å | 11.2940(10) |
| *b*/ Å | 14.7991(13) |
| *c*/ Å | 10.8625(10) |
| α (°) | 90 |
| β (°) | 102.960(2) |
| γ (°) | 90 |
| *V*/ $Å_3$ | 1769.3(3) |
| *Z* | 4 |
| *Dc*/ $mg \cdot m^{-3}$ | 1.458 |
| Reflns. collected | 16726 |
| Reflns. unique | 3116 |
| R(*int*) | 0.0234 |
| Index ranges | -13<=h<=13, -17<=k<=17, -12<=h<=12 |
| Refinement method | Full-matrix, least squares on $F^2$ |

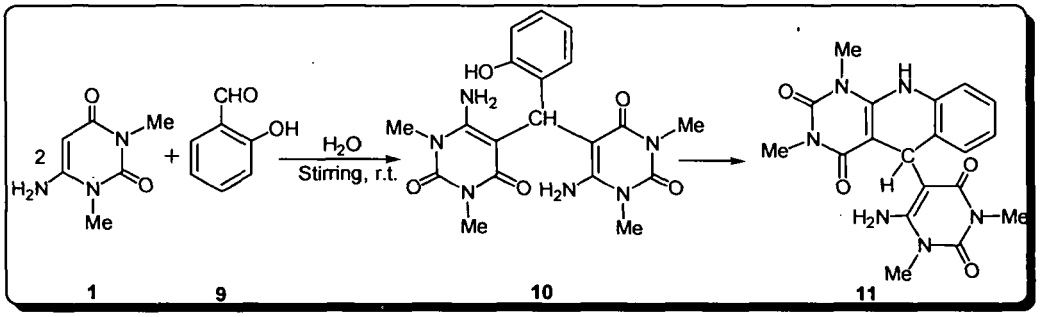
$wR_2$ 0.1091 $R_1$ 0.0388 *GoF* 1.036

In the same way, when the reaction of (**1**) with cinnamaldehyde was performed, from the NMR spectrum, formation of both *cis* / *trans* bis-uracil (**7**) & (**8**) (**Scheme 3**) adducts were observed. However, attempt to separate the *trans* or *cis* product by column or thin layer chromatographic technique failed. But, upon recrystallisation, only the *trans* product recrystallized out in the form of square size transparent crystals. Finally, the product was confirmed by single crystal X-ray analysis. The ORTEP diagram of compound (**8**) is shown in **Fig. 5.**




**Scheme 3:** Synthesis of *cis* / *trans* bis-uracil (**7**) & (**8**)

Interestingly, when salicyldehyde was reacted with (**1**), 5-(6-amino-1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxopyrimidin-5-yl)-1,3-dimethylpyrimido[4,5-*b*]-quinoline -2,4(1*H*,3*H*,5*H*,10*H*)-dione (**11**) formed and reaction proceeded via bis-uracil adduct (**10**) (**Scheme 4**). The structure of the compound was confirmed by single crystal X-ray analysis. NMR and IR spectroscopy, mass spectrometry and elemental analysis support the structure. The ORTEP diagram of compound (**11**) is shown in **Fig. 6.**




**Scheme 4:** Synthesis of pyrimido[4,5-*b*]quinoline-2,4-dione derivative (**11**)

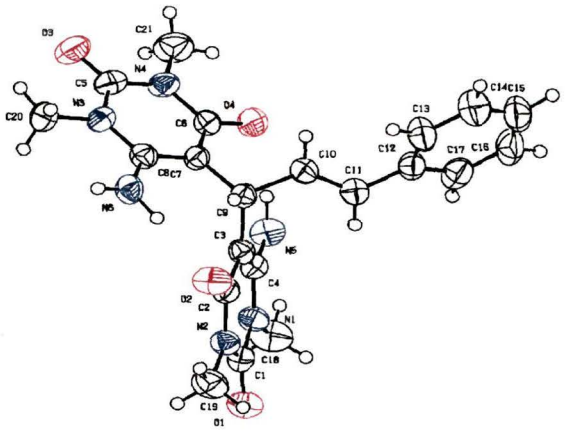


**Fig. 5:** ORTEP diagram of compound **(8)**

**Table 6**: Detail crystallographic data of **(8)**

| | |
|---|---|
| Formula | $C_{21}H_{24}N_6O_4$ |
| M | 424.5 |
| Crystal system | Monoclinic |
| Temperature/K | 293 K |
| Space group | $P2_1/c$ |
| *a*/ Å | 11.4666(9) |
| *b*/ Å | 12.3330(11) |
| *c*/ Å | 14.7670(12) |
| α (°) | 90 |
| β (°) | 99.632(8) |
| γ (°) | 90 |
| *V*/ $Å_3$ | 2058.9(5) |
| *Z* | 4 |
| *Dc*/ $mg \cdot m^{-3}$ | 1.37 |
| Reflns. collected | 9676 |
| Reflns. unique | 1147 |
| R(*int*) | 0.072 |
| Index ranges | -15<=h<=15, -14<=k<=16, -20<=h<=18 |
| Refinement method | Full-matrix, least squares on $F^2$ |

$wR_2$ 0.2154 $R_1$ 0.082 *GoF* 0.974

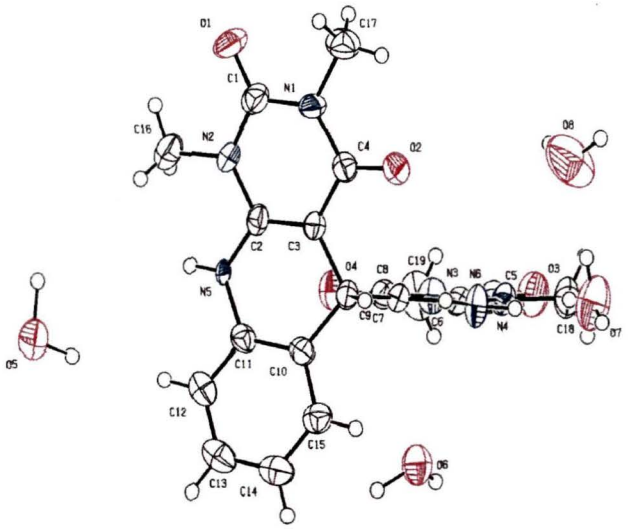
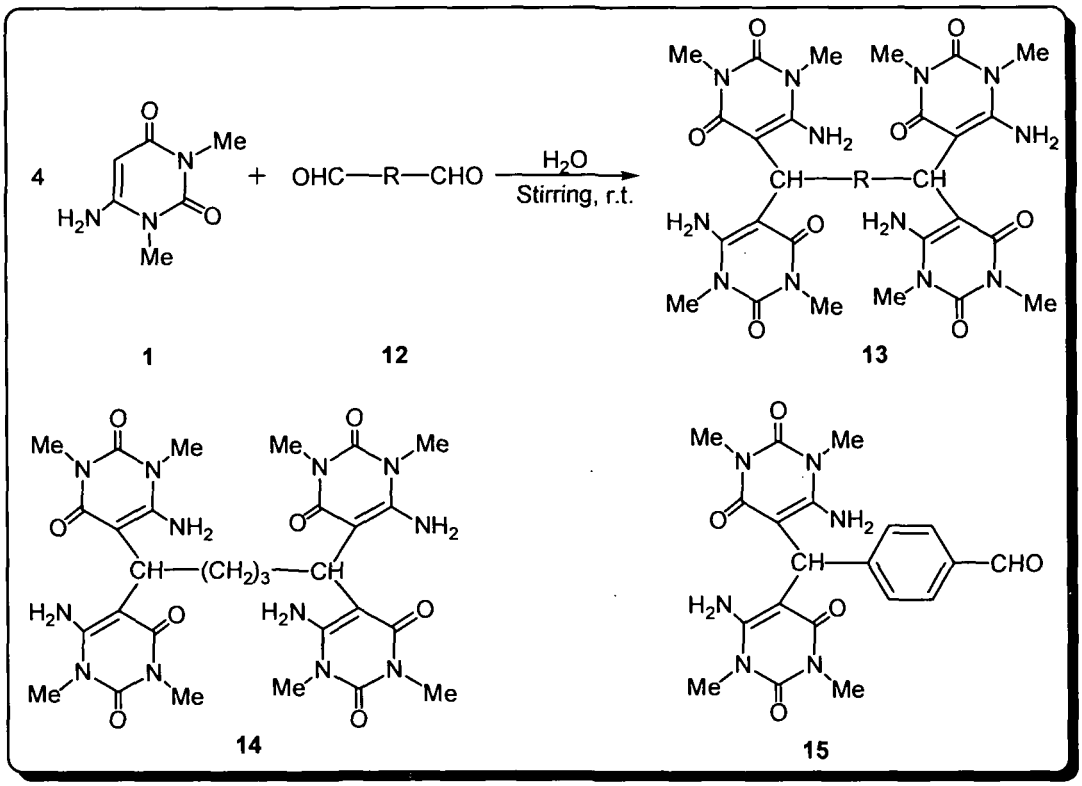



**Fig. 6:** ORTEP diagram of compound **(11)**

**Table 7**: Detail crystallographic data of **(11)**

| | |
|---|---|
| Formula | $2(C_{19}H_{20}N_6O_4)\cdot 7(H_2O)$ |
| M | 918.93 |
| Crystal system | Monoclinic |
| Temperature/K | 293 K |
| Space group | C 2/c |
| *a*/ Å | 29.993(4) |
| *b*/ Å | 7.9105(6) |
| *c*/ Å | 21.458(3) |
| α (°) | 90 |
| β (°) | 119.860(16) |
| γ (°) | 90 |
| *V*/ $Å_3$ | 4415.2(12) |
| *Z* | 4 |
| *Dc*/ $mg\cdot m^{-3}$ | 1.379 |
| Reflns. collected | 5906 |
| Reflns. unique | 1756 |
| R(*int*) | 0.0766 |
| Index ranges | -40<=h<=40, -10<=k<=10, -29<=h<=29 |
| Refinement method | Full-matrix, least squares on $F^2$ |
| $wR_2$ 0.2358 | *GoF* 1.028 |

To extend the scope of the reaction one step further, we also studied the reaction of 6-amino-1,3-dimethyluracil (**1**) with dicarbaldehyde (**12**) (**Scheme 5**). The results are reported in **Table 8** (entries **a** and **b**). In the case of an aliphatic dicarbaldehyde, glutaraldehyde (entry **b**, **Table 8**), we obtained the tetrakis-uracil adduct (**14**), but in the case of *p*-benzenedicarbaldehyde (entry **a**, **Table 8**), we obtained the bis-uracil adduct (**15**) instead of the tetrakis-uracil adduct, as evidenced by the appearance of the aldehydic peak at $\delta$ = 9.96 ppm in the $^{1}$H NMR spectrum and at $\delta$ = 192.04 ppm in the $^{13}$C NMR spectrum of (**15**).




**Scheme 5:** Synthesis of tetrakisuracil derivatives (**13**)

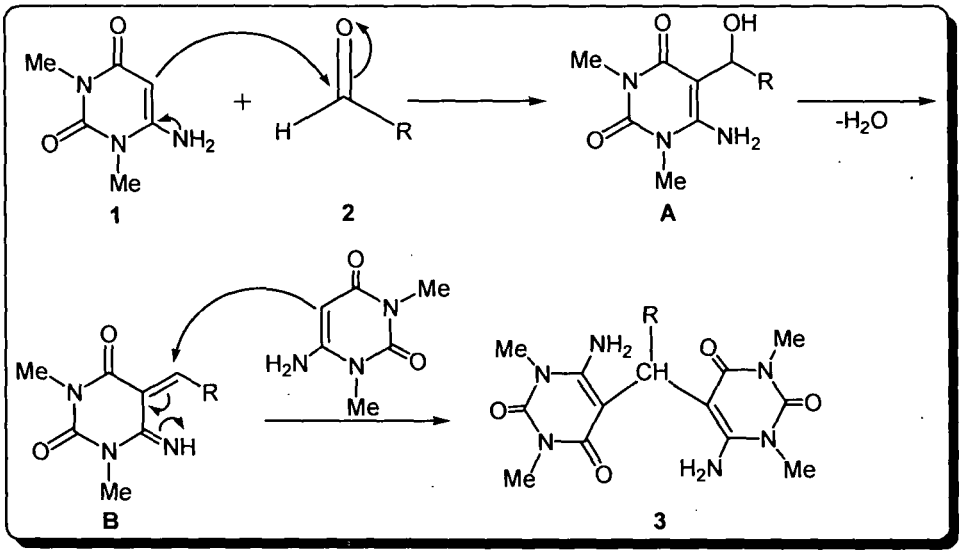
Only a few examples of the nucleophilic nature[38] of the 5-position of uracil and its metal-binding capacity have been reported.[39] Indole also shows similar nucleophilic behaviour at the 3-position and reacts with the carbonyl moiety to produce bis-indolyl compounds.[40]

**Table 8:** Synthesis of tetrakisuracil derivatives (**13**)

| Entry | Carbonyl Compounds (**12**) | Time/h | m.p. (°C) | Yield (%)[a] |
|---|---|---|---|---|
| a | *p*-(CHO)$C_6H_4$(CHO) | 7 | 301-303 | 86 |
| b | CHO$(CH_2)_5$CHO | 7 | 194-195 | 89 |

[a] Isolated yield

At this stage, the detailed mechanism is not fully understood. A plausible mechanism for the reaction is shown in **Scheme 6**. Mechanistically, the nucleophilic 5-position of 6-amino-1,3-dimethyluracil (**1**) attacks the carbon centre of the aldehyde (**2**) and is followed by elimination of a water molecule. A second molecule of (**1**) then attacks at the nucleophilic 5-position to afford the product (**3**). 6-Amino-1,3-dimethyluracil (**1**) is water-soluble, when the aldehyde, which is insoluble, is added to an aqueous solution of uracil (**1**) the reaction probably occurs at the water-organic layer interface. As soon as the product forms, it precipitates owing to its hydrophobic nature, which might be the driving force for the reaction.




**Scheme 6:** Plausible reaction mechanism for the synthesis of bis-uracil (**3**)

**Bioactivity tests:**

**Antioxidant assay:**

**Fig. 7** shows the antioxidant effect of 5 pyrimidine compounds **(8), (14), (3g), (3h), (3l)** respectively and gallic acid as a function of various concentrations. The strong antioxidant activities of the compounds were revealed in **Table 9**.

**Table 9:** $IC_{50}$ value of 5 Pyrimidine compounds

| Pyrimidine compound no. | $IC_{50}$ value ($\mu$gm/ml) |
|---|---|
| **8** | 372.1±4.3 |
| **14** | 198±3.2 |
| **3g** | 40.76±0.89 |
| **3l** | 62.21± 5.9 |
| **3h** | 302.5± 2.2 |

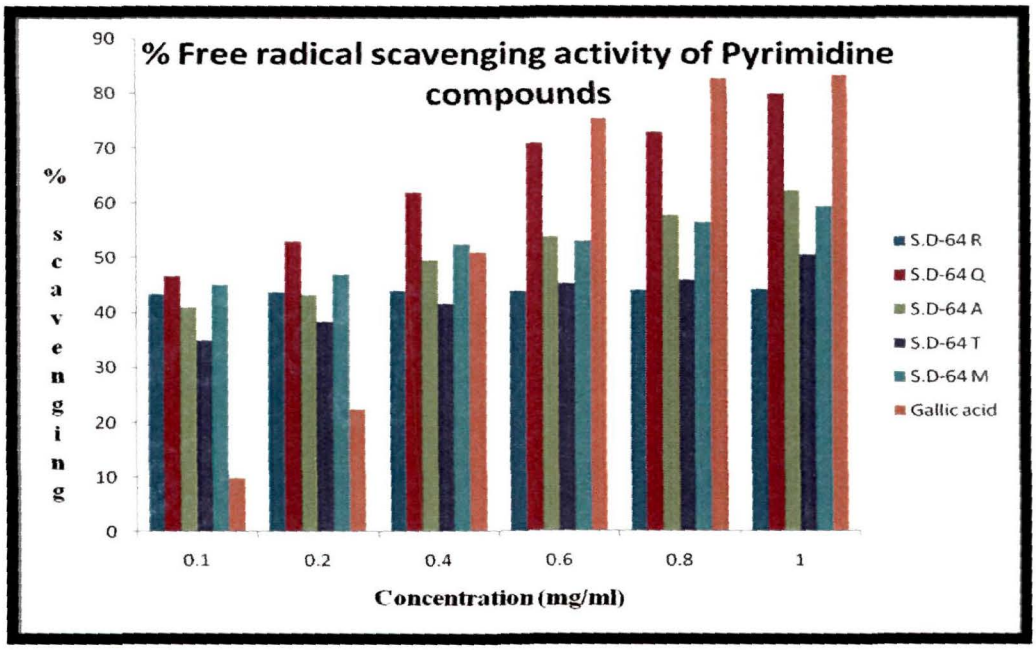



**Fig. 7**: Antioxidant activity of pyrimidine compounds (5 nos.) in DPPH assay

From the graph (**Fig. 7**) we observe that with respect to gallic acid, (**13b**) shows highest scavenging activity among all the pyrimidine compounds and $IC_{50}$ of the compound is 198±3.2 $\mu$gm/ml gallic acid equivalent.

*B. subtilis* (ATCC 11774) *P. aeruginosa* (MTCC 7814)

**Fig. 8:** Zone of inhibition of compound **(8)**

**Antibacterial assay:**

Antibacterial activities were studied against seven nos. of non-pathogenic bacteria; *Staphylococcus aureus* (ATCC 11632), *Bacillus subtilis* (ATCC 11774), *E. coli* (ATCC 9637), *Pseudomonas aeruginosa* (MTCC 7812), *Pseudomonas aeruginosa* (MTCC 7814), *Pseudomonas aeruginosa* (MTCC 7815) and *Pseudomonas aeruginosa* (MTCC 7816). Compounds were found to be equally active against both the gram (+) and gram (-) bacteria. All the tested compounds showed high to moderate to good antibacterial activities. The zone of inhibition of the compound **(8)** is shown in **Fig. 8**. The zone of inhibition of the compounds against various bacteria is shown in **Table 10**. The compounds having good activities against particular bacteria, we further calculated the MIC values (**Table 11**) for them. The MIC value for the compounds ranges from 0.0125-0.1 mg.

**Table 10:** Zone of inhibition of bisuracil derivatives **(3a-m), (8), (11)** and **(14)** against various bacterial strains

| Entry | Zone of Inhibition against | | | | | | |
|---|---|---|---|---|---|---|---|
| | *SA* | *BS* | *EC* | *PA1* | *PA2* | *PA3* | *PA4* |
| 3a | + | ++ | ± | +++ | - | + | + |
| 3b | - | + | ++ | ++ | - | - | +++ |
| 3c | + | - | ± | + | + | +++ | ± |
| 3d | - | - | - | ++ | - | - | + |
| 3e | - | - | + | - | + | - | ++ |
| 3f | - | - | + | + | - | + | - |
| 3g | ± | - | + | ± | ± | - | + |
| 3h | +++ | ± | +++ | ± | - | +++ | ± |
| 3i | ± | - | ± | ± | - | - | ± |
| 3j | - | ± | ± | ± | + | ± | ++ |
| 3k | + | ± | ++ | ++ | - | ++ | + |
| 3l | ++ | +++ | ± | ± | ++ | ± | +++ |
| 3m | + | ++ | - | - | ± | - | ± |
| 8 | ++ | +++ | +++ | + | +++ | + | +++ |
| 11 | +++ | ± | + | + | - | + | ++ |
| 14 | +++ | +++ | ++ | ++ | ++ | - | +++ |

*SA= Staphylococcus aureus; BS= Bacillus subtilis; EC= E. coli; PA1= Pseudomonas aeruginosa* (MTCC 7812); *PA2=Pseudomonas aeruginosa* (MTCC 7814); *PA3=Pseudomonas aeruginosa* (MTCC 7815); *PA4=Pseudomonas aeruginosa* (MTCC 7816), Sign: - (Inactive); ± (Negligible); + (Poor); ++ (Good); +++ (Very good)

**Table 11:** MIC value of those compounds which showed very good zone of inhibition

| Entry | MIC values against (mg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | *SA* | *BS* | *EC* | *PA1* | *PA2* | *PA3* | *PA4* |
| 3a | -- | -- | -- | 0.006 | -- | -- | -- |
| 3b | -- | -- | -- | -- | -- | -- | 0.0125 |
| 3c | -- | -- | -- | -- | -- | 0.025 | -- |
| 3h | 0.05 | -- | 0.025 | -- | -- | 0.0125 | -- |
| 8 | -- | 0.05 | 0.0125 | -- | 0.0125 | -- | 0.025 |
| 11 | 0.1 | -- | -- | -- | -- | -- | -- |
| 14 | 0.05 | 0.0125 | -- | -- | -- | -- | 0.0125 |

*SA= Staphylococcus aureus; BS= Bacillus subtilis; EC= E. coli; PA1= Pseudomonas aeruginosa* (MTCC 7812); *PA2=Pseudomonas aeruginosa* (MTCC 7814); *PA3=Pseudomonas aeruginosa* (MTCC 7815); *PA4=Pseudomonas aeruginosa* (MTCC 7816)

**Conclusion:**

The experimental procedure for the electrophilic substitution is remarkably simple without the need for any dry solvents, an inert atmosphere or reflux conditions. The product was purified by simple filtration without the need for further separation technique, that is, neither chromatography (TLC/column chromatography) nor extraction using organic solvent. Importantly, the separation step in any synthesis involves most of the capital and operating costs (60–80%) of the overall cost. Different steps (reaction, separation and purification) contribute to the environmental footprint of the process.[41] Hence, our method is environmentally benign (eliminating the use of organic solvents in both the reaction and purification), safe and cost effective. Note that no dehydrating agent, heat (hence energy is saved), catalyst, surfactant or additive is required. Moreover, protection of the $-NH_2$ group is also not required. The present method opens a new avenue for the synthesis of bis-uracils, which might be useful for exploring their bioactivities.

In conclusion, we have developed a mild, chemoselective, highly efficient, clean and truly "green" methodology for the synthesis of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimethyluracil-5-yl)methanes by condensation of 6-amino-1,3-dimethyluracil with all types of aldehydes (aromatic, aliphatic and heterocyclic) in water at room temperature.

Some of the synthesized bis-uracil/tetrakis-uracil derivatives showed very good antioxidant properties, and moderate to good antibacterial activities, but we did not

observed good antifungal properties. The MIC values of pyrimidine derivatives against various bacteria were 0.05-0.0125 mg and $IC_{50}$ values of pyrimidine derivatives in DPPH redical methods were 40.76±0.89-372±4.3 $\mu$gm/ml.

**Experimental Section:**

**General procedure for the synthesis of bis-uracil (3):**

Distilled water (30 mL) was added to 6-amino-1,3-dimethyluracil (**1**; 155 mg, 1 mmol) in a 100 mL round-bottomed flask and the mixture was stirred at room temperature until all the 6-amino-1,3-dimethyluracil had dissolved. Benzaldehyde (**2a**, 54 mg, 0.5 mmol) was added drop wise to the 6-amino-1,3-dimethyluracil solution with constant stirring and then after a few minutes the product appeared as a white precipitate and stirring was continued for a further 1 h so that all of the reactants were converted into product. The white precipitate was filtered and collected to provide the pure product **3a** in 95% yield. A small amount of the product was dissolved in distilled ethanol (98%) and then warmed. Then the solution was filtered, allowed to cool and evaporated at room temperature to give square shaped white shining transparent crystals. The crystals were collected and dried; m.p. 296–299 °C. The crystals are stable at room temperature and also in an open environment for several days.

The same procedure was followed for the other substrates.

**General procedure for the synthesis of bis- and tetrakis-uracil (14) and (15):**

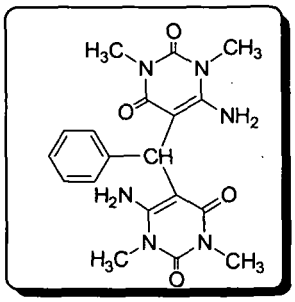
Distilled water (30 mL) was added to 6-amino-1,3-dimethyluracil (**1**) (155 mg, 1 mmol) in a 100 mL round-bottomed flask and the mixture was stirred at room temperature until all the 6-amino-1,3-dimethyluracil had dissolved. Then glutaraldehyde (25 mg, 0.25 mmol; entry **12b**, **Table 2**) was added dropwise to the 6-amino-1,3-dimethyluracil solution with constant stirring. After 3 h a yellowish white precipitate appeared and stirring was continued for further 4 h so that all of the reactants were converted into product. The yellowish white precipitate was filtered and collected. On drying we obtained the product (**8**) in 89% yields. A small amount of the product was dissolved in distilled ethanol (98%) and warmed. Then the solution was filtered, allowed to cool and

evaporated at room temperature to give square-shaped yellow shining transparent crystals. The crystals were collected and dried; m.p. 194–195 °C. The crystals are stable at room temperature and also in an open environment for several days.

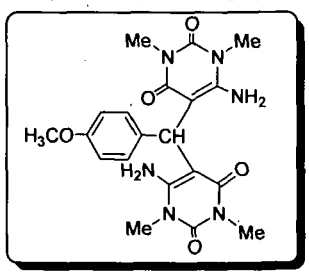
The same procedure was followed for **(12a)**.

**Physical and spectral data:**

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(benzylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (3a):** Colour- white shining transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3453.37, 3383.38, 3197.94, 2993.08, 1695.96, 1654.14, 1587.29; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.27 (s, 3H, $NCH_3$), 3.36 (s, 3H, $NCH_3$), 3.44 (s, 6H, $NCH_3$), 5.79 (s, 1H, CH), 6.48 (br s, 2H, $NH_2$), 6.69 (br s, 2H, $NH_2$), 7.14-7.25 (m, 5H, arom) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.4 ($NCH_3$), 28.8 ($NCH_3$), 29.1 ($NCH_3$), 29.4 ($NCH_3$), 35.7 (CH), 87.4 & 88.8 (C-5 & C′-5), 125.7 (C-4, arom), 126.6 (C-3 & C-5, arom), 127.9 (C-2 & C-6, arom), 138.1 (C-1, arom), 150.9 (C-6 & C′-6), 153.1 & 154.4 (C-2 & C′-2), 163.2 & 164.8 (C-4 & C′-4) ppm. MS, *m/z* 398 ($M^+$); Anal. Calcd (%) for $C_{19}H_{22}N_6O_4$: C, 57.28; H, 5.57; N, 21.07. Found C, 57.31; H, 5.56; N, 21.01.

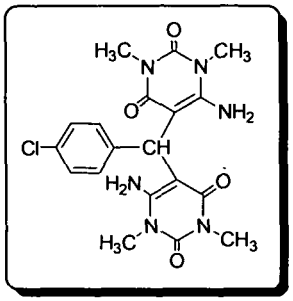


**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(4-methoxybenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (3b):** Colour- white crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3358.89, 3195.07, 3083.58, 2952.68, 1688.34, 1593.50, 1503.18, 1448.50; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.29 (s, 3H, $NCH_3$), 3.36 (s, 3H, $NCH_3$), 3.45 (s, 6H, $NCH_3$), 3.77 (s, 3H, $OCH_3$), 5.74 (s, 1H, CH), 6.49 (br s, 2H, $NH_2$), 6.70 (br s, 2H, $NH_2$), 6.80 (d, *J*=8.72 Hz, 2H, arom), 7.04 (d, *J*=8.24 Hz, 2H, arom) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$)

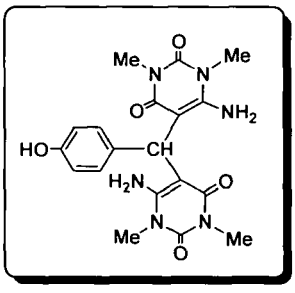


$\delta_c$ 29.2 ($NCH_3$), 29.3 ($NCH_3$), 29.3 ($NCH_3$), 29.4 ($NCH_3$), 35.1 (CH), 55.2 ($OCH_3$), 87.9 & 89.1 (C-5 & C′-5), 113.4 (C-1, arom), 127.7 (C-3 & C-5, arom), 129.9 (C-2 & C-6, arom), 151.0 (C-6 & C′-6), 153.4 & 154.6 (C-2 & C′-2), 157.6 (C-4, arom), 163.3 & 165.2 (C-4 & C′-4) ppm; MS, *m/z* 393 ($M^+$); Anal. Calcd (%) for $C_{20}H_{24}N_6O_5$: C, 56.07; H, 5.65; N, 19.62. Found C, 56.10; H, 5.68; N, 19.65.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(4-chlorobenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (3c):** Colour- white transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3346.99, 3156.01, 3040.98, 2951.22, 1692.61, 1589.72, 1495.72; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.27 (s, 3H, $NCH_3$), 3.38 (s, 3H, $NCH_3$), 3.45 (s, 6H, $NCH_3$), 5.74 (s, 1H, CH), 6.48 (br s, 2H, $NH_2$), 6.68 (br s, 2H, $NH_2$), 7.08 (d, *J*=21.08 Hz, 2H, arom), 7.26 (d, *J*=21.08 Hz, 2H, arom) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 29.2 ($NCH_3$), 29.3 ($NCH_3$), 29.4 ($NCH_3$), 29.7 ($NCH_3$), 35.6 (CH), 88.5 & 88.6 (C-5 & C′-5), 127.9 (C-4, arom), 128.1 (C-3 & C-5, arom), 131.4 (C-2 & C-6, arom), 136.8 (C-1, arom), 150.9 (C-6 & C′-6), 153.2 & 154.5 (C-2 & C′-2), 163.3 & 164.6 (C-4 & C′-4) ppm; MS, *m/z* 432 ($M^+$); Anal. Calcd (%) for $C_{19}H_{21}ClN_6O_4$: C, 52.72; H, 4.89; N, 19.42. Found C, 52.73; H, 4.86; N, 19.38.

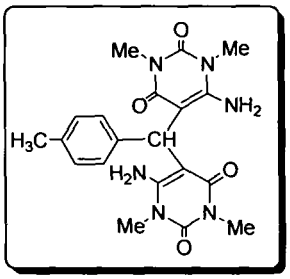



**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(4-hydroxybenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (3d):** Colour- transparent crystalline solid. Solubility- soluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3393.35, 3118.08, 2956.74, 1664.76, 1601.04, 1498.95; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 2.98 (s, 3H, $NCH_3$), 3.01 (s, 3H, $NCH_3$), 3.04 (s, 3H, $NCH_3$), 3.47 (s, 3H, $NCH_3$), 5.69 (s, 1H, CH), 7.16 (br s, 2H, $NH_2$), 7.41 (br s, 2H, $NH_2$), 6.72 (d, *J*=8.72 Hz, 2H, arom), 6. 96 (d, *J*=8.24 Hz, 2H, arom), 8.58 (s, 1H, OH)

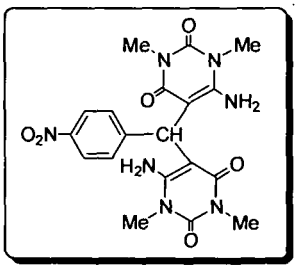


ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta_c$ 29.52 ($2NCH_3$), 29.55 ($2NCH_3$), 34.89 (CH), 114.85 (C-4, arom), 127.52 (C-3 & C-5, arom), 127.55 (C-2 & C-6, arom), 129.16 (C-1, arom), 151.07 (C-6 & C′-6), 154.79 (C-2 & C′-2) ppm; MS, *m/z* 414 ($M^+$); Anal. Calcd (%) for $C_{19}H_{22}N_6O_5$: C, 55.07; H, 5.35; N, 20.28. Found C, 55.04; H, 5.33; N, 20.25.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(4-methylbenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (3e):** Colour- transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3358.52, 3152.81, 3053.07, 2929.70, 1690.23, 1591.04, 1498.48, 1447.64; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.30 (s, 3H, $CH_3$), 3.28 (s, 3H, $NCH_3$), 3.37 (s, 3H, $NCH_3$), 3.45 (s, 6H, $NCH_3$), 5.75 (s, 1H, CH), 6.49 (br s, 2H, $NH_2$), 6.69 (br s, 2H, $NH_2$), 7.01-7.08 (m, 4H, arom) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 20.8 ($CH_3$), 28.3 ($NCH_3$), 28.3 ($NCH_3$), 29.6 ($NCH_3$), 29.6 ($NCH_3$), 35.6 (CH), 86.8 & 88.4 (C-5 & C′-5), 126.4 (C-4, arom), 128.4 (C-3 & C-5, arom), 134.4 (C-2 & C-6, arom), 135.5 (C-1, arom), 150.9 (C-6 & C′-6), 153.5 & 154.5 (C-2 & C′-2), 163.2 & 164.4 (C-4 & C′-4) ppm; MS, *m/z* 412 ($M^+$); Anal. Calcd (%) for $C_{20}H_{24}N_6O_4$: C, 58.24; H, 5.87; N, 20.38. Found C, 58.21; H, 5.84; N, 20.31.

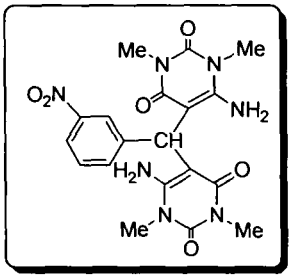



**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(4-nitrobenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (3f):** Colour- brown transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3394.02, 3189.69, 1676.16, 1615.01, 1505.16; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.30 (s, 3H, $NCH_3$), 3.36 (s, 3H, $NCH_3$), 3.39 (s, 3H, $NCH_3$), 3.42 (s, 3H, $NCH_3$), 5.84 (s, 1H, CH), 6.53 (br s, 2H, $NH_2$), 6.68 (br s, 2H, $NH_2$), 8.08 (d, $J$=8.72 Hz, 2H, arom), 8.40 (d, $J$=8.72 Hz, 2H, arom) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.6 ($NCH_3$), 29.1 ($NCH_3$), 29.4 ($NCH_3$), 29.7 ($NCH_3$), 35.9 (CH), 86.7 & 88.2 (C-5 & C′5), 122.1 (C-2 & C-6, arom), 128.1 (C-3 & C-5, arom), 141.2 (C-1, arom), 148.6 (C-4, arom), 150.9

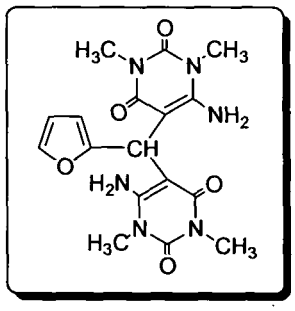


(C-6 & C′-6), 153.4 & 154.9 (C-2 & C′-2), 163.3 & 165.1 (C-4 & C′-4) ppm; MS, *m/z* 443 ($M^+$); Anal. Calcd (%) for $C_{19}H_{21}N_7O_6$: C, 51.47; H, 4.77; N, 22.11. Found C, 51.43; H, 4.78; N, 22.13.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(3-nitrobenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (3g):** Colour- brown transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic volatile solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3396.10, 3196.67, 1681.06, 1607.07, 1503.67, 1451.58; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.36 (s, 3H, $NCH_3$), 3.45 (s, 3H, $NCH_3$), 3.50 (s, 3H, $NCH_3$), 3.52 (s, 3H, $NCH_3$), 5.81 (s, 1H, CH), 6.51 (br s, 2H, $NH_2$), 6.69 (br s, 2H, $NH_2$), 7.23-7.24 (m, 1H, arom), 7.41-7.46 (m, 2H, arom), 7.95-8.08 (m, 1H, arom) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.6 ($NCH_3$), 29.1 ($NCH_3$), 29.4 ($NCH_3$), 29.7 ($NCH_3$), 35.9 (CH), 86.7 & 88.2 (C-5 & C′-5), 121.2 (C-4, arom), 122.1 (C-2, arom), 128.9 (C-5, arom), 133.3 (C-6, arom), 141.2 (C-1, arom), 148.6 (C-3, arom), 151.0 (C-6 & C′-6), 153.4 & 154.9 (C-2 & C′-2), 163.4 & 165.1 (C-4 & C′-4) ppm; MS, *m/z* 443 ($M^+$); Anal. Calcd (%) for $C_{19}H_{21}N_7O_6$: C, 51.47; H, 4.77; N, 22.11. Found C, 51.42; H, 4.74; N, 22.11.

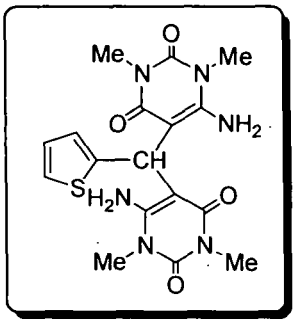



**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(furyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (3h):** Colour- transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3351.56, 3190.48, 3074.22, 2951.20, 1698.55, 1592.24, 1497.74; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.29 (s, 3H, $NCH_3$), 3.42 (s, 9H, $NCH_3$), 5.65 (s, 1H, CH), 5.98 (1H, furan), 6.28 (1H, furan), 6.51 (br, s, 2H, $NH_2$), 6.63 (br, s, 2H, $NH_2$), 7.24 (1H, furan) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.6 ($NCH_3$), 28.6 ($NCH_3$), 29.3 ($NCH_3$), 29.3 ($NCH_3$), 31.8 (CH), 87.6 (C-5 & C′5), 105.9 (C-3, furan), 110.2 (C-4, furan), 141.0 (C-6 & C′-6), 150.9 (C-5, furan), 152.4 & 153.5 (C-2 & C′-2), 164.1



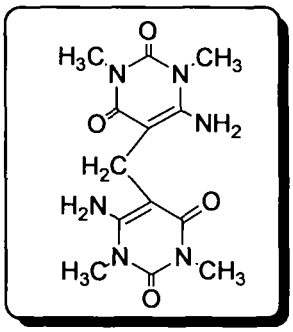
(C-4 & C′-4) ppm; MS, *m/z* 388 ($M^+$); Anal. Calcd (%) for $C_{17}H_{20}N_6O_5$: C, 52.57; H, 5.19; N, 21.64. Found C, 52.55; H, 5.20; N, 21.62.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(thiophenyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (3i):** Colour- transparent crystalline solid. Solubility- insoluble in water, sparingly



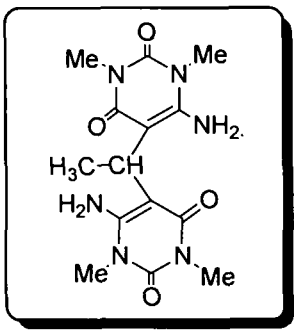
soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3361.56, 3327.33, 3057.73, 2982.55, 1681.87, 1632.89, 1573.09; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.31 (s, H, $NCH_3$), 3.33 (s, H, $NCH_3$), 3.42 (s, H, $NCH_3$), 3.45 (s, H, $NCH_3$), 5.93 (s, 1H, CH), 6.54 (br s, 2H, $NH_2$), 6.68 (1H, thiophene), 6.87 (1H, thiophene), 6.91 (br s, 2H, $NH_2$), 7.13 (1H, thiophene) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 29.32 ($NCH_3$), 29.38 ($NCH_3$), 29.41 ($NCH_3$), 29.44 ($NCH_3$), 33.3 (CH), 89.0 & 89.3 (C-5 & C′5), 123.4 & 124.0 (C-6 & C′-6), 126.3 (C-3, furan), 144.4 (C-4, furan), 150.9 (C-5, furan), 153.2 & 153.9 (C-2 & C′-2), 163.5 & 164.5 (C-4 & C′-4) ppm; MS, *m/z* 404.13 ($M^+$); Anal. Calcd (%) for $C_{17}H_{20}N_6O_4S$: C, 50.48; H, 4.98; N, 20.78. Found C, 50.46; H, 4.99; N, 20.77.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(methyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (3j):** Colour- white transparent crystalline solid. Solubility- insoluble in water,

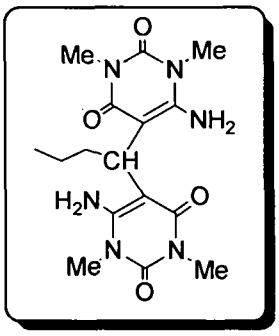


common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3386.70, 3155.75, 2927.21, 1682.97, 1619.94, 1504.20; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 3.19 (s, 3H, $NCH_3$), 3.28 (s, 3H, $NCH_3$), 3.37 (s, 6H, $NCH_3$), 4.13 (s, 2H, $CH_2$), 7.51 (br s, 2H, $NH_2$), 7.70 (br s, 2H, $NH_2$) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta_c$ 19.53 ($CH_2$), 29.96 ($2NCH_3$), 30.07 ($2NCH_3$), 85.2 & 85.41 (C-5 & C′-5), 151.08 (C-6 & C′-6), 154.30 & 154.36 (C-2 & C′-2), 164.00 (C-4 & C′-4) ppm; MS, *m/z* 322 ($M^+$); Anal. Calcd (%) for $C_{13}H_{18}N_6O_4$: C, 48.44; H, 5.63; N, 26.07. Found C, 48.42; H, 5.61; N, 26.08.

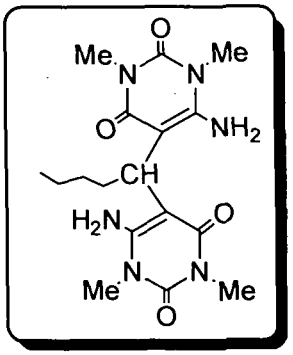
**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(ethyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (3k):** Colour- white transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. IR (KBr) ($\nu_{max}/cm^{-1}$) 3410.87, 3172.15, 2997.44, 2949.39, 1680.66, 1621.14, 1493.60; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.66 (3H, $CH_3$), 3.31 (s, 6H, $NCH_3$), 3.46 (s, 6H, $NCH_3$), 4.43-4.45 (m, 1H, CH), 6.48 (br s, 2H, $NH_2$), 6.91 (br s, 2H, $NH_2$) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 16.1 ($CH_3$), 26.3 (CH), 28.3 ($NCH_3$), 28.8 ($NCH_3$), 29.2 ($NCH_3$), 29.5 ($NCH_3$), 90.1 & 91.3 (C-5 & C′-5), 151.0 (C-6 & C′-6), 152.9 & 153.6 (C-2 & C′-2), 164.2 & 164.4 (C-4 & C′-4) ppm; MS, *m/z* 336.15 ($M^+$); Anal. Calcd (%) for $C_{14}H_{20}N_6O_4$: C, 49.99; H, 5.99; N, 24.99. Found C, 49.99, H, 5.98; N, 24.97.




**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(butyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (3l):** Colour- white transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. IR (KBr) ($\nu_{max}/cm^{-1}$) 3401.23, 3146.61, 2955.29, 1666.63, 1606.54, 1493.54; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 0.91-0.93 (m, 3H, $CH_3$), 1.25-1.27 (m, 2H, $CH_2$), 2.16-2.21 (m, 2H, $CH_2$), 3.31 (s, 3H, $NCH_3$), 3.35 (s, 3H, $NCH_3$), 3.45 (s, 6H, $NCH_3$), 4.20 (t, *J*=8 Hz, 1H, CH), 6.54 (br s, 2H, $NH_2$), 6.67 (br s, 2H, $NH_2$) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 13.7 ($CH_3$), 21.7 (CH), 27.8 ($CH_2$), 28.4 ($NCH_3$), 28.7 ($NCH_3$), 29.1 ($NCH_3$), 31.3 ($NCH_3$), 31.6 ($CH_2$), 88.6 & 89.6 (C-5 & C′-5), 150.6 & 150.7 (C-6 & C′-6), 152.5 & 153.6 (C-2 & C′-2), 163.7 & 164.3 (C-4 & C′-4) ppm; MS, m/z 364.19 ($M^+$); Anal. Calcd (%) for $C_{16}H_{24}N_6O_4$: C, 52.74; H, 6.64; N, 23.06. Found C, 52.72; H, 6.66; N, 23.08.

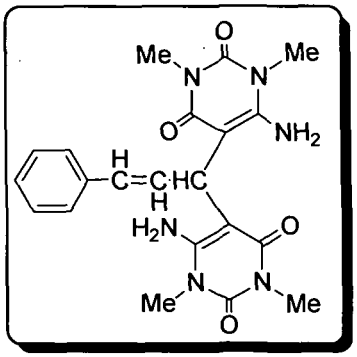



**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(pentyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (3m):** Colour- white transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. IR (KBr) ($\nu_{max}/cm^{-1}$) 3396.53, 3106.74, 2952.08, 2859.75, 1661.83, 1606.21, 1490.77; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 0.84-0.88 (m, 3H, $CH_3$),



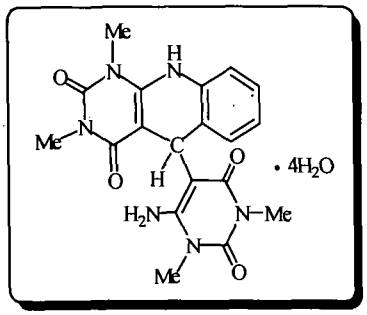
1.26-1.30 (m, 4H, $CH_2$-$CH_2$), 2.05-2.21 (m, 2H, $CH_2$), 3.34 (s, 3H, $NCH_3$), 3.36 (s, 3H, $NCH_3$), 3.46 (s, 3H, $NCH_3$), 3.47 (s, 3H, $NCH_3$), 4.13-4.18 (m, 1H, CH), 6.52 (br s, 2H, $NH_2$), 6.94 (br s, 2H, $NH_2$) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 13.3 ($CH_3$), 21.8 (CH), 27.4 ($CH_2$), 27.9 ($CH_2$), 28.2 ($NCH_3$), 28.4 ($NCH_3$), 28.6 ($NCH_3$), 30.4 ($NCH_3$), 31.4 ($CH_2$), 88.2 & 89.2 (C-5 & C′-5), 150.1 & 150.2 (C-6 & C′-6), 151.9 & 153.0 (C-2 & C′-2), 163.2 & 163.8 (C-4 & C′-4) ppm; MS, m/z 378.2 ($M^+$); Anal. Calcd (%) for $C_{17}H_{26}N_6O_4$: C, 53.96; H, 6.93; N, 22.21. Found C, 53.97; H, 6.93; N, 22.20.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(cinnamylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (8):** Colour- yellowish transparent crystalline solid. Solubility- insoluble in water,



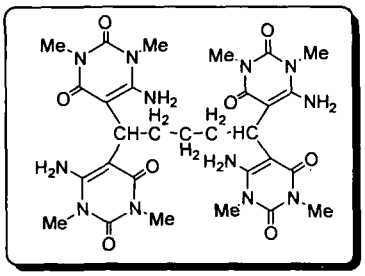
sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3390.54, 3214.46, 3097.30, 2941.31, 1688.03, 1594.43, 1497.32; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.31 (s, 3H, $NCH_3$), 3.35 (s, 3H, $NCH_3$), 3.42 (s, 6H, $NCH_3$), 5.16 (s, 1H, CH), 6.27 (br s, H, $NH_2$), 6.31 (br s, H, $NH_2$), 6.70 (d, *J*=4.56 Hz, 1H, CH=CH), 6.74 (d, *J*=4.56 Hz, 1H, CH=CH), 7.15-7.34 (m, 5H, arom) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.0 ($NCH_3$), 28.1 ($NCH_3$), 29.1 ($NCH_3$), 29.2 ($NCH_3$), 34.2 (CH), 36.1 (CH=CH), 39.3 (CH=CH), 86.3 & 86.8 (C-5 & C′-5), 126.2 (C-4, arom), 128.4 (C-3 & C-5, arom), 128.9 (C-2 & C-6, arom), 137.7 (C-1, arom), 145.3 (C-6 & C′-6), 150.5 & 150.9 (C-2 & C′-2), 162.1 & 165.0 (C-4 & C′-4) ppm; MS, *m/z* 424 ($M^+$); Anal. Calcd (%) for $C_{21}H_{24}N_6O_4$: C, 59.42; H, 5.70; N, 19.80. Found C, 59.45; H, 5.75; N, 19.78.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(2-hydroxybenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (11):** Colour- transparent crystalline solid. Solubility- soluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3457.17, 3297.16, 3195.14, 2973.08, 1694.16, 1657.21, 1583.23; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.09 (s, 3H, $NCH_3$), 3.32 (s,



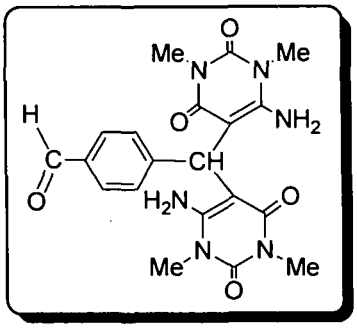
3H, $NCH_3$), 3.48 (s, 3H, $NCH_3$), 3.57 (s, 3H, $NCH_3$), 4.84 (s, 1H, CH), 5.75 (s, 1H, OH), 6.41 (br s, 4H, $NH_2$), 7.05-7.25 (m, 5H, arom) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 27.6 ($NCH_3$), 28.1 ($NCH_3$), 29.2 ($NCH_3$), 29.3 ($NCH_3$), 30.1 (CH), 87.8 & 93.3 (C-5 & C′-5), 115.5 (C-4, arom), 123.4 & 125.2 (C-3 & C-5, arom), 127.8 & 128.3 (C-2 & C-6, arom), 150.4 & 150.5 (C-6 & C′-6), 151.3 & 151.5 (C-2 & C′-2), 154.3 (C-1, arom), 161.2 & 164.0 (C-4 & C′-4) ppm; MS, *m/z* 414 ($M^+$); Anal. Calcd (%) for $C_{19}H_{22}N_6O_5$: C, 55.07; H, 5.35; N, 20.28. Found C, 55.10; H, 5.33; N, 20.22.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(glutarylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione] (14):** Colour- yellow transparent crystalline solid. Solubility- insoluble in water,



sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3409.63, 3215.74, 2938.88, 1664.95, 1603.34, 1495.23, 1446.66; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 0.81-0.94 (m, 2H, $CH_2$), 1.22-1.34 (m, 4H, $2CH_2$), 3.09 (s, 3H, $NCH_3$), 3.25 (s, 3H, $NCH_3$), 3.34 (s, 18H, $NCH_3$), 3.94 (s, 1H, CH), 4.12 (s, 1H, CH), 6.78 (br s, 2H, $NH_2$), 6.88 (br s, 2H, $NH_2$), 7.19 (br s, 2H, $NH_2$), 7.67 (br s, 2H, $NH_2$) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$+ DMSO-$d_6$) $\delta_c$ 22.7 (CH), 23.5 (CH), 27.4 ($CH_2$), 27.7 ($CH_2$), 28.0 ($NCH_3$), 28.3 ($NCH_3$), 28.7 ($NCH_3$), 29.2 ($NCH_3$), 29.4 ($NCH_3$), 29.5 ($NCH_3$), 29.7 ($NCH_3$), 30.1 ($NCH_3$), 31.7 ($CH_2$), 85.9, 87.9, 89.3 & 89.4 (C-5, C-5, C′-5 & C′-5), 128.2, 128.6, 129.7 & 130.8 (C-6, C-6, C′-6 & C′-6), 150.8, 150.9, 153.0 & 154.2 (C-2, C-2, C′-2 & C′-2), 162.8, 163.4 & 164.0 (C-4, C-4, C′-4 & C′-4) ppm; MS, *m/z* 684.31 ($M^+$); Anal. Calcd (%) for $C_{29}H_{40}N_{12}O_8$: C, 50.87; H, 5.89; N, 24.55. Found C, 50.88 H, 5.90; N, 24.54.

**6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(terepthaldibenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (15):** Colour- transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3420.99, 3110.18, 2924.07, 1683.96,



1603.00, 1496.68; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.27 (s, 3H, $NCH_3$), 3.39 (s, 3H, $NCH_3$), 3.47 (s, 3H, $NCH_3$), 3.50 (s, 3H, $NCH_3$), 5.84 (s, 1H, CH), 6.51 (br s, 2H, $NH_2$), 6.69 (br s, 2H, $NH_2$), 7.32 (d, $J$=8 Hz, 2H, arom), 7.78 (d, $J$=8 Hz, 2H, arom), 9.96 (s, 1H, CHO) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.5 ($NCH_3$), 29.0 ($NCH_3$), 29.4 ($NCH_3$), 29.7 ($NCH_3$), 36.3 (CH), 86.7 & 88.7 (C-5 & C′-5), 127.4 (C-4, arom), 129.7 (C-3 & C-5, arom), 134.3 (C-2 & C-6, arom), 146.3 (C-1, arom), 150.9 (C-6 & C′-6), 153.3 & 154.8 (C-2 & C′-2), 165.1 (C-4 & C′-4), 192.0 (CHO) ppm; MS, m/z 426.17 ($M^+$); Anal. Calcd (%) for $C_{20}H_{22}N_6O_5$: C, 56.33; H, 5.20; N, 19.17. Found C, 56.33; H, 5.21; N, 19.18

# Section B

## Nucleophilic addition of 6-aminouracil derivatives using 'green surfactant' isolated from *P. aeruginosa* OBP1 in water at room temperature

**Introduction:**

Nucleophilic nature of the C5-position of uracil and its derivatives is well known.[30-34, 39] Electron density distributions of uracil and their derivatives also show that C5-position is the only carbon centre susceptible to attack by electrophiles.[43] Depending on the nature of substituted or unsubstituted uracil derivatives the nucleophilicity of C5-position also changes. It is observed that the nucleophilicity of C5-position increases in the presence of amino group at C6-position instead of unsubstituted uracil.[29]

In the recent years, the surface active molecules (e.g. biosurfactant) produced by microbes are getting popularity. Such molecules tend to accumulate at the interface of two mediums such as air-liquid, liquid-liquid and liquid-solid and form a thin molecular film at the junction of the two mediums that ultimately lower the interfacial tension and surface tension.[44] At the interface of two mediums, such molecules interact with each other with different degrees of polarity and hydrogen bonding between the molecules causes a decrease in the surface tension and critical micelle concentration (CMC). These properties create micro-emulsions leading to micelle formation in which hydrocarbons can solubilize in water or water in hydrocarbons. These properties make the surfactant more applicable in industrial processes like wetting and phase dispersion, detergency, foaming, emulsification etc.[45] The use of biosurfactant seems to offer more potential than chemical surfactants, due to their structural diversity, biodegradability, biocompatibility, ecofriendly, reusable, stability over the different extreme conditions such as high temperature, pH and salinity, less toxicity, and production from the renewable sources, mainly from wastes.[46] In this chapter, we have used the rhamnolipids (mixture of mono-rhamnolipids and di-rhamnolipids) (**Fig. 9**), one major class of biosurfactant, which is most often produced by *P. aeruginosa* OBP1. The active ingredient, rhamnolipid

biosurfactant, is a transparent liquid with a light to dark amber tint and a mild, sweet soapy odor. The biosurfactant used in our experiment is a mixture of two rhamnolipid molecules that we recently published.[47]

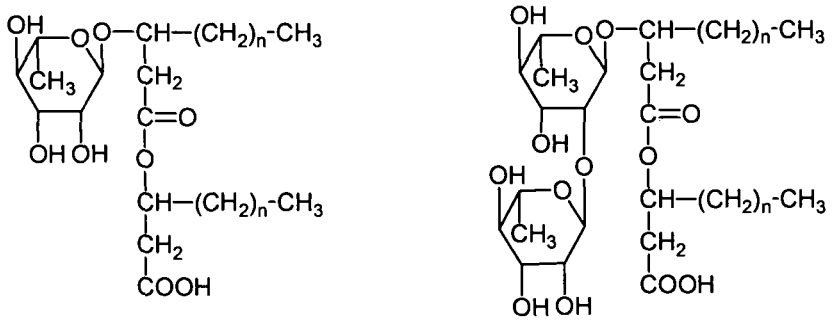


**Fig. 9**: Structure of rhamnolipids (monorhamnolipid & dirhamnolipid)

**Materials and Methods:**

Melting points were determined on a Büchi 504 apparatus and are uncorrected. IR spectra were recorded as KBr pallets on a Nicolet (Impact 410) FT-IR spectrophotometer. $^{1}H$ NMR & $^{13}C$ NMR spectra were recorded on a JNM ECS 400 MHz NMR spectrophotometer (JEOL) using Tetramethylsilane (TMS) as the internal standard. X-ray intensity data were collected on a Bruker SMART APEX CCD area- detector diffractometer with Mo Kα radiation (λ= 0.71073 Å). The structures were solved by *SHELX97* and refined by full-matrix least squares on $F^2$ (*SHELX97*).[42] Reactions were monitored by thin layer chromatography using aluminium sheets with silica gel 60 $F_{254}$ (Merck). Elemental analyses were carried out in a Perkin Elmer CHN analyzer (2400 series II). Mass spectra were recorded on a Waters Q-TOF Premier & Aquity UPLC spectrometer. All the chemicals were used as received.

**Crystal structure of (17d):**

Single crystals of **(17d)** were grown from their 98% ethanol solution. The intensity data were collected at 296 K on a Bruker SMART APEX CCD area-detector diffractometer with Mo *K*α radiation (λ= 0.71073 Å). Crystallographic and experimental data are listed in **Table 13**. Hydrogen site location is inferred from neighboring sites, H atoms are

treated by a mixture of independent and constrained refinement. They were positioned geometrically and treated as riding on their parent C-atoms.

**Biosurfactant recovery:**

The collected supernatant was first centrifuged at 10,000 rpm for 20 min at 4 °C to remove the bacterial cells. The culture supernatant was acidified to pH 2 with 6 *N* HCl and allowed to stand overnight at 4 °C to precipitate the rhamnolipid. The precipitate was harvested by centrifugation at 10,000 rpm for 15 min at 4 °C. The recovered precipitate was extracted thrice with ethyl acetate at room temperature. The organic phase was collected in a round bottom flask and connected to a rotary evaporator (Eyela, CCAS-1110, Tokyo, Rikakikai Co. Ltd.) to remove the solvent. The process yielded a viscous honey-coloured biosurfactant.[48] The thick residue was washed twice with n-hexane to remove any residual n-hexadecane. Finally the yellowish product was dissolved in ethyl acetate, filtered and concentrated using a rotary evaporator.

The isolated biosurfactant was further analyzed by thin layer chromatography (TLC) on silica gel 60 G (Merck) in chloroform: methanol: water (65:25:4, *v/v/v*). The plates were sprayed with orcinol-$H_2S0_4$ solution[49] and developed at 100°C for 5 min. Positive spots were scraped and extracted with 3 ml of chloroform: methanol (2:1, *v/v*) in an elution column.

The surface tension of the supernatant was measured with a Krüss K12 tensiometer (Tensiometer K9, Krüss K9 ETS-S) accordingly to the De Noüy ring method.[50] At the end of each measurement, the platinum ring was rinsed thrice with water followed by 3 times with acetone and was allowed to dry in air. Culture supernatant 25 ml was taken into a glass beaker of volume of 50 ml and placed onto the platform of tensiometer. The surface tension was measured at 25 °C ± 1 °C. After introducing the platinum ring in the culture supernatant, it was slowly allowed to touch the liquid-air interface, to measure the surface tension. The platinum ring was kept in the supernatant for a while in order to attain equilibrium conditions. The measurement was repeated thrice and an average value was obtained.

**Determination of CMC of biosurfactant:**

For the determination of CMC value, the culture supernatant was serially diluted and surface tension for each dilution was measured. The CMC value, expressed in mg/l, was estimated from the break point of the surface tension versus the concentration. For calibrating the instrument, it was subjected to the determination of surface tension of the pure water at 25 °C ± 1 °C.

**Results and Discussion:**

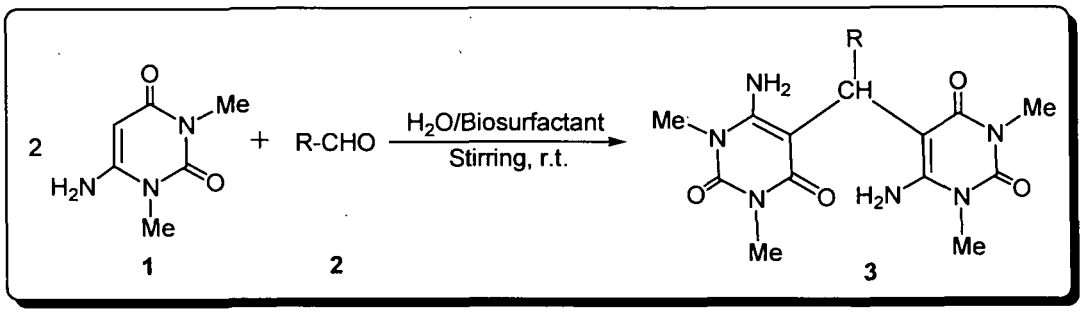
Application of biosurfactant in chemistry, specially, in organic transformation is still not explored and to the best of our knowledge, for the first time we have introduced the use of biosurfactant in organic transformation, we believe this methodology will open a new vista in synthetic methodology. In our organic synthesis programme, we are interested in the synthesis of bioactive uracil derivatives.[24] In this connection, recently, we have reported the formation of bis-uracil derivatives by exploiting the nucleophilic behavior of C-5 position.[51] Here, nucleophilic addition of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione to aldehydes in water was described.

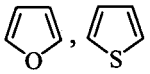
In continuation to our earlier work,[51] to explore the reaction further, here, we report the nucleophilic addition of two uracil derivatives via C-5 position with aldehydes (aromatic, aliphatic and heterocyclic) by varying their C6-position using biosurfactant in water at room temperature.

Following the same procedure as described,[51] we tried the reaction of 6-[(dimethylamino)methyleneamino]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione with aldehydes in water. But, the reaction did not proceed under that reaction condition. Using *p*-Toluene sulphonic acid (*p*-TSA) as catalyst or applying heat did not help the reaction to proceed at all. Interestingly, when we carried out the reaction using biosurfactant in water, the reaction proceeded but with very low yield. However, when we added *p*-TSA (15 mol%) as catalyst, the yield of the product was found to increase.

Delighted with this result, we wanted to check our earlier reported work in the presence of biosurfactant. Accordingly, as a model reaction, when we simply stirred 6-amino-1,3-dimethyluracil (**1**) and benzaldehyde (**2a**; R = Ph) using biosurfactant (at CMC) in water at room temperature, we ended up with the formation of 5,5'-

phenylmethylenebis(1,3-dimethyl-6-aminopyrimidine-2,4-dione) (**3a**) within 15 min in 99% yield (**Scheme 7**). The reaction was monitored by TLC. The reaction was very clean providing only one product, that is, (**3a**). The structure was confirmed by IR, $^{1}H$ and $^{13}C$ NMR spectroscopy, mass spectrometry, elemental analyses and single crystal X-ray analysis.




R= Ph, CH=CH-Ph, *p*-$OCH_3$Ph, *p*-ClPh, *p*-OHPh, *o*-OHPh, *p*-$CH_3$Ph, *p*-$NO_2$Ph, *m*-$NO_2$Ph, H, $CH_3$, $CH_3$-$(CH_2)_3$, $CH_3$-$(CH_2)_2$, 

**Scheme 7:** Synthesis of bisuracil derivatives (**3**) in the presence of biosurfactant

In the presence of biosurfactant, the solubility of uracil increases and more over in some cases aldehydes also become soluble. The best solubility was observed at critical micelle concentration (CMC). Here, 8 ml of water is sufficient instead of 30-35 ml water in comparison to our earlier report. Encouraged by this interesting result, to study the scope and limitations of the reaction further, we extended the reaction to other differently substituted aromatic, aliphatic, heterocyclic aldehydes and ketones (**2a-r**) under the same optimized reaction condition. The results are summarized in **Table 12** (entries **a-r**). To our delight, all the aldehydes (entries **2a-o**, **Table 12**) reacted with equal ease within short times, furnishing the products, bis-uracils (**3a-o**) in good to excellent yields (90-99%) and with no side products formation. In comparison to our earlier report, here, we have shown that the yields of product have increased and the reaction time has reduced drastically along a reduction of the amount of water used from 30-35 ml to 8ml.

Instead of 1,3-dimethyl-6-aminopyrimidine-2,4(1*H*,3*H*)-dione (**1**), we studied the reaction between 6-[(dimethylamino)methyleneamino]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**16**) and aldehyde (**2a**) using biosurfactant in water. The formation of

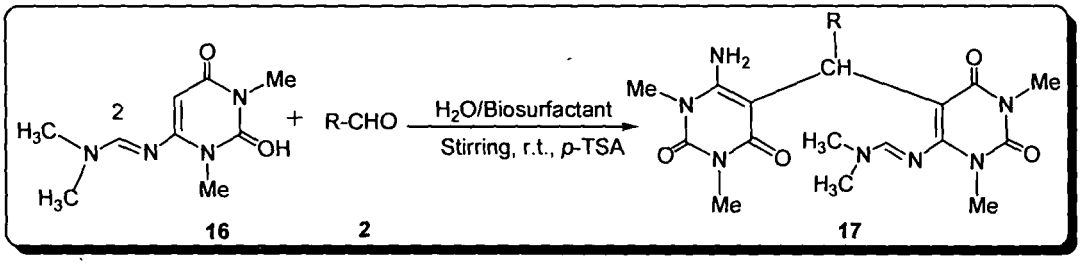
product were observed in poor yield and to increase the yields of the products we added 15 mol % of *p*-TSA. We observed the formation of two products which were separated by column chromatography, further purified through recrystallization. The reaction was very clean providing only one product, that is, **(17a) (Scheme 8)**. The structure was confirmed by IR, $^{1}H$ and $^{13}C$ NMR spectroscopy, mass spectrometry, elemental analyses and single crystal X-ray analysis.

**Table 12:** Synthesis of bisuracil derivatives **(3)** in the presence of biosurfactant

| Entry | Carbonyl Compounds **2** | Time/h | | m.p. (°C) | Yield (%)[a] | |
|---|---|---|---|---|---|---|
| | | In presence of biosurfactant | In absence of biosurfactant | | In presence of biosurfactant | In absence of biosurfactant |
| a | $C_6H_5CHO$ | 0.25 | 1 | 296-298 | 99 | 95 |
| b | $C_6H_5CH=CHCHO$ | 0.91 | 4 | 266-269 | 95 | 93 |
| c | *p*-$OMeC_6H_4CHO$ | 1.83 | 4 | 274-275 | 97 | 91 |
| d | *p*-$ClC_6H_4CHO$ | 0.25 | 0.41 | 268-271 | 99 | 99 |
| e | *p*-$OHC_6H_4CHO$ | 2 | 7 | 245-248 | 91 | 75 |
| f | *o*-$OHC_6H_4CHO$ | 2 | 7 | 246-247 | 90 | 73 |
| g | *p*-$MeC_6H_4CHO$ | 0.33 | 0.41 | 275-276 | 99 | 99 |
| h | *p*-$NO_2C_6H_4CHO$ | 2.33 | 10 | 228-229 | 93 | 73 |
| i | *m*-$NO_2C_6H_4CHO$ | 2.25 | 5 | 224-225 | 91 | 82 |
| j | 2-furaldehyde | 0.33 | 1 | 247-249 | 99 | 99 |
| k | Thiophene-2-carbaldehyde | 0.75 | 6 | 309-311 | 99 | 87 |
| l | Paraformaldehyde | 0.41 | 1 | 328-331 | 93 | 91 |
| m | $CH_3CHO$ | 0.75 | 3 | 253-255 | 94 | 92 |
| n | $CH_3(CH_2)_2CHO$ | 0.83 | 3 | 232-233 | 95 | 93 |
| o | $CH_3(CH_2)_3CHO$ | 0.83 | 3 | 159-161 | 95 | 90 |
| p | $(CH_3)_2CO$ | 48 | 48 | -- | --[b] | --[b] |
| q | $CH_3COPh$ | 48 | 48 | -- | --[b] | --[b] |
| r | PhCOPh | 48 | 48 | -- | --[b] | --[b] |

[a] Isolated yield [b] No product formation

The $^1$H NMR peaks at $\delta$ = 2.80 (3 H), 3.02 (3 H), 3.33 (3 H), 3.36 (3 H), 3.38 (3 H), 3.39 ppm are due to the six *N*-methyl groups, the peak at $\delta$ = 5.69 ppm is due to the -CH proton, the broad singlet peaks at $\delta$ = 5.93 ppm is due to one $-NH_2$ protons, the peaks within $\delta$ = 7.09–7.24 ppm are due to five aromatic protons and $\delta$ = 8.41 ppm is due to –N=CH . The $-NH_2$ groups were confirmed by shaking with $D_2O$, that is, the $-NH_2$ peak disappeared from the $^1$H NMR spectrum upon shaking with $D_2O$. The structure was further confirmed by single-crystal X-ray analysis. Suitable crystals were obtained by slow evaporation from ethanol solution. ORTEP diagram for compound (**17d**) is shown in **Fig. 10.**




R= Ph, CH=CH-Ph, *p*-$OCH_3$Ph, *p*-ClPh, *o*-OHPh, *p*-$CH_3$Ph, *p*-$NO_2$Ph, *m*-$NO_2$Ph, H, $CH_3$,

**Scheme 8:** Synthesis of bisuracil derivatives (**17**) in the presence of biosurfactant

Encouraged by this interesting result, we extended the reaction to other differently substituted aromatic, aliphatic, heterocyclic aldehydes and ketones (**2a-k**) under the same optimized reaction condition. The results are summarized in **Table 14** (entries **a-k**). To our delight, all the aldehydes (entries **2a-h**, **Table 14**) reacted with equal ease within short times, furnishing the products, 6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(phenylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] derivatives (**17a-h**) in good to good yields (59-71%) and with no side products formation.

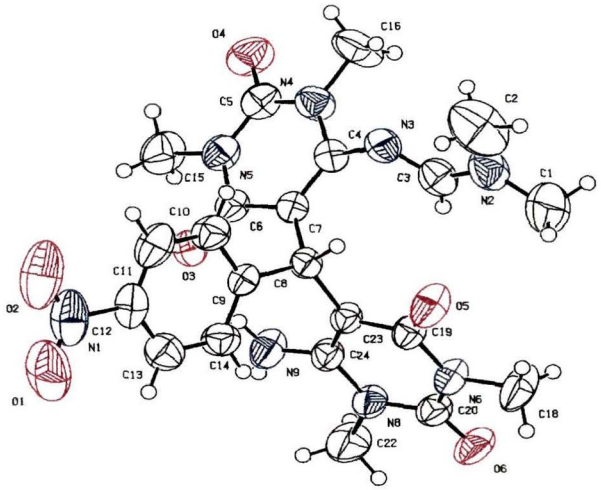



**Fig. 10**: ORTEP diagram of compound **(17d)**

**Table 13**: Detail crystallographic data of **(17d)**

| | |
|---|---|
| Formula | $C_{22}H_{26}N_8O_6$ |
| M | 498.51 |
| Crystal system | Triclinic |
| Temperature/K | 296 K |
| Space group | Pbca |
| *a*/ Å | 9.7878(5) |
| *b*/ Å | 15.0944(7) |
| *c*/ Å | 32.5855(16) |
| α (°) | 90 |
| β (°) | 90 |
| γ (°) | 90 |
| *V*/ $Å_3$ | 4814.2(4) |
| *Z* | 8 |
| *Dc*/ $mg \cdot m^{-3}$ | 1.376 |
| Reflns. collected | 5754 |
| Reflns. unique | 6017 |
| R(*int*) | 0.0591 (3168) |
| Index ranges | -13<=h<=13, -20<=k<=20, -43<=h<=43 |
| Refinement method | Full-matrix, least squares on $F^2$ |
| $wR_2$ 0.1930 (5754) | *GoF* 1.037 |

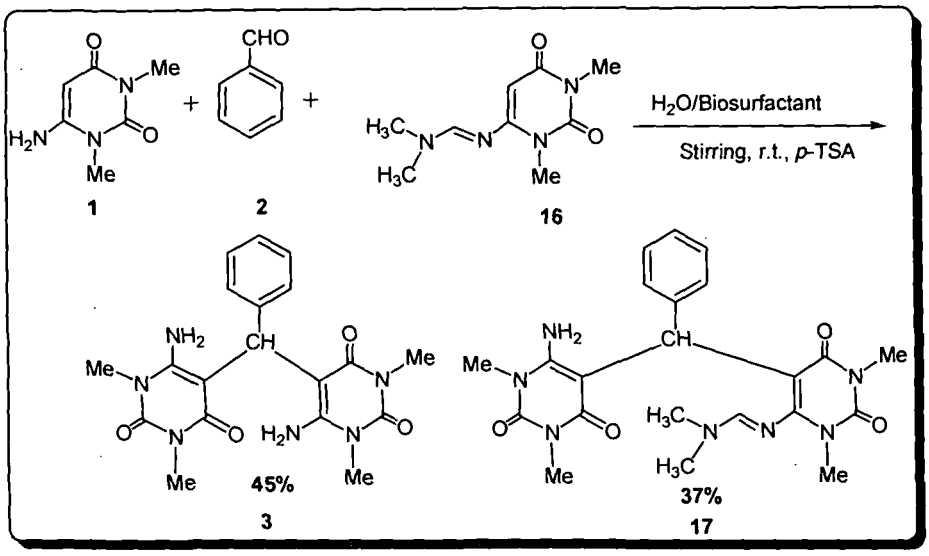
**Table 14**: Synthesis of bisuracil derivatives (**17**) in the presence of biosurfactant

| Entry | Carbonyl Compounds (**2**) | Time/h | m.p. (°C) | Yield (%)[a] |
|---|---|---|---|---|
| a | $C_6H_5CHO$ | 3.5 | 110 | 70 |
| b | *p*-$ClC_6H_4CHO$ | 2 | 176 | 85 |
| c | *p*-$MeC_6H_4CHO$ | 4 | 191 | 66 |
| d | *p*-$NO_2C_6H_4CHO$ | 4.5 | 189 | 62 |
| e | *m*-$NO_2C_6H_4CHO$ | 4.5 | 207 | 65 |
| f | 2-furaldehyde | 3 | 129 | 60 |
| g | Paraformaldehyde | 8 | 235 | 61 |
| h | $CH_3CHO$ | 9 | 360 | 59 |
| i | $(CH_3)_2CO$ | 48 | -- | --[b] |
| j | $CH_3COPh$ | 48 | -- | --[b] |
| k | PhCOPh | 48 | -- | --[b] |

[a] Isolated yield [b] No product formation

To understand the reaction mechanism we also studied the three component reaction between 6-amino-1,3-dimethyluracil (**1**), 6-[(dimethylamino)methyleneamino]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**16**) and benzaldehyde (**2a**). In that case, we also observed the formation of two products (**3**) and (**17**) (**Scheme 9**). After addition of benzaldehyde in biosurfactant, uracil derivatives became soluble, immediately we observed the formation of product (**3**) followed by the formation of product (**17**).

There are two main reasons (the use of biosurfactant in water), to increase the yields, solubility and reduce the reaction times. Firstly, the equilibrium of the nucleophilic reaction in shifted far to right in the reaction carried out in presence of biosurfactant and catalyst was observed to be high yielding, having as much as 99% of product formation. The aldehyde molecules being hydrophobic in nature, forms highly structured solvation layer in aqueous environment, in absence of biosurfactant. This results in reduce entropy condition with high free energy content in the existing physiochemical environment. Introducing surfactant gradually to an extant of CMC led to formation of hydrophobic pocket within the bulk water solvent due to hydrophobic interior of micelles, which facilities bringing reactant components to close proximity for product formation. The biosurfactant usually interferes in the existing solubility of




**Scheme 9:** Competitive reaction between (**1**) and (**16**)

solvation layer, which led to other probable interactions and chemical events that may be responsible of release of binding energy. The binding energy in turn helps in expediting the reaction towards completion in presence of catalyst. Secondly, from our possible reaction mechanism we also observed that the nucleophilic 5-position of 6-[(dimethylamino)methyleneamino]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**16**) attacks the carbon centre of the aldehyde (**2**) followed by elimination of water molecule, a second molecule of uracil derivatives then attacks via its nucleophilic 5-position affording the product (**17**). Use of biosurfactant, dehydration has been successfully developed in water due to the hydrophobic nature of their interior (**Fig. 11**).[52]

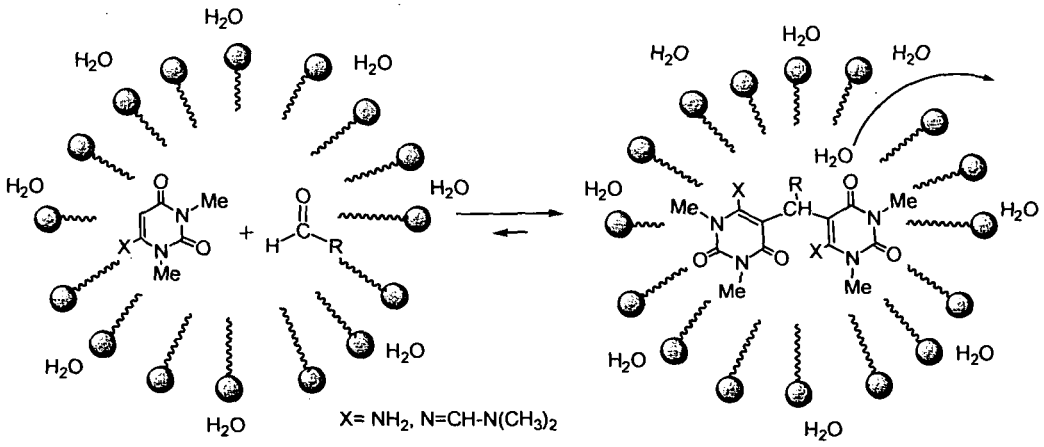
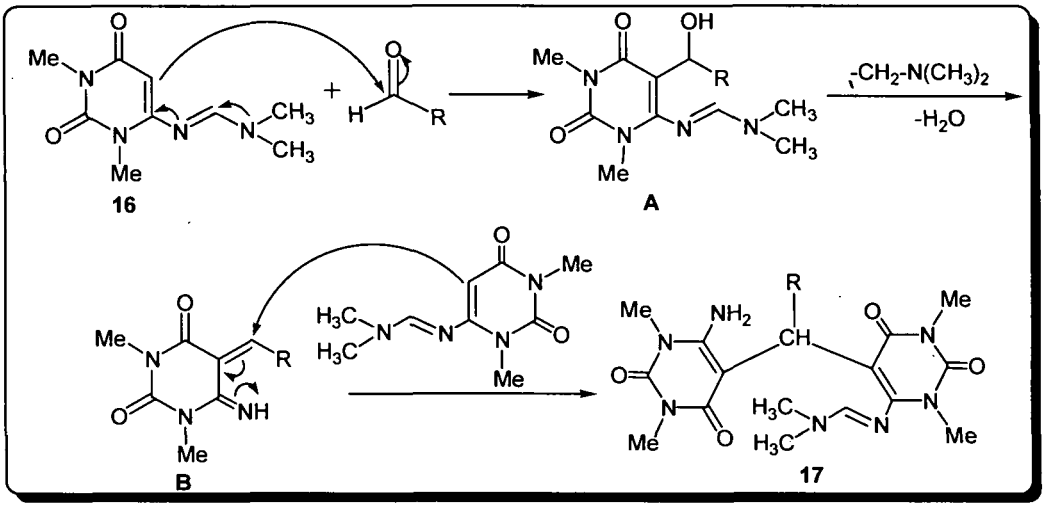



**Fig. 11:** Dehydration in the presence of a biosurfactant in water

One of the most economic points in context of green chemistry is the recyclability. We have recycled the biosurfactant for several times and again reused it.

A plausible mechanism for the reaction is described in the **Scheme 10**. Mechanistically, the nucleophilic 5-position of 6-[(dimethylamino)methyleneamino]-1,3-dimethylpyrimidine-2,4 -(1*H*,3*H*)-dione (**16**) attacks the carbon centre of the aldehyde (**2**) followed by elimination of water molecule. A second molecule of uracil derivatives then reacts via its nucleophilic 5-position affording the product (**17**).




**Scheme 10**: Plausible reaction mechanism

**Conclusion:**

Our method is environmentally benign (eliminating the use of organic solvents, neither for the reaction nor for the purification), safe and cost effective. Moreover use of biosurfactant seems to offer more potential than chemical surfactants, due to their structural diversity, biodegradability and biocompatibility related to the synthesis of surfactants, stability over the different extreme conditions such as high temperature, pH and salinity, less toxicity, and along with most importantly the eco-friendly nature.

In conclusion, we have first reported a mild, chemoselective, highly efficient, clean and truly 'green' methodology for the synthesis of bis-uracil derivatives by condensation of 6-aminouracil derivatives (**1** and **4**) and aldehydes (**2a-h**) using biosurfactant in water at room temperature.

**Experimental Section:**

**General procedure for the synthesis of bis-uracil (3):**

Distilled water (8 ml) containg biosurfactant (0.36 mg) was added to 6-amino-1,3-dimethyluracil (155 mg, 1 mmol) in a 100 mL round-bottomed flask and the mixture was stirred at room temperature until all the 6-amino-1,3-dimethyluracil dissolved. Benzaldehyde (54 mg, 0.5 mmol) was added drop wise to the 6-amino-1,3-dimethyluracil solution with constant stirring and then after a few minutes the product appeared as a white precipitate and stirring was continued for a further 1 h so that all of the reactants were converted into product. The white precipitate was filtered and collected to provide the pure product in 99% yield (**Fig. 12**). A small amount of the product was dissolved in distilled ethanol (98%) and then warmed. Then the solution was filtered, allowed to cool and evaporated at room temp. to give squareshaped white shining transparent crystals. The crystals were collected and dried; m.p. 296–299 °C. The crystals are stable at room temp. and also in an open environment for several days.

The same procedure was followed for the other substrates.

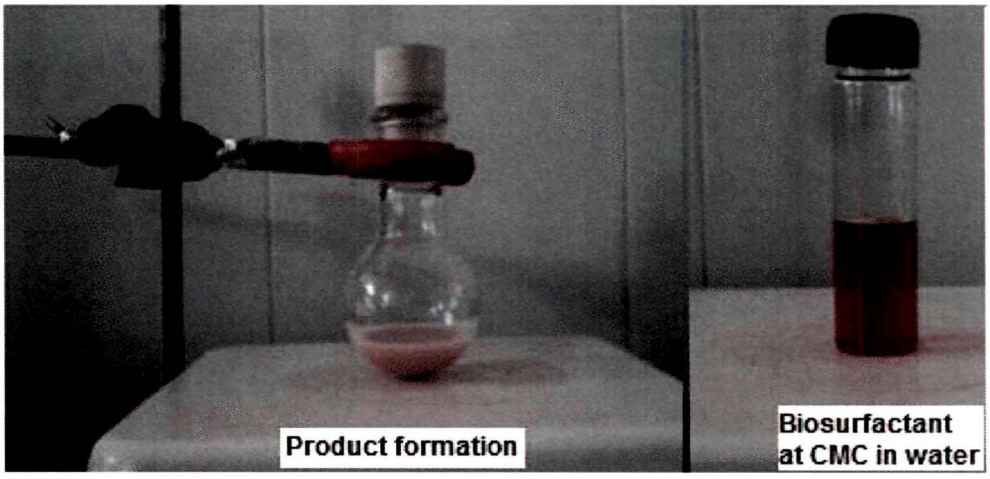



**Fig. 12:** Product formation in the presence of biosurfactant

**General procedure for the synthesis of (17):**

Distilled water (8 ml) containing biosurfactant (0.36 mg) was added to 6-[(dimethylamino)methylene]1,3-aminouracil (210 mg, 1 mmol) in a 100 mL round-

bottomed flask and the mixture was stirred at room temperature until all the 6-[(dimethylamino)methylene]1,3-aminouracil dissolved. 15 mol% *p*-TSA is added to get good yield. Benzaldehyde (108 mg, 1 mmol) was added drop wise to the solution with constant stirring and after few minutes, we get the pure product in 70% yield. The product was separated through TLC technique, was dissolved in distilled ethanol (98%) and then warmed. Then the solution was filtered, allowed to cool and evaporated at room temp. to give squareshaped yellow shining transparent crystals. The crystals were collected and dried; m.p. 110 °C. The crystals are stable at room temp. and also in an open environment for several days.

**Recycling procedure of biosurfactant:**

The precipitate bis-uracil derivatives were recovered by centrifugation at 3000 rpm for 10 min. The reaction mixture was further extracted twice with 30 ml chloroform to obtain the traces of bis-uracil derivatives left. For the recovery of biosurfactant, the reaction mixture was acidified to pH 2 using 6N HCl and kept overnight at 4 °C to precipitate and recovered by centrifugation at 10, 000 rpm for 15 min at 4 °C. Further the precipitate was dissolved in phosphate buffer saline (pH 7.2) solution, which is ready to be reutilized (**Fig. 13**).

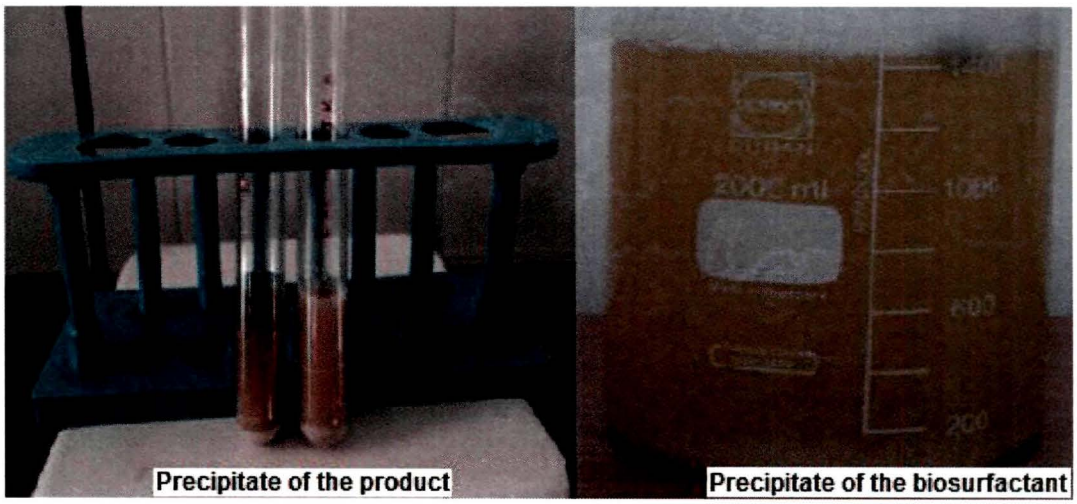
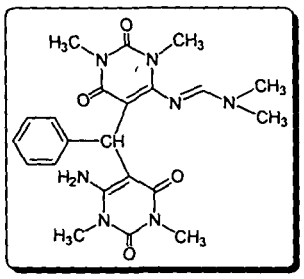



**Fig. 13:** Biosurfactant recycling

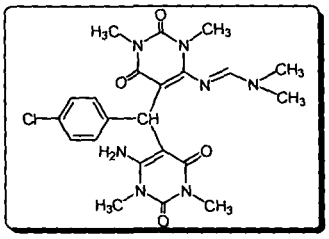
**Physical and spectral data:**

**6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(benzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (17a)**



Colour: yellowish crystalline solid. Solubility: Insoluble in water, soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO and DMAc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3400.15, 2925.34, 1713.15, 1609.60, 1342.04; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.80 (s, 3H, $NCH_3$), 3.02 (s, 3H, $NCH_3$), 3.28 (s, 3H, $NCH_3$), 3.33 (s, 3H, $NCH_3$), 3.36 (s, 3H, $NCH_3$), 3.38 (s, 3H, $NCH_3$), 5.69 (s, 1H, CH), 5.93 (s, 2H, $NH_2$), 7.09-7.24 (m, 5H, arom), 8.41 (s, 1H, N=CH) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_C$ 28.4 ($NCH_3$), 28.5 ($NCH_3$), 29.0 ($NCH_3$), 29.1 ($NCH_3$), 34.4 ($NCH_3$), 38.4 ($NCH_3$), 40.4 (CH), 89.7 (C-5 and C`-5), 125.9 (C-4 arom), 127.1 (C-3 and C-5), 128.3 (C-2 and C-6), 141.1 (C-1 arom), 151.4 (C-6 and C`-6), 152.8 and 154.2 (C-2 and C`-2), 160.4 (C=N), 165.3 (C-4 and C`-4) ppm. MS, *m/z* 453.21 ($M^+$). Anal. Calcd (%) for $C_{22}H_{27}N_7O_4$: C 58.22, H 6.03, N 21.65. Found C 58.27, H 6.00, N 21.62.

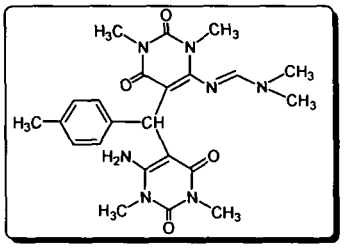
**6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(4-chlorobenzylidene)-bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (17b)**



Colour: yellowish crystalline solid. Solubility: Insoluble in water, soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO and DMAc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3409.67, 2929.33, 1707.99, 1605.90, 1342.77; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.91 (s, 3H, $NCH_3$), 3.07 (s, 3H, $NCH_3$), 3.31 (s, 3H, $NCH_3$), 3.35 (s, 3H, $NCH_3$), 3.39 (s, 3H, $NCH_3$), 3.41 (s, 3H, $NCH_3$), 5.72 (s, 1H, CH), 6.18 (s, 2H, $NH_2$), 7.02-7.28 (m, 4H, arom), 8.24 (s, N=CH) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_C$ 28.4 ($NCH_3$), 28.5 ($NCH_3$), 29.1 ($NCH_3$), 31.5 ($NCH_3$), 34.4 ($NCH_3$), 37.7 ($NCH_3$), 40.4 (CH), 89.8 and 98.5 (C-5 and C`-5), 128.3 (C-4 arom), 128.4 (C-3 and C-5), 131.4 (C-2 and C-6), 140.0 (C-1 arom), 151.3 and 151.6 (C-6 and C`-6), 152.9 and 154.0 (C-2 and C`-2), 160.4 (C=N), 162.5 and

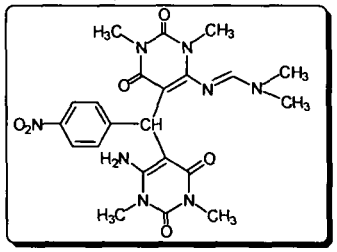
165.3 (C-4 and C`-4) ppm. MS, *m/z* 487.17 ($M^+$). Anal. Calcd (%) for $C_{22}H_{26}ClN_7O_4$: C 54.13, H 5.36, N 20.07. Found C 54.15, H 5.37, Cl 7.27, N 20.09.

**6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(4-methylbenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] [17c]**



Colour: yellowish crystalline solid. Solubility: Insoluble in water, soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO and DMAc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3401.13, 2921.09, 1705.03, 1609.11, 1342.90; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.17 (s, 3H, $CH_3$), 2.82 (s, 3H, $NCH_3$), 3.03 (s, 3H, $NCH_3$), 3.34 (s, 3H, $NCH_3$), 3.38 (s, 3H, $NCH_3$), 3.41 (s, 3H, $NCH_3$), 3.51 (s, 3H, $NCH_3$), 5.65 (s, 1H, CH), 6.10 (s, 2H, $NH_2$), 6.97-7.30 (m, 4 H, arom), 7.63 (s, N=CH) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_C$ 21.7 ($CH_3$), 28.3 ($NCH_3$), 28.5 ($NCH_3$), 29.2 ($NCH_3$), 29.3 ($NCH_3$), 34.4 ($NCH_3$), 37.7 ($NCH_3$), 40.4 (CH), 89.8 and 98.5 (C-5 and C`-5), 128.3 (C-4 arom), 128.4 (C-3 and C-5), 131.4 (C-2 and C-6), 140.0 (C-1 arom), 151.3 and 151.6 (C-6 and C`-6), 152.9 and 154.0 (C-2 and C`-2), 161.6 (C=N), 165.4 (C-4 and C`-4) ppm. MS, *m/z* 467.23 ($M^+$). Anal. Calcd (%) for $C_{23}H_{29}N_7O_4$: C 50.08, H 6.23, N 20.95. Found C 59.09, H 6.25, N 20.97.

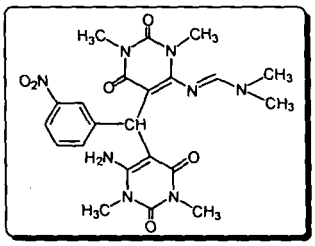
**6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(4-nitrobenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (17d)**



Colour: yellowish crystalline solid. Solubility: Insoluble in water, soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO and DMAc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3422.31, 2932.98, 1706.11, 1625.76, 1353.63, 1253.76; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.94 (s, 3H, $NCH_3$), 3.06 (s, 3H, $NCH_3$), 3.28 (s, 3H, $NCH_3$), 3.35 (s, 3H, $NCH_3$), 3.39 (s, 3H, $NCH_3$), 3.47 (s, 3H, $NCH_3$), 5.85 (s, 1H, CH), 6.40 (s, 2H, $NH_2$), 7.16-7.26 (m, 4H, arom), 8.07 (s, N=CH) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_C$ 28.5 ($NCH_3$), 28.6 ($NCH_3$), 29.2 ($NCH_3$), 31.6 ($NCH_3$), 34.4 ($NCH_3$), 38.1 ($NCH_3$), 40.5 (CH), 88.8 and 98.4 (C-5 and C`-5), 123.5 (C-4 arom), 127.5 (C-3 and C-5), 146.0 (C-2 and C-6), 149.9 (C-1

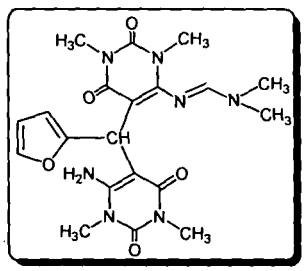
arom), 151.2 and 151.4 (C-6 and C`-6), 153.1 and 153.8 (C-2 and C`-2), 160.5 (C=N), 162.2 and 165.4 (C-4 and C`-4) ppm. MS, *m/z* 498.20 ($M^+$). Anal. Calcd (%) for $C_{22}H_{26}N_8O_6$: C 53.02, H 5.27, N 22.47. Found C 53.01, H 5.26, N 22.48.

**6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(3-nitrobenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (17e)**



Colour: yellowish crystalline solid. Solubility: Insoluble in water, soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO and DMAc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3403.15, 2923.77, 1706.95, 1613.76, 1331.62, 1250.42; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.92 (s, 3H, $NCH_3$), 3.04 (s, 3H, $NCH_3$), 3.24 (s, 3H, $NCH_3$), 3.33 (s, 3H, $NCH_3$), 3.38 (s, 3H, $NCH_3$), 3.40 (s, 3H, $NCH_3$), 5.83 (s, 1H, CH), 6.39 (s, 2H, $NH_2$), 7.15-7.39 (m, 4H, arom), 7.87 (s, N=CH) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_C$ 28.5 ($NCH_3$), 29.2 ($NCH_3$), 29.3 ($NCH_3$), 31.6 ($NCH_3$), 34.4 ($NCH_3$), 37.7 ($NCH_3$), 40.5 (CH), 88.7 and 98.3 (C-5 and C`-5), 121.0 and 121.8 (C-3 and C-5), 128.9 (C-4 arom), 133.2 (C-1 arom), 144.0 and 148.5 (C-2 and C-6), 151.3 and 151.5 (C-6 and C`-6), 153.2 and 153.8 (C-2 and C`-2), 161.6 (C=N), 162.2 and 165.4 (C-4 and C`-4) ppm. MS, *m/z* 498.20 ($M^+$). Anal. Calcd (%) for $C_{22}H_{26}N_8O_6$: C 53.03, H 5.27, N 23.45. Found C 53.01, H 5.26, N 23.48.

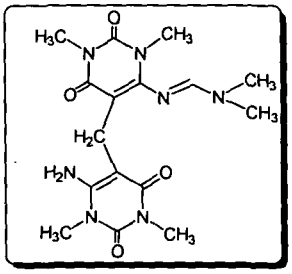
**6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(2-furyl)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (17f)**



Colour: yellowish crystalline solid. Solubility: Insoluble in water, soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO and DMAc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3412.45, 2923.90, 1702.87, 1607.11, 1345.12; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.94 (s, 3H, $NCH_3$), 3.06 (s, 3H, $NCH_3$), 3.32 (s, 3H, $NCH_3$), 3.33 (s, 3H, $NCH_3$), 3.36 (s, 3H, $NCH_3$), 3.43 (s, 3H, $NCH_3$), 5.63 (s, 1H, CH), 5.96 (1H, furan), 6.20 (1H, furan), 6.25 (s, 2H, $NH_2$), 6.26 (1H, furan), 8.07 (s, N=CH) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.4 ($NCH_3$), 29.1 ($NCH_3$), 29.9 ($NCH_3$), 31.4 ($NCH_3$), 33.4 ($NCH_3$), 34.4 ($NCH_3$), 40.5 (CH),

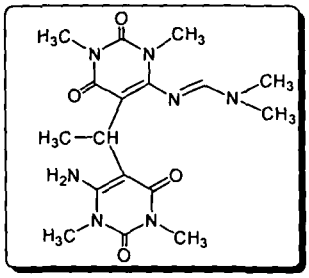
89.1 and 97.5 (C-5 & C′5), 105.9 (C-3, furan), 110.2 (C-4, furan), 141.1 (C-6 & C′-6), 152.9 (C-5, furan), 154.0 & 154.6 (C-2 & C′-2), 161.3 (CH=N), 165.1 (C-4 & C′-4) ppm; MS, *m/z* 443.19 ($M^+$). Anal. Calcd (%) for $C_{20}H_{25}N_7O_5$: 54.20, H 5.71, N 22.09. Found C 54.17, H 5.68, N 22.11.

**6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(methylene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (17g)**



Colour: yellowish crystalline solid. Solubility: Insoluble in water, soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO and DMAc. IR (KBr) ($\nu_{max}$/$cm^{-1}$) 3407.09, 2920.71, 1709.33, 1601.33, 1349.09; $^1$H NMR (400 MHz, $CDCl_3$): $\delta_H$ 2.80 (s, 3H, $NCH_3$), 2.96 (s, 3H, $NCH_3$), 3.26 (s, 3H, $NCH_3$), 3.28 (s, 3H, $NCH_3$), 3.40 (s, 3H, $NCH_3$), 3.56 (s, 3H, $NCH_3$), 4.15 (s, 2H, $CH_2$), 7.51 (s, 2H, $NH_2$), 8.27 (s, N=CH) ppm. $\delta_C$ 19.5 ($CH_2$), 29.1 ($NCH_3$), 29.2 ($NH_3$), 29.3 ($NH_3$), 29.9 ($NCH_3$), 30.0 ($NCH_3$), 31.2 ($NH_3$), 89.7 & 97.3 (C-5 & C′-5), 151.0 & 151.2 (C-6 & C′-6), 154.2 & 154.3 (C-2 & C′-2), 160.4 (CH=N), 162.5 & 165.3 (C-4 & C′-4) ppm; MS: *m/z* = 377 ($M^+$). $C_{16}H_{27}N_7O_4$: 50.56, H 6.49, N 21.69. found C 50.55, H 6.47, N 21.72.

**6-amino-6′-(dimethylamino)methyleneamino-1,1',3,3'-tetramethyl-5,5'-(ethylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione] (17h)**



Colour: yellowish crystalline solid. Solubility: Insoluble in water, soluble in common organic solvents, soluble in aprotic polar solvent like DMF, DMSO and DMAc. IR (KBr) ($\nu_{max}$/$cm^{-1}$) 3403.99, 2923.70, 1703.03, 1607.77.11, 1342.09; $^1$H NMR (400 MHz, $CDCl_3$): $\delta_H$ 1.66-1.68 (m, 3H, $CH_3$), 2.93 (s, H, $NCH_3$), 3.21 (s, H, $NCH_3$), 3.38 (s, H, $NCH_3$), 3.46 (s, H, $NCH_3$), 3.49 (s, H, $NCH_3$), 3.74 (s, H, $NCH_3$), 4.41-4.45 (m, 1H, CH), 6.48 (s, 2H, $NH_2$), 9.17 (s, N=CH) ppm; $\delta_C$ 21.1 ($CH_3$), 26.7 (CH), 28.4 ($NCH_3$), 28.5 ($NCH_3$), 29.2 ($NCH_3$), 29.5 ($NCH_3$), 31.5 ($NCH_3$), 31.7 ($NCH_3$), 89.7 & 90.3 (C-5 & C′-5), 151.0 & 151.4 (C-6 & C′-6), 152.8 & 153.6 (C-2 & C′-2), 162.1 (CH=N), 164.4 & 165.3 (C-4 &

C′-4) ppm; MS, *m/z* 391.2 ($M^+$). $C_{17}H_{25}N_7O_4$: 52.17, H 6.47, N 25.02. Found C 52.16, H 6.44, N 25.05.

**References:**


1. Szent-Gyorgyi, A. In *Cell-Associated Water*, Ed: Drost-Hansen, W.; Cleg, J.S. (Academic Press, New York, 1979).
2. a) Grieco, P.A. In *Organic Synthesis in Water* (Blackie Academic and Professional, London, 1998). b) Li, C.J.; Chan, T.H. In *Organic Reactions in Aqueous Media* (John Wiley & Sons, New York, 1997). c) Hirai, Y.; Uozumi, Y. Clean synthesis of triarylamines: Buchwald-Hartwig reaction in water with amphiphilic resin-supported palladium complexes, *Chem. Commun.* **46**, 1103-1105 (2010). d) Savant, M.M. *et al* Water mediated construction of trisubstituted pyrazoles/isoxazoles library using ketene dithioacetals, *J. Comb. Chem.* **12**, 176-180 (2010). e) Carril, M.; SanMartin, R.; Tellitu, I.; Dominguez, E. On-Water chemistry: Copper-catalyzed straightforward synthesis of benzo[*b*]furan derivatives in neat water, *Org. Lett.* **8**, 1467-1470 (2006). f) Li, C.J.; Chen, L. Organic chemistry in water, *Chem. Soc. Rev.* **35**, 68-82 (2006). g) Pratt, L.R. Introduction: Water, *Chem. Rev.* **102**, 2625-2626 (2002).
3. a) Anastas, P.; Warner, J.C. In *Green Chemistry: Theory and Practice* (Oxford University Press: Oxford, 1998). b) Tundo, P. *et al* Synthetic pathways and processes in green chemistry. Introductory overview, *Pure Appl. Chem.* **72**, 1207-1228 (2000). c) Manabe, K.; Iimura, S.; Sun, X.; Kobayashi, S. Dehydration reactions in water. Brønsted acid-surfactant-combined catalyst for ester, ether, thioether, and dithioacetal formation in water, *J. Am. Chem. Soc.* **124**, 11971-11978 (2002). d) Tsukinoki, T.; Nagashima, S.; Mitoma, Y.; Tashiro, M. Organic reaction in water. Part 4. New synthesis of vicinal diamines using zinc powder-promoted carbon-carbon bond formation, *Green Chem.* **2**, 117-119 (2000). e) Bigi, F.; Conforti, M.L.; Maggi, R.; Piccinno, A.; Sartori, G. Clean synthesis in water: Uncatalysed preparation of ylidenemalononitriles, *Green Chem.* **2**, 101-103 (2000).

4. a) Head-Gordon, T.; Hura, G. Water structure from scattering experiments and simulation, *Chem. Rev.* **102**, 2651-2670 (2002). b) Lindstrom, U.M. Stereoselective organic reactions in water, *Chem. Rev.* **102**, 2751-2772 (2002). c) Hayashi, Y. "In Water" or "In the presence of water"?, *Angew. Chem. Int. Ed. Eng.* **45**, 8103-8104 (2006). d) Tan, B. *et al* Water-more than just a green solvent: A stereoselective one-pot access to all-chiral tetrahydronaphthalenes in aqueous media, *Chem. A Eur. J.* **16**, 3842-3848 (2010). e) Lu, Z. *et al* Water-controlled regioselectivity of Pd-catalyzed domino reaction involving a C-H activation process: Rapid synthesis of diverse carbo- and heterocyclic skeletons, *Org. Lett.* **12**, 480-483 (2010). f) Akagawa, K.; Fujiwara, T.; Sakamoto, S.; Kudo, K. Efficient asymmetric $\alpha$-oxyamination of aldehydes by resin-supported peptide catalyst in aqueous media, *Org. Lett.* **12**, 1804-1807 (2010). g) Fioroni, G.; Fringuelli, F.; Pizzo, F.; Vaccaro, L. Epoxidation of $\alpha,\beta$-unsaturated ketones in water. An environmentally benign protocol, *Green Chem.* **5**, 425-428 (2003). h) Bonollo, S.; Fringuelli, F.; Pizzo, F.; Vaccaro, L. A green route to $\beta$-amino alcohols *via* the uncatalyzed aminolysis of 1,2-epoxides by alkyl- and arylamines, *Green Chem.* **8**, 960-964 (2006). i) Bonollo, S.; Fringuelli, F.; Pizzo, F.; Vaccaro, L. $Zr(DS)_4$ as an efficient catalyst for the aminolysis of epoxides in water, *Synlett.* **17**, 2683-2686 (2007).

5. a) Most recent review: Butler, R.N.; Coyne, A.G. Water: Nature's reaction enforcer-comparative effects for organic synthesis "in-water" and "on-water", *Chem. Rev.* **110**, 6302-6337 (2010). b) Li, C.J. Organic reactions in aqueous media with a focus on carbon-carbon bond formations: A decade update, *Chem. Rev.* **105**, 3095-3165 (2005). c) Brogan, A.P.; Dickerson, T.J.; Janda, K.D. Enamine-based aldol organocatalysis in water: Are they really "all wet"?, *Angew. Chem. Int. Ed. Eng.* **45**, 8100-8102 (2006). d) Lipshutz, B.H.; Aguinaldo, G.T.; Ghorai, S.; Voigtritter, K. Olefin cross-metathesis reactions at room temperature using the nonionic amphiphile "PTS": Just add water, *Org. Lett.* **10**, 1325-1328 (2008). e) Das, B.; Satyalakshmi, G.; Suneel, K.; Damodar, K. Organic reactions in water: A distinct novel approach for an efficient synthesis of $\alpha$-amino

phosphonates starting directly from nitro compounds, *J. Org. Chem.* **74**, 8400–8402 (2009).

6. a) Klijn, J.E.; Engberts, B.F.N. Organic chemistry: Fast reactions 'on water', *Nature* **435**, 746-747 (2005). b) Narayan, S. *et al* "On water": Unique reactivity of organic compounds in aqueous suspension, *Angew. Chem. Int. Ed. Eng.* **44**, 3275-3279 (2005). c) Chanda, A.; Fokin, V.V. Organic synthesis "On Water", *Chem. Rev.* **109**, 725-748 (2009). d) Duplais, C.; Krasovskiy, A.; Wattenberg, A.; Lipshutz, B.H. Cross-couplings between benzylic and aryl halides "on water": synthesis of diarylmethanes, *Chem. Commun.* **46**, 562-564 (2010).
7. Recent articles: a) Nishikata, T.; Lipshutz, B.H. Aminations of allylic phenyl ethers via micellar catalysis at room temperature in water, *Chem. Commun.* 6472-6474 (2009). b) Nishikata, T.; Lipshutz, B.H. Allylic ethers as educts for Suzuki-Miyaura couplings in water at room temperature, *J. Am. Chem. Soc.* **131**, 12103-12105 (2009). c) Krasovskiy, A.; Duplais, C.; Lipshutz, B.H. Zn-mediated, Pd-catalyzed cross-couplings in water at room temperature without prior formation of organozinc reagents, *J. Am. Chem. Soc.* **131**, 15592-15593 (2009). d) Nishikata, T.; Lipshutz, B.H. Cationic Pd(II)-catalyzed Fujiwara-Moritani reactions at room temperature in water, *Org. Lett.* **12**, 1972-1975 (2010). e) Lipshutz, B.H.; Ghorai, S. PQS-2: Ring-closing- and cross-metathesis reactions on lipophilic substrates; in water only at room temperature, with in-flask catalyst recycling, *Tetrahedron* **66**, 1057-1063 (2010). f) Nishikata, T.; Abela, A.R.; Lipshutz, B.H. Room temperature C-H activation & cross-coupling of aryl ureas in water, *Angew. Chem. Int. Ed. Eng.* **49**, 781-784 (2009).
8. a) Siskin, M.; Katritzky, A.R. Reactivity of organic compounds in hot water: Geochemical and technological implications, *Science* **254**, 231-237 (1991). b) Akiya, N.; Savage, P.E. The roles of water for chemical reactions in high-temperature water, *Chem. Rev.* **102**, 2725-2750 (2002). c) Siskin, M.; Katritzky, A.R. Reactivity of organic compounds in superheated water: general background, *Chem. Rev.* **101**, 825-836 (2001).
9. a) Rideout, D.C.; Breslow, R. Hydrophobic acceleration of Diels-Alder reactions, *J. Am. Chem. Soc.* **102**, 7817-7818 (1980). b) For review: Breslow, R.

Determining the geometries of transition states by use of antihydrophobic additives in water, *Acc. Chem. Res.* **37**, 471-478 (2004).

10. a) Jiménez-Gonźalez, C.; Curzons, A.D.; Constable, D.J.C.; Cunningham, V.L. Cradle-to-gate life cycle inventory and assessment of pharmaceutical compounds, *Int. J. Life Cycle Assess.* **9**, 114-121 (2004). b) Jiménez-Gonźalez, C.; Curzons, A.D.; Constable, D.J.C.; Cunningham, V.L. Expanding GSK's solvent selection guide-application of life cycle assessment to enhance solvent selections, *Clean Technol. Environ. Policy*, **7**, 42-50 (2005).
11. Abraham, M.A.; Moens L. Eds. in *Clean Solvents: Alternative Media for Chemical Reactions and Processing* (ACS Symposium Series 819, Washington, D.C., 2002).
12. a) Fathalla, M.; Lawrence, C.M.; Zhang, N.; Sessler, J.L.; Jayawickramarajah, J. Base-pairing mediated non-covalent polymer, *Chem. Soc. Rev.* **38**, 1608-1620 (2009). b) Bradshaw, T.K.; Hutchinson, D.W. 5-Substituted pyrimidine nucleosides and nucleotides, *Chem. Soc. Rev.* **6**, 43-62 (1977). c) Sivakova, S.; Rowan, S.J. Nucleobases as supramolecular motifs, *Chem. Soc. Rev.* **34**, 9-21 (2005). d) Powner, M.W.; Gerland, B.; Sutherland, J.D. Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. *Nature* **459**, 239-242 (2009). e) Pedersen, O.S.; Pedersen, E.B. Non-nucleoside reverse transcriptase inhibitors: The NNRTI boom, *Antiviral Chem. Chemother.* **10**, 285-314 (1999).
13. a) Dinner, A.R.; Blackburn, G.M.; Karplus, M. Uracil-DNA glycosylase acts by substrate autocatalysis, *Nature* **413**, 752-755 (2001). b) Noia, J.D.; Neuberger, M.S. Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase, *Nature* **419**, 43-48 (2002). c) Katritzky, A.R.; Todadze, E. Heterocycles in the service of humankind, *Arkivoc* **ix**, 248-268 (2009).
14. Recent articles: a) Lundqvist T. *et al* Exploitation of structural and regulatory diversity in glutamate racemases, *Nature* **447**, 817-822 (2007). b) Plant, A.; Thompson, P.; Williams, D.M. Application of the Ugi reaction for the one-pot synthesis of uracil polyoxin C analogues, *J. Org. Chem.* **74**, 4870-4873 (2009). c) Manta, S. *et al* Synthesis of 4,6-dideoxy-3-fluoro-2-keto-beta-D-glucopyranosyl

analogues of 5-fluorouracil, *N*6-benzoyl adenine, uracil, thymine, N4-benzoyl cytosine and evaluation of their antitumor activities, *Bioorg. Chem.* **38**, 48-55 (2010).

15. a) Parker, J.B. *et al* Enzymatic capture of an extrahelical thymine in the search for uracil in DNA, *Nature* **449**, 433-437 (2007). b) Okamoto, A. Chemical approach toward efficient DNA methylation analysis, *Org. Biomol. Chem.* 7, 21-26 (2009). c) McCarthy, O. *et al* Design, synthesis and evaluation of novel uracil acetamide derivatives as potential inhibitors of plasmodium falciparum dUTP nucleotidohydrolase, *Eur. J. Med. Chem.* **44**, 678-688 (2009). d) Herman, B.D.; Votruba, I.; Holý, A.; Sluis-Cremer, N.; Balzarini, J. The acyclic 2,4-diaminopyrimidine nucleoside phosphonate acts as a purine mimetic in HIV-1 reverse transcriptase DNA polymerization, *J. Biol. Chem.* **285**, 12101-12108 (2010).
16. a) Raddatz, P.; Bergmann, R. Ger. Pat., 360731, 1998; Chem. Abstr. **108**, 54786 (1988). b) Nair, V. *et al* A heterocyclic molecule with significant activity against dengue virus, *Bioorg. Med. Chem. Lett.* **19**, 1425-1427 (2009). c) Samanta, A. *et al* Morpholino, piperidino, and pyrrolidino derivatives of pyrimidine nucleosides as inhibitors of ribonuclease A: Synthesis, biochemical, and crystallographic evaluation, *J. Med. Chem.* **52**, 932-942 (2009). d) Rico-Gomez, R.M. *et al* Synthesis of new mannosyl, galactosyl and glucosyl theophylline nucleosides with potential activity as antagonists of adenosine receptors. DEMA-induced cyclization of glycosylideneiminouracils, *Carbohydrate Res.* **343**, 855-864 (2008).
17. a) Tucci, F.C. *et al* 3-(2-Aminoalkyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methyluracils as orally bioavailable antagonists of the human gonadotropin releasing hormone receptor, *J. Med. Chem.* **47**, 3483-3486 (2004). b) Sutherlin, D.P. *et al* Discovery of (thienopyrimidin-2-yl)aminopyrimidines as potent, selective, and orally available pan-PI3-kinase and dual pan-PI3-kinase/mTOR inhibitors for the treatment of cancer, *J. Med. Chem.* **53**, 1086-1097 (2010).

18. Heidelberger, C. In *Pyrimidine and Pyrimidine Antimetabolites in Cancer Medicine,* Eds., Holland, J.F.; Frei, E.; Febiger, L. (Philadelphia, 801, 1984).
19. Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide, *Science* **254**, 1497-1500 (1991).
20. Thureau, P.; Ancian, B.; Viel, S.; Thévand, A. Determining chemical exchange rates of the uracil labile protons by NMR diffusion experiments, *Chem. Commun.* **2**, 200-202 (2006).
21. a) Bansal, R. *et al* Synthesis of a series of 8-(substituted-phenyl)xanthines and a study on the effects of substitution pattern of phenyl substituents on affinity for adenosine A(1) and A(2A) receptors, *Eur. J. Med. Chem.* **44**, 2122-2127 (2009). b) Drabczynska, A. *et al* N9-Benzyl-substituted 1,3-dimethyl- and 1,3-dipropyl-pyrimido[2,1-*f*]purinediones: Synthesis and structure–activity relationships at adenosine $A_1$ and $A_{2A}$ receptors, *Bioorg. Med. Chem.* **15**, 5003-5017 (2007).
22. Valderrama, J.A. *et al* Studies on quinones. Part 44: Novel angucyclinone *N*-heterocyclic analogues endowed with antitumoral activity, *Bioorg. Med. Chem.* **16**, 10172-10181 (2008).
23. Alpha, B.; Lehn, J.M.; Perathoner, S.; Sabbatini, N. Luminescence Probes: The $Eu^{3\oplus}$- and $Tb^{3\oplus}$-cryptates of polypyridine macrobicyclic ligands, *Angew. Chem., Int. Ed. Eng.* **26**, 1266-1267 (1987).
24. a) Thakur, A.J.; Saikia, P.; Prajapati, D.; Sandhu, J.S. Studies on 6-[(dimethyl - amino)methylene]aminouracils: A facile one-pot synthesis of pyrimido[4,5-*d*]pyrimidines and pyrido[2,3-*d*]pyrimidines, *Synlett.* **8**, 1299-1301 (2001). b) Prajapati, D.; Thakur, A.J. Studies on 6-[(dimethylamino)methylene]aminouracil: A facile one-pot synthesis of novel pyrimido[4,5-*d*]pyrimidine derivatives, *Tetrahedron Lett.* **46**, 1433-1436 (2005). c) Prajapati, D.; Gohain, M.; Thakur, A.J. Regiospecific one-pot synthesis of pyrimido[4,5-*d*]pyrimidine derivatives in the solid state under microwave irradiations, *Bioorg. Med. Chem. Lett.* **16**, 3537-3540 (2006). d) Das, S.; Saikia, B.K.; Sridhar, B.; Thakur, A.J. 6-[(Dimethyl - amino)methyleneamino]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione dihydrate, *Acta Cryst.* **E64**, o1662 (2008). e) Thakur, A.J.; Das, S.; Phukan, A.K. Replay of

amide type resonance in 6-[(dimethylamino)methylene]1,3-dimethylaminouracil: A dynamic NMR and density functional theory study, *J. Mol. Struc.* **929**, 134-140 (2009).

25. Brand-Williams, W.; Cuvelier, M.E.; Benset, C. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* **28**, 25-30 (1995).
26. Boakye-Yiadom, I.K. Antimicrobial activity of two flavonone isolates from the Cameroonian plant *Enythiana sigmoides. Planta Medicia.* **54**, 126-212 (1979).
27. Banso, A.; Adeyemo, S.O. Phytochemical and antimicrobial evaluation of ethanolic extracts *Draclena mannii* bark. *Nig. J. Biotech.* **18,** 27-32 (2007).
28. Radhika, P.; Sastry, B.S.; Harica, B.M. Antimicrobial screening of *Andrographis paniculata* (Acanthaceae) root extracts. *Res. J. Biotech.* **3**, 62-63 (2008)
29. Lam, B.L.; Pridgen, L.N. An acid-catalyzed hydroxyalkylation of uracil: A facile synthesis of 5-(arylhydroxymethyl)uracils, *J. Org. Chem.* **51**, 2592-2594 (1986).
30. Azizian, J.; Mohammadizadeh, M.R.; Teimouri, F.; Mohammadi, A.A.; Karimi, A.R. Reactions of 6-aminouracils: The first simple, fast, and highly efficient synthesis of bis(6-aminopyrimidonyl)methanes (BAPMs) using thermal or microwave-assisted solvent-free methods, *Synth. Commun.* **36**, 3631-3638 (2006).
31. Bansal, R. *et al* Formation of aryl-bis (6-amino-1,3-dimethyluracil-5-yl) methanes by reaction of 6-amino-1,3-dimethyluracil with aromatic aldehydes, *J. Heterocycl. Chem.* **45**, 1789-1795 (2008).
32. Spacilova, L. *et al* Synthesis and cytotoxic activity of various 5-[alkoxy-(4-nitro-phenyl)-methyl]uracils in their racemic form, *Bioorg. Med. Chem. Lett.* **17**, 6647-6650 (2007).
33. Dabiri, M.; Arvin-Nezhad, H.; Khavasi, H.R.; Bazgir, A. A novel and efficient synthesis of pyrimido[4,5-*d*]pyrimidine-2,4,7-trione and pyrido[2,3-*d*:6,5-*d*]dipyrimidine-2,4,6,8-tetrone derivatives, *Tetrahedron* **63**, 1770-1774 (2007).
34. Shi, D.; Shi, J.; Rong, S. A facile and clean synthesis of pyrimidine derivatives via three-component reaction in aqueous media, *Chin. J. Chem.* **28**, 791-796 (2010).
35. Narender, T.; Khaliq, T.; Srivastava, M.N. Naturally occurring uracil dimer from the Marine sea hare *Dolabella auricularia*, *Nat. Prod. Commun.* **2**, 71-73 (2007).

36. a) Semenov, V.E. *et al* New type of pyrimidinophanes with $\alpha,\omega$-bis(uracil-1-yl)alkane and bis(uracil-5-yl)methane units, *Mendeleev Commun.* **11(3)**, 96-97 (2001). b) Semenov, V.E. *et al* Synthesis and antimicrobial activity of pyrimidinophanes with two uracil units and bridging nitrogen atoms, *Pharm. Chem. J.* **43**, 448-453 (2009). c) Semenov, V.E.; Giniyatullin, R.K.; Reznik, V.S. Heterocyclophanes with 6-methyluracyl and hydantoin fragments. Ring contraction in 1,3-bis(5-bromopentyl)-5-nitrouracyl, *Russ. J. Org. Chem.* **46**, 309-310 (2010).

37. Kong, L.Q.; Zhao, J.; Fan, L.; Yang, D.C. The facile synthetic method of 5-hydroxymethyluracil, *Chin. Chem. Lett.* **20**, 314-316 (2009).

38. a) Das, S.; Saikia, B.K.; Das, B.; Saikia, L.; Thakur, A.J. 6,6′-Diamino-1,1′,3,3′-tetramethyl-5,5′-(4-chlorobenzylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione, *Acta Cryst.* **65E**, o2416-o2417 (2009). b) Jursic, B.S.; Neumann, D.M.; Martin, K.L.; Stevens, E.D.; Barbituric acid initiated rearrangement of 2,2′-pyridil into 5,5′-(2-pyrilidine)bisbarbituric acid, *Org. Lett.* **4**, 811-813 (2002). c) Kivekas, R.; Sundberg, M.R.; Ruiz, J.; Colacio, E. 6-Amino-1,3-dimethyl-5-(2-ethylphenyl - azonio)uracil bromide dihydrate, *Acta Cryst.* **C47**, 1512-1515 (1991). d) Low, J.N.; Ferguson, G.; Moreno-Carretero, M.N.; Hueso-Urena, F. 6-Amino-5-hydroxyiminomethyl-1,3-dimethyluracil, *Acta Cryst.* **C50**, 1328-1329 (1994). e) Dabiri, M.; Azimi, S.C.; Khavasi, H.R.; Bazgir, A. A novel reaction of 6-aminouracils and isatins, *Tetrahedron* **64**, 7307-7311 (2008).

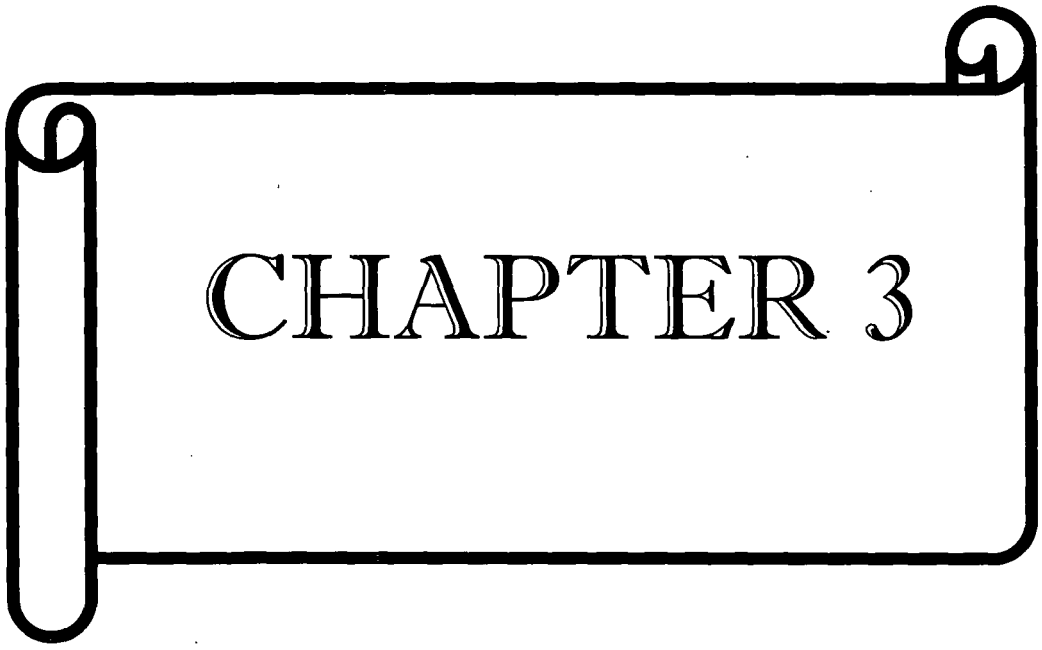
39. a) Hopp, M.; Erxleben, A.; Rombeck, I.; Lippert, B. The uracil C(5) position as a metal binding site: Solution and X-ray crystal structure studies of Pt(II) and Hg(II) compounds, *Inorg. Chem.* **35**, 397-403 (1996). b) Zamora, F.; Sabat, M.; Lippert, B. (1,3-Dimethyluracil-5-yl)mercury(II): Preparative, structural, and NMR spectroscopic studies of an analog of CH(3)Hg(II), *Inorg. Chem.* **35**, 4858-4864 (1996). c) Low, J.N.; Howie, R.A.; Hueso-Urena, F.; Moreno-Carretero, M.N. Structures of 6-amino-1,3-dimethyl-5-nitrosouracil monohydrate and 6-amino-5-formyl-1,3-dimethyluracil monohydrate, *Acta Cryst.* **C48**, 145-147 (1992).

40. a) Shiri, M.; Zolfigol, M.A.; Kruger, H.G.; Tanbakouchian, Z. Bis- and trisindolylmethanes (BIMs and TIMs), *Chem. Rev.* **110**, 2250-2293 (2010). b) Ji, S.J. *et al* Efficient Fe(III)-catalyzed synthesis of bis(indolyl)methanes in ionic liquids, *Eur. J. Org. Chem.* 1584-1587 (2004).
41. Pollet, P.; Hart, R.J.; Eckert, C.A.; Liotta, C.L. Organic aqueous tunable solvents (OATS): A vehicle for coupling reactions and separations, *Acc. Chem. Res. 43*(9), 1237-1245 (2010).
42. Sheldrick, G.M. A short history of SHELX, *Acta. Cryst.* **A64**, 112-122 (2008).
43. Shishkin, O.V.; Gorb, L.; Leszczynski, J. Modeling of the hydration shell of uracil and thymine, *Int. J. Mol. Sci.* **1**, 17-27 (2000).
44. Bodour, A.A.; Maier, R.M.; Application of a modified drop collapse technique for surfactant quantification and screening of biosurfactant-producing microorganisms. *J. Microbiol. Methods* **32**, 273-280 (1998).
45. Desai, J.D.; Banat, I.M.; Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Bio. Rev.* **61**, 47-56 (1997).
46. a) Bharali, P.; Das, S.; Konwar, B.K.; Thakur, A.J. Crude biosurfactant from thermophilic *Alcaligenes faecalis*: Feasibility in petro-spill bioremediation, *Int. Biodeter. Biodegr.* 2011 (in press). b) Benincasa, M.; Abalos, A.; Oliveira, I.; Manresa, A. Chemical structure, surface properties and biological activities of the biosurfactant produced by *Pseudomonas aeruginosa* LBI from soapstock, *Antonie Van Leeuwenhoek* **85**, 1-8 (2004). c) Banat, I.M.; Makkar, R.S.; Cameotra, S.S.; Potential commercial application of microbial surfactants, *Applied Microbiol. Biotech.* **53**, 495-508 (2000).
47. Bharali, P.; Konwar, B.K. Production and physico-chemical characterization of a biosurfactant produced by *Pseudomonas aeruginosa* OBP1 isolated from petroleum sludge, *Appl .Biochem .Biotechnol.* 2011 (in press).
48. Wu, J.Y. *et al* Rhamnolipid production with indigenous *Pseudomonas aeruginosa* EM1 isolated from oil-contaminated site, Bioresource Technology, **99**, 1157–1164 (2008).
49. Chandrasekaran, E.V.; Bemiller, J.N. In *Methods in Carbohydrate Chemistry*, Ed: Wrhiste, L.; Wolfrom, M. L. (Academic, New York, pp. 89–97, 1980).

50. Bodour, A.A.; Maier, R.M. Application of a modified drop collapse technique for surfactant quantification and screening of biosurfactant-producing microorganisms. *J. Microbiol. Methods* **32**, 273-280 (1998).

51. Das, S.; Thakur, A. J. A clean, highly efficient and one-pot green synthesis of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimethyluracil-5-yl)methanes in water, *Eur. J. Org. Chem.* 2301-2308 (2011).

52. Manabe, K.; Iimura, S.; Sun, X.M.; Kobayashi, S. Dehydration reactions in water. Broensted acid-surfactant-combined catalyst for ester, ether, thioether, and dithioacetal formation in water, *J. Am. Chem. Soc.* **124**, 11971-11978 (2002).


CHAPTER 3

# Replay of amide type resonance in 6-[(dimethylamino)methylene]1,3-dimethylaminouracil:A dynamic NMR and density functional theory study

**Introduction:**

The biological activity of a protein is largely governed by the restricted rotation of the C-N bond of peptide linkage.[1] The key to the understanding of this hindered C-N rotational barrier in amides, the prototype of the peptide bond in proteins, is provided by 'amide resonance'.[2] There is a large body of literature concerning the importance of resonance in predicting this rotational barrier in other related systems.[3] Uracil, the nucleobase of pyrimidine family, combined with adenine (AU) comprises one of the major motifs present in the biopolymer RNA. Uracil is also involved in the self-assembly of RNA.[4] The versatility of uracil and its derivatives as exhibited by their wide range of biological activities is well recognized by synthetic[5] as well as biological chemists.[6] With the development of uracil based anticancer and antiviral drugs (e.g. AZT, DDI, DDC, BVDU), there is a renewed interest in the synthesis and design of hetero-aromatic species of uracil origin having biological significances.[7] Several patents have been reported describing the synthesis of such heterocycles, derivatives of which are useful as vasodilators, bronchodilators, antiallergic, antihypertensive, and anticancer agents.[8] Uracils having N-3 side chains derived from various amino alcohols were designed and synthesized as potent human gonadotropin releasing hormone receptor antagonists.[9] Many drug candidates have been modelled on these compounds, particularly for cancer and virus research. 5-Substituted uracils and their nucleosides are widely used in the chemotherapy of cancer.[10] The chemistry of uracil moiety and its derivatives were extensively studied in the past decades mainly because of its mechanistic, synthetic and biological importance which made them of substantial experimental and theoretical interest.[11] Here, we present a combined variable temperature-dynamic nuclear magnetic resonance (VT-DNMR) spectroscopy and density functional theory (DFT) study about the C-N bond rotation of 6-[(dimethylamino)methylene]1,3-dimethylaminouracil (**1**), which consists of an uracil ring and an amidine side chain.

**Materials and Methods:**

$^1$H NMR spectra were recorded on a VARIAN 300 MHz spectrometer ($^1$H operating frequency at 299.942 MHz), equipped with a variable temperature unit, which has been calibrated using ethylene glycol. Spectra were recorded over a range of temperatures starting from 25 to 100 °C, using the residual solvent signal (*d* 5.98 ppm for the solvent 1,1,2,2-tetrachloroethane-$d_2$) as the reference. Sample of (**1**) was prepared in 1,1,2,2-tetrachloroethane-$d_2$ (Cambridge Isotope Laboratories) and placed in a 5 mm NMR tube (Wilmad). Samples for kinetic measurements were allowed to equilibrate in the NMR probe for 5 min before recording the spectra. Temperature fluctuations during the series of experiments were ±0.2 °C. All the experiments were performed three times.

**Crystal structure of (1):**

Single crystals of (**1**) were grown from their 98% ethanol solution. The intensity data were collected at 294 K on a Bruker SMART APEX CCD area-detector diffractometer with Mo *K*α radiation (λ= 0.71073 Å). Crystallographic and experimental data are listed in **Table 2**.[12] The structures were solved by SHELX97 and refined by full-matrix least squares on $F^2$ (SHELX97).[13] Hydrogen site location is inferred from neighboring sites, H atoms are treated by a mixture of independent and constrained refinement. They were positioned geometrically and treated as riding on their parent C-atoms.

**Computational details:**

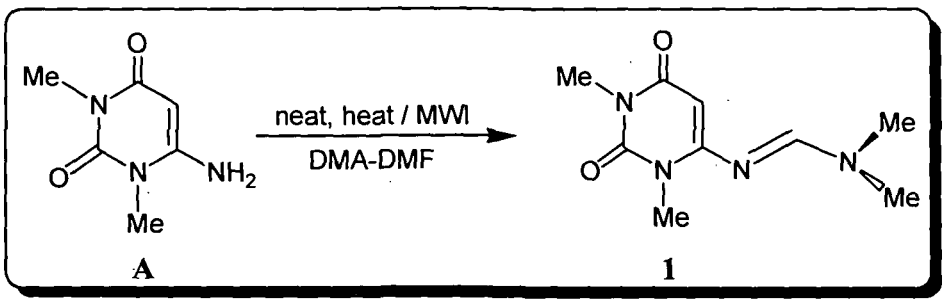
All the molecules were fully optimized at the B3LYP/6-31+G* level of density functional theory.[14] Analytical vibrational frequency calculations were then performed on the optimized geometry to ascertain the nature of the stationary point. The ground and transition states were characterized with zero and one imaginary frequency, respectively. In order to check the effect of the ring as well as substituents on the rotational barrier, we calculated the parent compounds without the uracil ring (**3** and **4**) for both ground state (GS) and transition state (TS) geometry using methyl and hydrogen as the substituents.

The effect of substituent is also probed for the parent compound in which the uracil ring is present (**2**). Further, structures were optimized without considering the two water molecules which were present in the X-ray structure. The electronic structures of all the molecules were analysed with the help of NBO partitioning scheme as implemented in the Gaussian 03 suite of program.[15] The transition state geometry of the actual molecule (1TS) was further verified by intrinsic reaction coordinate (IRC)[16] analysis at the same level of theory.

**Results and Discussion:**

Papesch *et al* in 1951 and Blicke *et al* in 1954 first introduced the synthetic procedure of 6-amino-1,3-dimethyluracil (**A**)[17,18] by the reaction between *N,N'*-dimethylurea[19] and cyanoacetic acid. The structure was further confirmed by $^{1}H$ & $^{13}C$ NMR & single crystal X-ray analysis. From single crystal X-ray analysis it is observed that compound is stable with intra- or intermolecular hydrogen bonding. **Fig. 1** represents the ORTEP diagram of 6-amino-1,3-dimethyluracil (**A**).

6-[(Dimethylamino)methylene]1,3-dimethylaminouracil (**1**) was readily obtained by the reaction of 6-amino-1,3-dimethylbarbituric acid with *N,N*-dimethyl formamide dimethylacetal (DMF-DMA) in the solventless condition for 2 h at refluxing temperature of DMF-DMA or under microwave irradiation (**Scheme 1**).




**Scheme 1**: Synthetic route for the synthesis of (**1**)

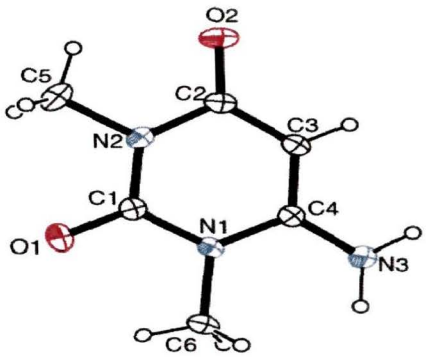


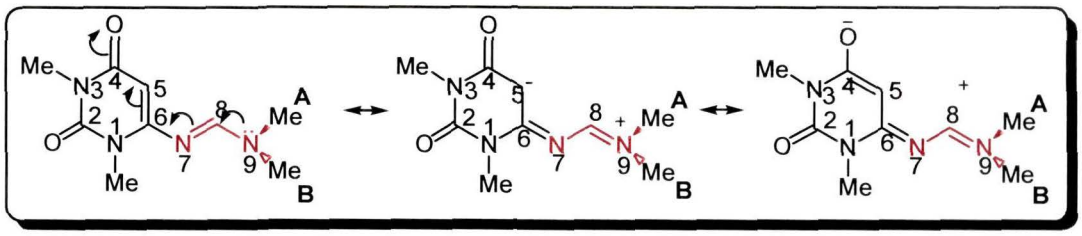

**Fig. 1:** ORTEP diagram of 6-amino-1,3-dimethyluracil (**A**)

**Table 1:** Detail crystallographic data of 6-amino-1,3-dimethyluracil (**A**)

| | |
|---|---|
| Formula | $C_6H_9N_3O_2$ |
| M | 155.16 |
| Crystal system | Pnma |
| Temperature/K | 293 K |
| Space group | Pnma |
| *a*/ Å | 14.7970(3) |
| *b*/ Å | 6.7049(2) |
| *c*/ Å | 7.1628(2) |
| α (°) | 90 |
| β (°) | 90 |
| γ (°) | 90 |
| *V*/ Å$^3$ | 710.64(3) |
| *Z* | 4 |
| *Dc*/ mg·m$^{-3}$ | 1.450 |
| Reflns. collected | 974 |
| Reflns. unique | 770 |
| R(*int*) | 0.0374 |
| Index ranges | -19<=h<=1x, -8<=k<=1x, -9<=h<=1x |
| Refinement method | Full-matrix, least squares on $F^2$ |
| $wR_2$ 0.1138 | *GoF* 1.080 |

The exocyclic imine bond (N7-C8) of (**1**), in conjugation with the double bond (C5-C6) of the pyrimidine ring, thus constituting a heterodiene (C5-C6-N7-C8) system. In addition, the exocyclic N7-C8-N9 system constitutes an enamine system which is responsible for the nucleophilic behaviour of C5 position (**Scheme 2**).

An understanding of the rotational barrier of the C8-N9 bond will help in understanding the diene behaviour of the C5-C6-N7-C8 system and intramolecular flexibilities.



**Scheme 2:** Resonance structures for (**1**)

**X-ray structure:**

The structure of (**1**) was determined by X-ray crystallographic analysis and it features a planar six-membered uracil ring attached to an exocyclic enamine side chain (**Fig. 2**). This side chain makes an angle of 174.3° with the plane of the uracil ring. The C6-N7, N7-C8 and C8-N9 bond lengths of 1.365, 1.299 and 1.320 Å, respectively, are in tune with those given for $Csp^2$-$Nsp^2$ bonds in the literature.[20] The side chain also features a planar tri-coordinate carbon atom in C8 (sum of the bonding angles: 359.9°). The crystal structure also contains two water molecules which form O-H- - - -O hydrogen bonds with the carbonyl O atoms of the uracil group.

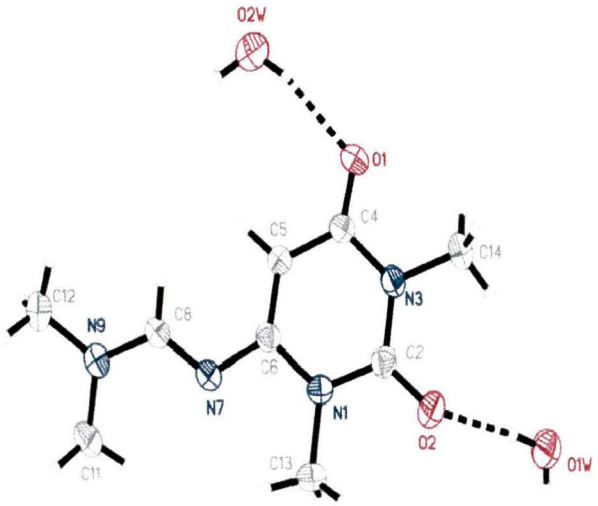



**Fig. 2:** ORTEP diagram of **(1)**

**Table 2**: Detail crystallographic data of **(1)**

| | |
|---|---|
| Formula | $C_9H_{14}N_4O_2 \cdot 2H_2O$ |
| M | 246.27 |
| Crystal system | Triclinic |
| Temperature/K | 294 (2) |
| Space group | *P*1 |
| *a*/ Å | 7.1310 (5) |
| *b*/ Å | 9.8571 (7) |
| *c*/ Å | 9.9160 (7) |
| α (°) | 92.921 (1) |
| β (°) | 101.916 (1) |
| γ (°) | 109.912 (1) |
| *V*/ $Å^3$ | 635.62 (8) |
| *Z* | 2 |
| *Dc*/ $mg \cdot m^{-3}$ | 1.287 |
| Reflns. collected | 6112 |
| Reflns. unique | 2231 |
| R(*int*) | 0.019 |
| Index ranges | -8<=h<=8, -11<=k<=11, -11<=h<=11 |
| Refinement method | Full-matrix, least squares on $F^2$ |
| $wR_2$ 0.155 | $R_1$ 0.051 *GoF* 1.07 |

**$^1$H VT-NMR studies:**

The most widely used NMR method for investigating internal rotation is the appearance of coalescence of peaks measured at variable temperatures.[21] In 1,1,2,2-tetrachloroethane-$d_2$ at 25°C, (**1**) shows six $^1$H NMR signals at $\delta$ 3.063 (s, 3H) and 3.102 (s, 3H) (N9-$Me_2$); 3.403 (s, 3H, N3-Me) and 3.298 (s, 3H, N1-Me); 5.027(s, 1H, C5-H); and 7.655 (s, 1H, N7=C8-H) ppm, respectively. The two N9-methyl (**A** and **B**) groups split into two sharp singlets (3.102 and 3.063 ppm) with equal intensity indicating their magnetically non equivalent character. This nonequivalence is attributed to the hindered rotation of the dimethylamino moiety about the C8-N9 bond. As the exocyclic C8-N9 bond rotates, chemical exchange of the two N9-$CH_3$ protons (**A** and **B**) will affect the NMR line shapes and intensity. A barrier is observable when exchange of the studied nuclei into different electronic environments is slow enough on the NMR timescale (usually in the range of 1-$10^6$ $s^{-1}$).[22] **Fig. 3** shows the series of partial $^1$H NMR spectra (showing only the four N-methyl groups attached to the three nitrogens, i.e. N1, N3 and N9) of (**1**) recorded at different selected temperatures and at different rates (slow, medium and fast) of chemical exchange. In the limit of slow exchange (lower temperatures), distinct and sharp resonances are observed for the two N9-$CH_3$ protons (labelled with asterisks, **Fig. 3**). At room temperature (25 °C), the four methyl peaks, each at N1, N3 and two at N9 has equal intensities. However, as the temperature is increased, the exchange rate increases and the two sharp N9-$CH_3$ peaks broaden, come closer, and eventually coalesce (3.093 ppm) at 89 °C (**Fig. 3**).

Appearance of coalescence of the exchanging peaks provides a quantitative measure of the rates. At this coalescence temperature, all the other $^1$H signals of (**1**) remain distinct and sharp, and, there is no overlap of peaks, except for the two N9-$CH_3$ signals. As the temperature is increased beyond 89 °C, the coalescence of the two peaks becomes sharper and sharper with increased intensity. Under the condition of fast exchange in the NMR timescale, the NMR instrument cannot distinguish the two N9-$CH_3$ peaks (**A** and **B**); it shows the time average picture only and hence, only one signal for both the N-$CH_3$ peaks (**Scheme 3**).

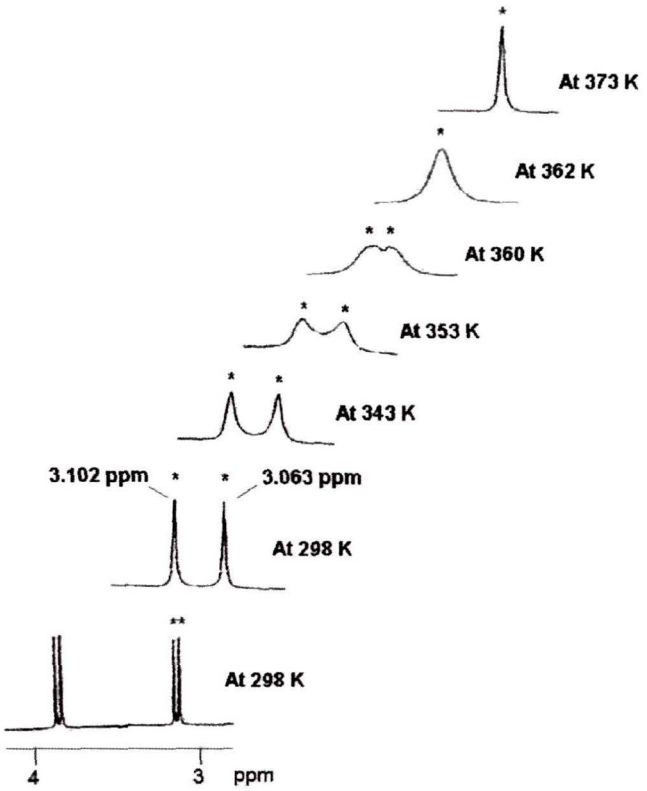
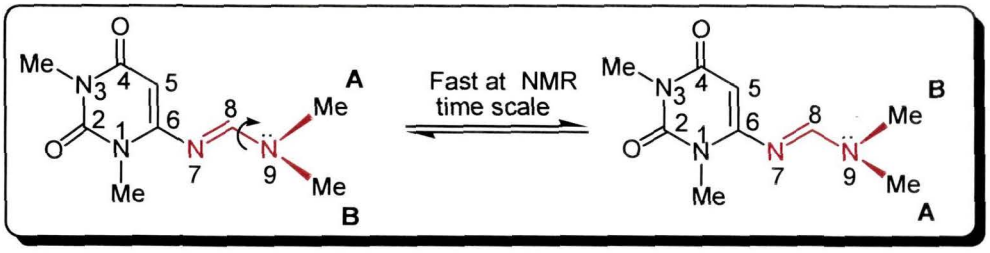



**Fig. 3:** Partial dynamic NMR spectra of (**1**) in 1,1,2,2-tetrachloroethane-$d_2$ (only two methyl groups marked by asterisks are shown)




**Scheme 3:** Situation under fast exchange condition in the NMR time scale

A number of solvents were screened to observe the dynamic process of (**1**) (**Table 3**) at variable temperatures including solvent effects, if any. Acetone-$d_6$, dichloromethane-$d_2$, chloroform-$d_3$, methanol-$d_4$, ethanol-$d_6$, THF-$d_8$, (entries **1-6**, **Table 3**) showed good solubility, but maximum attainable temperature in these solvents (restricted by their boiling points) were not high enough to reach the fast exchange region

or may be even coalescence of the signals due to the methyl groups of the C8-N9 bond. But, the spectra recorded in these solvents were very clean having sharp peaks with different chemical shift values with respect to the solvents. Acetonitrile-$d_3$, benzene-$d_6$, toluene-$d_8$, pyridine-$d_5$ (entries **7-10**, **Table 3**) did not work since the compound (**1**) was not soluble there in even at higher temperatures (up to their boiling points).

*N*,*N*-Dimethylformamide-$d_6$ was not selected as the solvent because the four methyl peaks of (**1**) overlap with the two *N*-methyl peaks of DMF. Moreover, DMF itself shows restricted rotation.[23] However, 1,1,2,2-tetrachloroethane-$d_2$ offers an excellent solvent because the solubility of (**1**) is very good in it and also, owing to its high boiling point, high temperature could be achieved, which is necessary for observing any possible coalescence. The barrier to rotation is interpreted using transition state theory,[18] which relates rate constant, $K_c$ to $\Delta G^{\ddagger}$, energy of activation (Eyring equation). The rate constant, $K_c$ for the present dynamic process was calculated at the coalescence temperature ($T_c$) using Gutowski–Holm equation.[24] Substituting $T_c$, $K_c$, $\Delta\gamma$ (the separation of the two N9-Me peaks in Hz) and taking the transmission coefficient as unity in the Eyring equation, the C-N rotational barrier ($\Delta G^{\ddagger}$) for (**1**) in 1,1,2,2-tetrachloroethane-$d_2$ is found to be

**Table 3:** List of solvents for dynamic study

| Entry | Solvent[a] | Highest temperature[b] checked | Coalescence |
|---|---|---|---|
| 1 | $(CH_3)_2CO$-$d_6$ | $53^0$C | Not observed |
| 2 | $CH_2Cl_2$-$d_2$ | $37^0$C | Not observed |
| 3 | $CHCl_3$- $d$ | $58^0$C | Not observed |
| 4 | $CH_3OH$- $d_4$ | $63^0$C | Not observed |
| 5 | $C_2H_5OH$-$d_6$ | $76^0$C | Not observed |
| 6 | $C_4H_8O$-$d_8$ | $62^0$C | Not observed |
| 7 | $CH_3CN$- $d_3$ | $77^0$C | Not observed |
| 8 | $C_6H_6$- $d_6$ | $77^0$C | Not observed |
| 9 | $C_7H_8$- $d_8$ | $108^0$C | Not observed |
| 10 | $C_5H_5N$- $d_5$ | $110^0$C | Not observed |
| 11 | $C_2H_2Cl_4$- $d_2$ | $100^0$C | Observed |

[a] all the solvents used are of highest purity available and as received [b] restricted by boiling points

18.97 kcal/mol. A change in temperature near coalescence temperature has very little effect on $\Delta G^{\ddagger}$. The difference in $\Delta G^{\ddagger}$ for the activation energy at 89 °C and at 86 °C (18.81 kcal/mol) is only 0.16 kcal/mol. Therefore any possible error in the measurement of coalescence temperature will have very little effect on the barrier.

**C-N Rotational energy barrier calculation:**

The rate constants, $K_c$ for the present dynamic process were calculated at the coalescence temperature ($T_c$) using Gutowski-Holm equation (1).

$$k_c = \frac{\pi\Delta\nu}{\sqrt{2}} \qquad \text{---------- (1)}$$

Experimental quantities that can be determined from Variable Temperature (VT) studies include $T_c$ and the separation of the peaks ($\Delta\nu$) at lowest temperature. When we substitute $K_c$ as determined by VT methods in the Eyring equation, it modifies into a useful form, equation (2).

$$\Delta G_c^{\ddagger} = 19.134 T_c \left[10.32 + \log\left(Tc/k_c\right)\right] \qquad \text{---------- (2)}$$

Putting the values of $K_c$ and $T_c$ into the equation (2) gives the energy of activation for the C-N rotational barrier ($\Delta G^{\ddagger}$) for **(1)** in 1,1,2,2-tetrachloroethane-$d_2$ :

$$\Delta G_c^{\ddagger} = 19.134 T_c \left[10.32 + \log\left(\frac{Tc * \sqrt{2}}{\pi\Delta\nu}\right)\right] \text{x}10^{-3}\ \text{KJmol}^{-1}$$

[Here $T_c$ = 362K, $\Delta\nu$ = 0.039x300 Hz = 11.7 Hz]

$$\Delta G_{362}^{\ddagger} = 19.134 * 362 \left[10.32 + \log\left(\frac{362 * \sqrt{2}}{\pi\Delta\nu}\right)\right] \text{x}10^{-3}\ \text{KJmol}^{-1}$$

$$= 6926.508 \left[10.32 + \log\left(\frac{362 * 1.414}{3.14159 * 11.7}\right)\right] \text{x}10^{-3}\ \text{KJmol}^{-1}$$

$$= 6926.508\,[10.32 + 1.14382]\text{x}10^{-3}\ \text{KJmol}^{-1}$$

$$= 79.40424\ \text{KJmol}^{-1}$$

$$= 18.97\ \text{Kcalmol}^{-1}$$

The optimized geometries of all the molecules in both ground state (GS) and transition state (TS) are shown in **Fig. 4** along with important geometrical parameters.

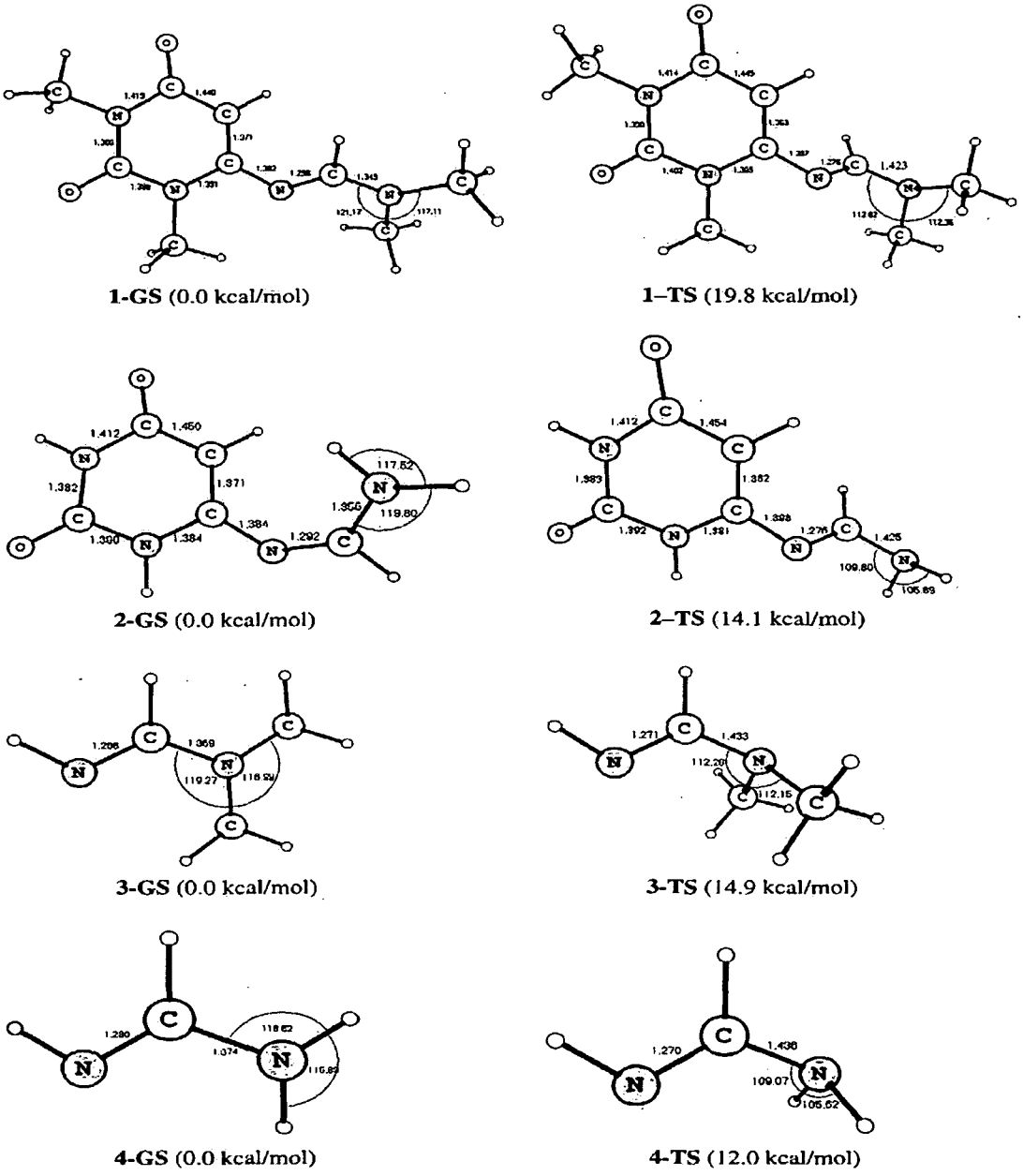



**Fig. 4:** DFT optimized ground and transition state geometries of **(1)**, **(2)**, **(3)**, and **(4)** with important bond lengths and bond angles. The energies are given within bracket. The GS structures are assigned a value of 0.0 kcal/mol and the energies of the TS are given relative to that of the GS.

The computed geometrical parameters of (**1**) are in excellent agreement with the X-ray crystal structure (**Table 4**). The uracil ring of (**1**) and (**2**) are planar in both GS and TS, and the geometrical parameters of the ring remain similar in both the geometries. The C7-N8 bond of all the molecules (**1-4**) does not change appreciably in both the geometries. However, the change in bond length in the GS and TS geometries is significant for the C8-N9 bond. It varies from 0.074 Å in (**1**) to 0.062 Å in (**4**). In the GS, the C8-N9 bond adopts partial double bond character (as indicated by the Wiberg Bond Index value of 1.255 and 1.260 for (**1**) and (**2**), respectively) but in the rotated TS, it becomes a single bond. Moreover, the end nitrogen atom adopts a planar geometry in the GS, but it gets pyramidalized in the TS.

**Table 4:** Comparison of computed[a] and experimental structural parameters of compound (**1**)

| Bonds | 1 (exptl.) | 1 (calcd.) |
|---|---|---|
| N1–C2 | 1.376 | 1.398 |
| C2–N3 | 1.373 | 1.386 |
| N3–C4 | 1.397 | 1.419 |
| C4–C5 | 1.408 | 1.440 |
| C5–C6 | 1.365 | 1.371 |
| C6–N1 | 1.388 | 1.391 |
| C6–N7 | 1.365 | 1.382 |
| N7–C8 | 1.299 | 1.296 |
| C8–N9 | 1.320 | 1.349 |
| C6–N7–C8 | 117.2 | 118.3 |
| N7–C8–N9 | 123.0 | 123.5 |
| C8–N9–C11 | 121.7 | 121.2 |
| C11–N9–C12 | 117.2 | 117.1 |

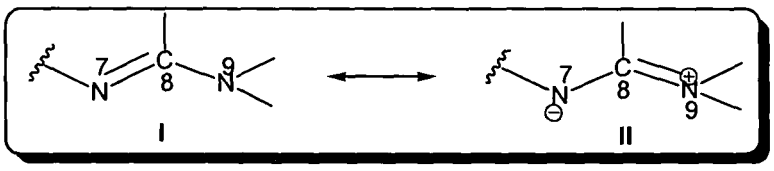
[a] B3LYP/6-31+G* level of theory, bond lengths in Å, angles in degrees

This is further supported by the calculated and observed C-N-H and H-N-H bond angles around the end nitrogen atom which correspond to $sp^2$ hybridized nitrogen atom in the GS (**Table 5**).

However, in the TS, the hybridization changes to $sp^3$ as indicated by the respective bond angles (**Fig. 4**). All the above data indicate that the actual ground state geometry can be envisaged as a resonance hybrid of two structures (**Scheme 4**, only the side chain is shown).

**Table 5:** Hybridization of the lone pair of electrons at N9, pyramidalization angle at N9 (for numbering, see **Scheme 2**) and rotational barrier computed at B3LYP/6-31+G* level of theory.

| Molecule | Occupancy | s (%) | p (%) | Pyramidalization angle, $\theta$ (in degrees) | Rotational barrier (kcal/mol) |
|---|---|---|---|---|---|
| 1-GS | 1.642 | 0.7 | 99.3 | 0.8 | 19.8 |
| 1-TS | 1.895 | 13.1 | 86.8 | 22.5 | --- |
| 2-GS | 1.729 | 1.7 | 98.2 | 1.7 | 14.1 |
| 2-TS | 1.957 | 19.0 | 80.8 | 34.5 | --- |
| 3-GS | 1.704 | 2.5 | 97.5 | 3.5 | 14.9 |
| 3-TS | 1.894 | 13.5 | 86.5 | 23.3 | --- |
| 4-GS | 1.798 | 6.7 | 93.2 | 9.4 | 12.0 |
| 4-TS | 1.958 | 19.6 | 80.3 | 36.3 | --- |




**Scheme 4**: Resonance structures of the side chain of (**1**)

Out of the two structures, structure II contains a double bond between C8 and N9, and it is the presence of this partial double bond, which is responsible for the observed barrier. Also, it is necessary to view both the ground and transition state structures of (**1**) from different perspectives to understand the geometrical changes caused by the rotation of the C8-N9 bond (**Fig. 5**). Since the above discussion about the geometry of all the complexes show that the geometrical parameters of the side chain of (**1**) and (**2**) in both the GS and TS are largely unaffected by the presence of the uracil ring, therefore emphasis will be laid only on the side chain so that a meaningful comparison can be made between all the computed structures. The insight obtained about the nature of the C-N bond of interest, i.e., the C8-N9 bond (for numbering of the atoms, look at **Scheme 4**) from NBO analysis agrees well with those acquired from geometrical parameters.

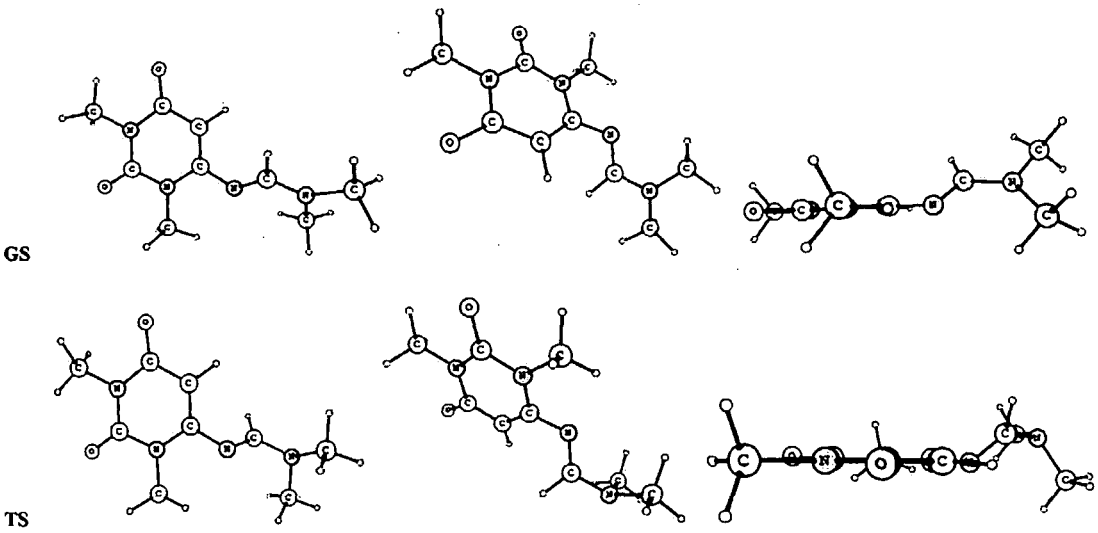



**Fig. 5**: Structures of GS and TS of (**1**) viewed from different angles. The top and bottom row represents the ground and transition state geometry of (**1**) with planar and pyramidal terminal nitrogen atom, respectively.

In the GS, it possesses partial double bond character where as in the TS, it is a single bond as evident from the hybridization of the nitrogen atom, which is $sp^2$ and $sp^3$ hybridized in the GS and TS, respectively (**Table 6**). The lone pair of electrons on N9 has a reduced occupancy in the GS than in the TS (**Table 5**). This is because in the GS, a part of the electron density of the lone pair gets delocalized into the anti-bonding orbital of the

**Table 6**: Hybridization of the N7-C8 and C8-N9 bonds at the B3LYP/6-31+G• level of theory using the NBO method

| Molecule | Bond X-Y | Occupancy | %X | %s (X) | %p (X) | %Y | %s (Y) | %p (Y) |
|---|---|---|---|---|---|---|---|---|
| 1-GS | σ (N7-C8) | 1.984 | 59.1 | 37.3 | 62.5 | 40.8 | 35.1 | 64.8 |
| | π (N7-C8) | 1.90 | 66.4 | 0.4 | 99.6 | 33.6 | 0.5 | 99.5 |
| | C8-N9 | 1.987 | 37.0 | 30.7 | 69.1 | 62.9 | 36.3 | 63.7 |
| 1-TS | σ (N7-C8) | 1.987 | 60.8 | 39.3 | 60.5 | 39.2 | 34.2 | 65.7 |
| | π (N7-C8) | 1.940 | 61.2 | 0.7 | 99.0 | 38.7 | 0.7 | 99.1 |
| | C8-N9 | 1.984 | 39.3 | 30.5 | 69.4 | 60.7 | 28.7 | 71.2 |
| 2-GS | σ (N7-C8) | 1.987 | 59.2 | 38.2 | 61.6 | 40.7 | 35.3 | 64.6 |
| | π (N7-C8) | 1.915 | 66.1 | 1.0 | 99.0 | 33.9 | 0.8 | 99.2 |
| | C8-N9 | 1.996 | 38.8 | 32.8 | 67.0 | 61.1 | 37.5 | 62.3 |
| 2-TS | σ (N7-C8) | 1.989 | 60.6 | 38.7 | 61.0 | 39.3 | 34.3 | 65.6 |
| | π (N7-C8) | 1.938 | 61.4 | 0.1 | 99.9 | 38.6 | 0.1 | 99.9 |
| | C8-N9 | 1.992 | 41.0 | 30.9 | 69.0 | 59.0 | 30.8 | 69.1 |
| 3-GS | σ (N7-C8) | 1.991 | 58.7 | 25.8 | 73.9 | 41.3 | 24.8 | 75.1 |
| | π (N7-C8) | 1.994 | 61.2 | 12.8 | 86.8 | 38.8 | 12.4 | 87.5 |
| | C8-N9 | 1.986 | 36.8 | 29.9 | 70.1 | 63.1 | 34.9 | 64.9 |
| 3-TS | σ (N7-C8) | 1.995 | 58.9 | 40.7 | 59.0 | 41.1 | 36.2 | 63.7 |
| | π (N7-C8) | 1.992 | 58.5 | 0.0 | 99.9 | 41.4 | 0.0 | 99.9 |
| | C8-N9 | 1.983 | 38.7 | 29.8 | 70.0 | 61.3 | 28.6 | 71.3 |
| 4-GS | σ (N7-C8) | 1.995 | 59.3 | 24.8 | 74.9 | 40.7 | 24.1 | 75.8 |
| | π (N7-C8) | 1.992 | 60.3 | 14.0 | 85.7 | 39.7 | 13.0 | 86.8 |
| | C8-N9 | 1.994 | 39.0 | 30.4 | 69.5 | 61.0 | 36.7 | 63.3 |
| 4-TS | σ (N7-C8) | 1.998 | 58.7 | 40.1 | 59.6 | 41.3 | 36.6 | 63.3 |
| | π (N7-C8) | 1.993 | 58.7 | 1.0 | 99.0 | 41.3 | 1.0 | 99.0 |
| | C8-N9 | 1.993 | 40.3 | 30.2 | 69.6 | 59.6 | 30.7 | 69.2 |

adjacent C-N double bond. Such delocalization is not possible in the TS as the participating orbitals are orthogonal to each other. The orbital housing the lone pair in the GS is an in-plane p orbital, but it attains some s character in the TS, thereby making the nitrogen atom more electronegative in the TS. The stabilization energies obtained from delocalization of the lone pair of electrons on N9 into the anti-bonding orbital of the adjacent C8-N7 double bond decreases from (**1**) to (**4**) (**Table 7**). Correspondingly, the rotational barrier also decreases from (**1**) to (**4**). It is this stabilizing interaction which is responsible for the partial double bond character of the C8-N9 single bond in the GS (structure II of **Scheme 4**) and hence the barrier. Natural charges computed at the same level of theory predict a higher negative charge for the N9 atom in the TS (**Table 8**). This

**Table 7**: NBO stabilization energies (kcal/mol) arising out of the delocalization of the lone pair of electrons at the terminal nitrogen atom

| Interactions | 1-GS | 1-TS | 2-GS | 2-TS | 3-GS | 3-TS | 4-GS | 4-TS |
|---|---|---|---|---|---|---|---|---|
| lpN9→σ*(N7–C8) | - | 10.4 | - | 10.7 | 9.5 | 10.1 | - | 9.8 |
| lpN9→π*(N7–C8) | 64.7 | - | 56.7 | - | 11.7 | - | 24.1 | - |

**Table 8**: Natural charges of the terminal heavy atoms calculated at the B3LYP/6-31+G* level of theory

| Molecule | N7 | C8 | N9 |
|---|---|---|---|
| 1-GS | -0.582 | +0.321 | -0.460 |
| 1-TS | -0.473 | +0.344 | -0.598 |
| 2-GS | -0.565 | +0.301 | -0.847 |
| 2-TS | -0.483 | +0.319 | -0.951 |
| 3-GS | -0.777 | +0.265 | -0.506 |
| 3-TS | -0.661 | +0.281 | -0.605 |
| 4-GS | -0.761 | +0.245 | -0.876 |
| 4-TS | -0.669 | +0.253 | -0.961 |

correlates well with the increased occupancy of N9 in the TS (**Table 7**). However, the charge on the C8 atom does not change appreciably in both the states. On the other hand, the N7 atom, which is double bonded to C8, carries a higher negative charge in the GS. All these data are consistent with our resonance based explanation about the origin of the rotational barrier about the C8-N9 bond. The computed barrier of 19.8 kcal/mol is in good agreement with the observed barrier of 18.97 kcal/mol. Interestingly, our explanation correlates well with those given for C-N rotational barrier in amides.[9]

**Bioactivity test:**

We also carried out the bioactivity tests of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**A**) and 6-[(Dimethylamino)methylene]1,3-dimethylaminopyrimidine-2,4(1*H*,3*H*)-dione (**1**) against four bacterial strains (both gram positive and gram negative) namely *Staphylococcus aureus* (ATCC 11632), *Bacillus subtilis* (ATCC 11774), *E. coli* (ATCC 9637), *Pseudomonas aeruginosa* (MTCC 7812) and two fungi strains namely *Candida albicans*, *Aspergillus niger*. Compounds were found to be inactive against both bacteria and fungi.

**Conclusion:**

The present study gives the C-N rotational energy barrier for the dimethylamino group in the exocyclic part of (**1**). As expected, being rigid, the pyrimidine ring does not undergo any change in its conformation. Changes occur only in the exocyclic part as the dynamic process of fast exchange is induced by the application of heat energy. Although from the classical viewpoint, we as certain the barrier arising from the partial double bond, yet there may be other factors responsible for the observed barrier. Theoretical calculations show that the origin of the barrier lies in delocalization of the lone pair of electrons on the end nitrogen atom into the anti-bonding orbital of the adjacent C-N double bond in the ground state. The computed barrier is in good agreement with the observed value.

**Experimental Section:**

**General Procedure for the synthesis of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (A):**

A mixture of 1,3-dimethylurea (8.81g, 0.1 mol), cyanoacetic acid (8.5g, 0.1 mol) and acetic anhydride (12.5 ml) were heated with exclusion of moisture under stirring at 60°C for 3h. The excess of acetic anhydride and acetic acid formed during the reaction were removed under reduced pressure. A 5% cold sodium hydroxide solution (50ml) was slowly added to the cooled residue with stirring whereby 1,3-dimethyl-6-aminouracil precipitated out. The precipitated was recrystallized from water to give a pure compound.

**General Procedure for the synthesis of 6-[(Dimethylamino)methylene]1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (1):**

A mixture of 1,3-dimethyl-6-amino uracil (1.55, 0.1 mol) and *N*,*N*-dimethyl formamide dimethyl acetal (DMF-DMA) (1.32 ml, 0.1 mol) were refluxed in heating mantle. 1,3-dimethyl-6-aminouracil is insoluble in DMF-DMA, but when we heated it up, it become soluble and after 1 h the 6-[(Dimethylamino)methylene]1,3-dimethylaminouracil precipitated out. The precipitate was recrystallized from ethanol (98%) to give a pure compound. The reaction proceeded more efficiently when carried out under microwave irradiation and takes only 3 min to complete the reaction in 95% yield.

**Geometry**:

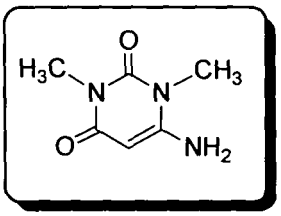
All e.s.d.'s (except the e.s.d. in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell e.s.d.'s are taken into account individually in the estimation of e.s.d.'s in distances, angles and torsion angles; correlations between e.s.d.'s in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell e.s.d.'s is used for estimating e.s.d.'s involving l.s. planes.

**Refinement:**

Refinement of $F^2$ against all reflections. The weighted *R*-factor *wR* and goodness of fit *S* are based on $F^2$, conventional *R*-factors *R* are based on *F*, with *F* set to zero for negative $F^2$. The threshold expression of $F^2 > \sigma(F^2)$ is used only for calculating *R*-factors *etc.* and is not relevant to the choice of reflections for refinement. *R*-factors based on $F^2$ are statistically about twice as large as those based on *F*, and *R*- factors based on all data will be even larger.

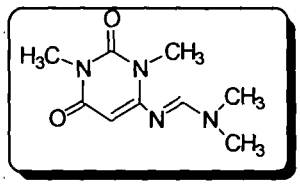
**Physical and spectral data:**

**6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (A)**



Yield 96%. m.p. 290-292 °C. Colour- Transparent middle crystalline solid. Solubility- Soluble in water, sparingly soluble aprotic polar solvents like DMF, DMSO, DMAc etc. IR $\nu_{max}$ (KBR)/cm$^{-1}$ 3451.81, 2993.08, 1705.00 1620.31, 1587.29; $^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C, TMS) $\delta_H$ 3.06 (s, 3H, $NCH_3$), 3.23 (s, 3H, $NCH_3$), 4.70 (s, 1H, CH), 6.79 (br s, 2H, $NH_2$) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$, 25°C, TMS) $\delta_C$ 27.1 ($NCH_3$), 29.3 ($NCH_3$), 75.0 (C-6), 151.7 (C-5), 155.0 (C–4), 161.6 (C–2) ppm; MS, *m/z* 155($M^+$); Anal. Calcd (%) for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found C, 46.52; H, 5.91; N, 27.09.

**6-[(Dimethylamino)methylene]1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (1)**



Yield 93%. mp 148-150 °C. Colour- Yellow shining transparent crystalline solid. Solubility- Soluble in water and common organic solvents; IR $\gamma_{max}$ (KBR)/cm$^{-1}$ 1700 and 1650 (C=O), 1630 (C=N); $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$ 2.99 (s, 3H, $NCH_3$), 3.12 (s, 3H, $NCH_3$), 3.23 (s, 3H, $NCH_3$), 3.3 (s, 3H, $NCH_3$), 4.9 (5-H), 7.49 (CH=N) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 158.20 (C-2), 152.41 (C-4), 154.21 (CH=N), 148.34(C-6), 144.67 (C-5), 29.81 ($NCH_3$), 29.32 ($NCH_3$), 28.96 ($NCH_3$), 27.91 ($NCH_3$) ppm. MS, *m/z* 210 ($M^+$); Anal. Calcd (%) for $C_9H_{14}N_4O_2$: C, 51.42, H, 6.66, N, 26.66. Found C, 51.52, H, 6.71, N, 26.30.

**References:**


1. Sivakova, S.; Rowan, S.J. Nucleobases as supramolecular motifs, *Chem. Soc. Rev.* **34**, 9-21 (2005)
2. a) Lunt, E. in *Comprehensive Organic Chemistry*, Ed: Barton, D.H.R.; Ollis, W.D. (Pergamon Press, Oxford, **4**, 493, 1979). b) Brown, D.J. in *Comprehensive Heterocyclic Chemistry*, Ed: Katritzky, A.R.; Rees, C.W. (Pergamon Press, Oxford, **3**, 57, 1984). c) Sasaki, T.; Minamoto, K.; Suzuki, T.; Yamashita, S. Search for a simpler synthetic model system for intramolecular 1,3-dipolar cycloaddition to the 5,6-double bond of a pyrimidine nucleoside, *Tetrahedron* **36,** 865-870 (1980).
3. a) Castle, R.N.; Philips, S.D. in *Comprehensive Heterocyclic Chemistry*, Ed: Katritzky, A.R.; Rees, C.W. (Pergamon Press, Oxford, **3**, 329, 1984). b) Jones, A.S.; Sayers, J.R.; Walker, R.T.; de Clercq, E. Synthesis and antiviral properties of (*E*)-5-(2-bromovinyl)-2'-deoxycytidine-related compounds, *J. Med. Chem.* **31** 268-271 (1988). c) Mitsuya, H.; Yarchoan, R.; Broder, S. Molecular targets for AIDS therapy, *Science* **249**, 1533-1544 (1990). d) Pontikis, R.; Monneret, C. Synthesis of deoxy analogs of HEPT involving a palladium (0) catalyzed coupling, *Tetrahedron Lett.* **35**, 4351-4354 (1994). e) Taylor, E.C.; Knof, R.J.; Meyer, R.F.; Holmes, A.; Hoefle, M.L. Pyrimido[4,5-*d*]pyrimidines. Part I, *J. Am. Chem. Soc.* **82**, 5711-5718 (1960).
4. a) Bradshaw, T.K.; Hutchinson, D.W. 5-Substituted pyrimidine nucleosides and nucleotides, *Chem. Soc. Rev.* **6**, 43-62 (1977). b) Figueroa-Villar, J.D.; Carneiro, C.L.; Cruz, E.R. Synthesis of 6-phenylaminofuro[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones from barbiturylbenzylidenes and isonitriles, *Heterocycles* **34**, 891-894 (1992). c) Campaigne, E.; Ellis, R.L.; Bradford, M.J.H., Synthesis of some ureidodihydrofurans and related pyrimidones as potential antimalarials, *J. Med. Chem.* **12**, 339-342 (1969). d) Pedersen, O.S.; Pedersen, E.B. Non-nucleoside reverse transcriptase inhibitors: The NNRTI boom, *Antiviral Chem. Chemother.* **10**, 285-314 (1999).

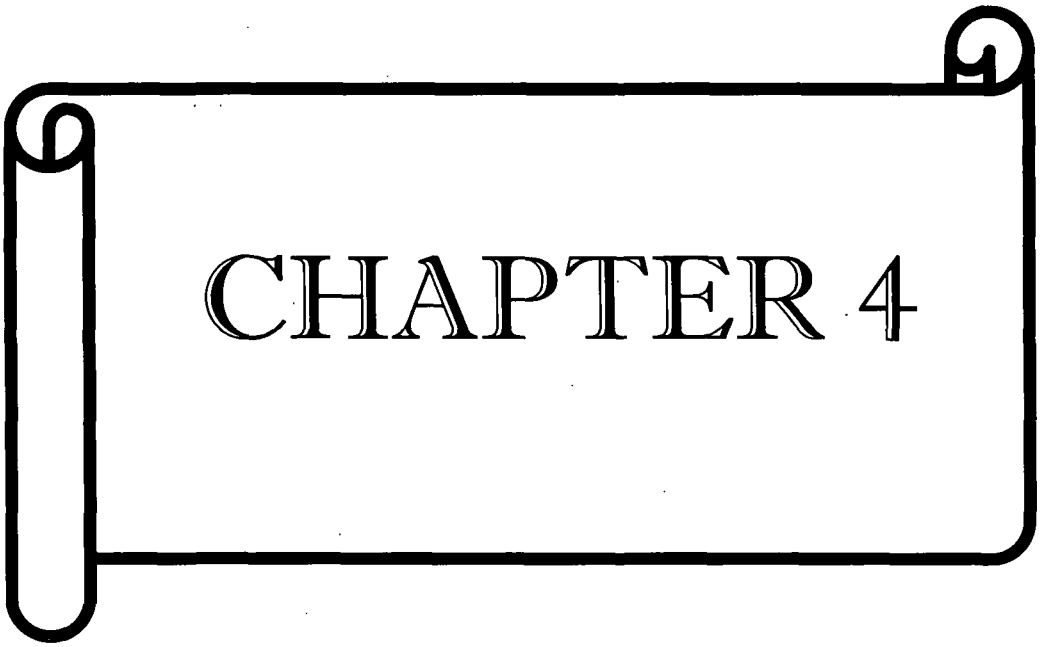
5. a) Coats, W.J. Eur. Pat., 351058, 1990; Chem. Abstr. **113**, 40711 (1990). b) Ramsey, A.A. US Apt. 3830812, 1974; FMC Corp. Chem. Abstr. **81**, 136174 (1974).
6. Kitamura, N.; Onishi, A. Eur. Pat., 163599, 1984; Chem. Abstr. **109**, 54786 (1984).
7. Raddatz, P.; Bergmann, R. Ger. Pat., 360731, 1998; Chem. Abstr. **109**, 54786 (1988).
8. a) Tucci, F.C. *et al* 3-(2-Aminoalkyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methyluracils as orally bioavailable antagonists of the human gonadotropin releasing hormone receptor, *J. Med. Chem.* **47**, 3483-3486 (2004). b) Heidelberger, C. in *Pyrimidine and Pyrimidine Antimetabolites in Cancer Medicine*, Ed: Holland, J.F.; Frei, E.; Febiger, L. (Philadelphia, 1984, pp. 801). c) Kurinovich, M.A.; Phillips, L.M.; Sharma, S.; Lee, J.K. The gas phase proton affinity of uracil: Measuring multiple basic sites and implications for the enzyme mechanism of orotidine 5′-monophosphate decarboxylase, *Chem. Commun.* **20,** 2354-2355 (2002). d) Thureau, P.; Ancian, B.; Viel, S.; Thévand, A. Determining chemical exchange rates of the uracil labile protons by NMR diffusion experiments, *Chem. Commun.* **2**, 200-202 (2006).
9. a) Wiberg, K.B. in *The Amide Linkage: Structural Significance in Chemistry, Biochemistry and Material Science*, Ed: Greenberg, A.; Brenemen, C.M.; Lieben, J.F. (Wiley-IEEE, New York, 33-46, 2002). b) Kemnitz, C.R.; Loewen, M.J. "Amide resonance" correlates with a breadth of C-N rotation barriers, *J. Am. Chem. Soc.* **129**, 2521-2528 (2007). c) Alkorta, I.; Cativiela, C.; Elguero, J.; Gil, A.M.; Jiménez, A.I. A theoretical study of the influence of nitrogen angular constraints on the properties of amides: Rotation/Inversion barriers and hydrogen bond accepting abilities of *N*-formylaziridine and -azirine, *New J. Chem.* **29**, 1450-1453 (2005). d) Wiberg, K.B.; Rush, D.J. Solvent effects on the thioamide rotational barrier: An experimental and theoretical study, *J. Am. Chem. Soc.* **123**, 2038-2046 (2001). e) Wiberg, K.B. The interaction of carbonyl groups with substituents, *Acc. Chem. Res.* **32**, 922-929 (1999). f) Laidig, K.E.; Cameron, L.M. Barrier to rotation in thioformamide: Implications for amide resonance, *J. Am.*

*Chem. Soc.* **118**, 1737-1742 (1996). g) Wiberg, K.B.; Rablen, P.R.; Rush, D.J.; Keith, T.A. Amides. 3. Experimental and theoretical studies of the effect of the medium on the rotational barriers for *N,N*-dimethylformamide and *N,N*-dimethylacetamide, *J. Am. Chem. Soc.* **117**, 4261-4270 (1995). h) Wiberg, K.B.; Rablen, P.R. Why does thioformamide have a larger rotational barrier than formamide?, *J. Am. Chem. Soc.* **117**, 2201-2209 (1995). i) Glendening, E.D.; Hrabal II, J.A., Resonance in formamide and its chalcogen replacement analogues: A natural population analysis/natural resonance theory viewpoint, *J. Am. Chem. Soc.* **119**, 12940-12946 (1997). j) Lauvergnat, D.; Hiberty, P.C. Role of conjugation in the stabilities and rotational barriers of formamide and thioformamide. An *ab initio* valence-bond study, *J. Am. Chem. Soc.* **119**, 9478-9482 (1997).

10. a) Spáčilová, L. *et al* Synthesis and cytotoxic activity of various 5-[alkoxy-(4-nitro-phenyl)methyl]uracils in their racemic form, *Bioorg. Med. Chem. Lett.* **17**, 6647-6650 (2007). b) Longley, D.B.; Harkin, D.P.; Johnston, P.G. 5-Fluorouracil: Mechanisms of action and clinical strategies, *Nat. Rev. Cancer* **3**, 330-338 (2003). c) Safarjalani, O.N.A. *et al* 5-(Phenylthio)acyclouridine: A powerful enhancer of oral uridine bioavailability: Relevance to chemotherapy with 5-fluorouracil and other uridine rescue regimens, *Cancer Chemother. Phamacol.* **55**, 541-551 (2005). d) Kennedy, B.J.; Torkelson, J.L.; Torlakovic, E. Uracil mustard revisited, *Cancer* **85**, 2265-2272 (1999).
11. a) Thakur, A.J.; Saikia, P.; Prajapati, D.; Sandhu, J.S. Studies on 6-[(dimethylamino)methylene]aminouracils: A facile one-pot synthesis of pyrimido[4,5-*d*]pyrimidines and pyrido[2,3-*d*]pyrimidines, *Synlett*, **8**, 1299-1301 (2001). b) Prajapati, D.; Gohain, M.; Thakur, A.J. Regiospecific one-pot synthesis of pyrimido[4,5-*d*]pyrimidine derivatives in the solid state under microwave irradiations, *Bioorg. Med. Chem. Lett.* **16**, 3537-3540 (2006). c) Thakur, A.J.; Das, S.; Phukan, A.K. Replay of amide type resonance in 6-[(dimethylamino) - methylene]-1,3-dimethylaminouracil: A dynamic NMR and density functional theory study, *J. Mol. Struc.* **929**, 134-140 (2009).

12. Das, S.; Saikia, B.K.; Sridhar, B.; Thakur, A.J. 6-[(Dimethylamino) - methyleneamino]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione dihydrate, *Acta Cryst.* **E64**, o1662 (2008).
13. Sheldrick, G.M. A short history of SHELX, *Acta. Cryst.* **A64**, 112-122 (2008).
14. a) B3LYP is Becke's three parameter hybrid method using the LYP correlation functional. See: Becke, A.D. Density-functional thermochemistry. III. The role of exact exchange, *J. Chem. Phys.* **98**, 5648-5652 (1993). b) Lee, C.; Yang, W.; Parr, R.G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, *Phys. Rev. B* **37**, 785-789 (1988). c) Vosko, S.H.; Wilk, L.; Nusair, M. Accurate spin-dependent electron liquid correlation energies for local spin density calculations: a critical analysis, *Can. J. Phys.* **58,** 1200-1211 (1980).
15. a) Frisch, M.J. *et al* Gaussian 03, Revision D.01, Gaussian Inc., Wallingford CT, 2004. b) Reed, A.E.; Weinstock, R.B.; Weinhold, F. Natural population analysis, *J. Chem. Phys.* **83**, 735-746 (1985). c) Reed, A.E.; Weinhold, F. Natural localized molecular orbitals, *J. Chem. Phys.* **83**, 1736-1740 (1985). d) Reed, A.E.; Weinhold, F.; Curtiss, L.A. Intermolecular interactions from a natural bond orbital, donor-acceptor viewpoint, *Chem. Rev.* **88**, 899-926 (1988).
16. a) Gonzalez, C.; Schlegel, B.H. An improved algorithm for reaction path following, *J. Chem. Phys.* **90**, 2154-2161 (1989). b) Gonzalez, C.; Schlegel, B.H. Reaction path following in mass-weighted internal coordinates, *J. Phys. Chem.* **94**, 5523-5527 (1990).
17. Papesch, V.; Schroeder, E.F. Synthesis of 1-mono and 1,3-disubstituted 6-aminouracils. Diuretic activity, *J. Org. Chem.* **16**, 1879-1890 (1951).
18. Blicke, F.F.; Godt, H.C. Reactions of 1,3-dimethyl-5,6-diaminouracil, *J. Am. Chem. Soc.* **76**, 2798-2800 (1954).
19. Das, S. *N,N'*-Dimethyl Urea (DMU), *Synlett spotlight*, **7**, 1138-1139 (2010).
20. Allen, F.H. *et al* Tables of bond lengths determined by X-ray and neutron diffraction. Part 1. Bond lengths in organic compounds, *J. Chem. Soc. Perkin Trans.* **2**, S1-S19 (1987).

21. Sandström, J. *Dynamic NMR Spectroscopy* (Academic Press, New York, 81-84, 96, 1982).
22. Muertterties, E.L.; Jesson, J.P. in *Dynamic Nuclear Magnetic Resonance Spectroscopy*, Ed: Jackman, M.; Cotton F.A. (Academic Press, New York, 253, 1975).
23. a) Korn, A.; Rudolph-Bohner, S.; Moroder, L. On the synthesis of (2*S*)-aziridine-2-carboxylic acid containing peptides, *Tetrahedron* **50**, 1717-1730 (1994). b) Neuman, R.C.; Jonas, V. Studies of chemical exchange by nuclear magnetic resonance. IX. Rotation about the amide bond in *N,N*-dimethylformamide, *J. Org. Chem.* **39**, 925-928 (1974).
24. Gutowsky, H.S.; Holm, C.H. Rate processes and nuclear magnetic resonance spectra. II. hindered internal rotation of amides, *J. Chem. Phys.* **25**, 1228-1234 (1956).


CHAPTER 4

No one can deny the role and versatility of Diels - Alder reaction (DAR) in organic synthesis. The corresponding aza version of DAR, i.e. aza-Diels-Alder reaction (aza-DAR) is another most well-studied, synthetically useful organic transformation and provides a powerful methodology for the construction of nitrogen-containing six-membered ring compounds in a single step, which constitute a broad spectrum of natural products; alkaloids, pharmaceutically, biologically active compounds and polymeric materials.[1] It is also one of the most synthetically useful methods with high regio- and stereo-selectivity of the products under mild conditions.[2] Not only chemical synthesis, and total synthesis, but also biosynthesis of many natural products were reported via aza-DAR.[3] The inverse electron demand aza-DAR has increased it's dimension one step forward.

There are already compelling evidences that aza-dienes have low reactivity in normal DAR, that evidence was based on calculated energies of activation ($E_a$). Experimental ionization potentials, electron affinities and theoretical energies of HOMO and LUMO orbitals' for a number of aza-dienes have been reported.[4] This is one of the reasons of low reactivity of aza-dienes, that presence of nitrogen atom of the diene creates a $\pi$-electron-deficient system and thus lowers its reactivity in the normal $HOMO_{diene}$ controlled DARs with electron poor dienophiles.

Here, we have considered aza-DAR by choosing the diene 6-[(dimethylamino) methylene]-1,3-dimethylaminouracil (**4**), which is a very interesting pyrimidine derivative with an azadiene unit residing partly in the ring and partly in the exocyclic part. It was postulated that the dienophilic nature of the pyrimidine ring was rather limited, and the diene properties of vinylpyrimidines has not yet been established. It happened only if a vinylpyrimidine system was appropriately substituted with strong electron-donating groups, cycloaddition might occur with electron-deficient dienophiles. In our previous reports,[5] we described aza-DAR by exploiting the reaction of the diene, 6-[(dimethylamino)methylene]-1,3-dimethylaminouracil (**4**) with various dienophiles.

# Section A

## An efficient synthesis of pyrido[2,3-*d*]pyrimidine-2,4-dione derivatives by a one-pot, multi-component reaction and their biological assay

**Introduction:**

The multi-component reaction (MCR) has drawn much attention these days as one of the most powerful emerging synthetic tools to synthesise biologically active compounds and have become an important area of research in organic, combinatorial, and medicinal chemistry.[6] They also have significant advantages in terms of user and ecofriendliness, because of step reduction and atom economy associated to their use.[7] Here, we report the synthesis of fused uracil derivatives by using MCR strategy.

The importance of uracil and its derivatives has already been described in **Chapter-2** (references 12-24, page-104-107). By using uracil and their derivatives many pyrido[2,3-*d*]pyrimidine derivatives have been synthesized and those synthesized compounds have shown wide spectrum of bioactivities such as anticancer,[8] antiviral,[9] antimicrobial,[10] antioxidant,[11] antitumor,[12] antileishmanial agent,[13] antiinflammatory,[14] and potent phosphodiesterase 4 inhibitors.[15] Recently, pyrido[2,3-*d*]pyrimidine analogues of folic acid have been screened for adenosine receptor antagonists.[16] In view of the biological significances of pyrimidine compounds, we have taken up the synthesis of such pyrimidine derivatives.[17]

**Materials and Methods:**

Melting points were determined with a Büchi 504 apparatus. IR spectra were recorded as KBr pallets with a Nicolet (Impact 410) FT-IR spectrophotometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded with a JNM ECS 400 MHz NMR spectrophotometer (JEOL) using tetramethylsilane (TMS) as the internal standard. Reactions were monitored by thin-layer chromatography using aluminium sheets with silica gel $60F_{254}$ (Merck). Elemental analyses were carried out with a Perkin–Elmer CHN analyser (2400 series II). Mass

spectra were recorded with a Waters Q-TOF Premier and Aquity UPLC spectrometer. All the chemicals were used as received.

**Compounds:**

All the tested compounds were synthesized by our general reaction procedure. The structures of the compounds were confirmed on the basis of IR, $^{1}H$ and $^{13}C$ NMR spectroscopy, mass spectrometry and elemental analyses.

**Chemicals:**

All the chemicals used including the solvents, were of analytical grade. Bacterial strains were kindly provided by Dr. Tapash Medhi, Department of Molecular Biology & Biotechnology, Tezpur University, Assam, India.

**Preparation of standards for bioactivity tests:**

Each sample was dissolved in absolute ethanol (analytical grade) and stock solutions of 1mg/ml were prepared for the experiment. Concentrations of 25mg/mL, 50mg/mL, 100mg/mL, 150mg/mL, 200mg/mL, 250mg/mL and 500g/mL of uracil derivatives in sterilized DMSO were prepared. Sterilized DMSO without the test compound as negative control, another with gentamicin (1mg/mL) as positive control for the bacteria and for fungi clotrimazole (1mg/mL) were used to serve as positive control.

**Antibacterial assay procedure:**

The agar well diffusion technique was used in the present investigation, following the procedure described by Boakye-Yiadom,[18] Banso and Adeyemo,[19] and Radhika *et al*[20] Five (5) wells, 8mm each were made on solidified nutrient agar and Sabouraud Dextrose Agar (SDA) media plates, respectively with the help of a sterile cork borer. 200$\mu$l of the log phase culture of the test microbes which includes *S. aureus* (ATCC 11774) and *E. coli* (ATCC 9637) were seeded on the surface of the nutrient agar medium, using swab stick. The cut agar discs were removed with the aid of sterile forceps. Concentrations of 25mg/mL, 50mg/mL, 100mg/mL, 150mg/mL, 200mg/mL, 250mg/mL and 500mg/mL of

the compound in the sterilized DMSO were separately introduced into separate wells. Two (2) control holes were set up, one filled with sterilized DMSO without the test compound as negative control, another with gentamicin as positive control for the bacteria, clotrimazole was used to serve as positive control in case of fungi. The plates were incubated at 37 °C for 24 h and 15 days at 27 °C respectively for the bacterial cultures. The observed zones of inhibition were measured using transparent metric ruler.

**MIC assay procedure:**

Same as described in **Chapter 2** (Section A, Page No. 55).

**MTT assay for cytotoxicity tests:**

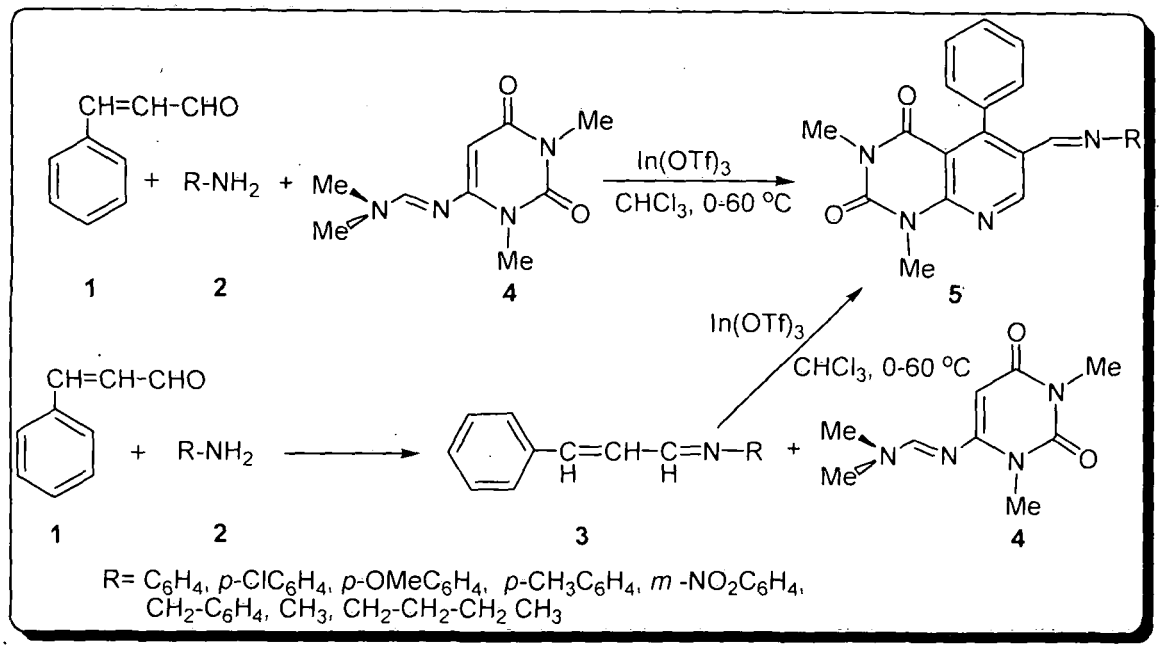
To test the cytotoxicity of the pyrido[2,3-*d*]pyrimidine derivatives, MTT assay was done in lymphocyte cell culture. The principle of this cytotoxicity test is that the yellow MTT (3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. Therefore the number of surviving cells can be determined indirectly by MTT reduction.

**CYP450 induction study:**

The *in vitro* CYP450 induction study was carried out in cells isolated from Goat liver. Isolated hepatocytes were tested for P450 induction using pyrido[2,3-*d*]pyrimidine derivatives having low MIC value and Rifampicin as a control. Finally, the samples were spectrophotometrically screened for the presence of CYP450 between 400 to 500 nm.

**Results and Discussion:**

Pyrido[2,3-*d*]pyrimidine derivatives (**5**) were synthesized by using MCR of 6-[(dimethylamino)methylene]amino-1,3-dimethyluracil (**4**), cinnamaldehyde (**1**) and amino moieties (**2**) (both aromatic and aliphatic) at 0-60 °C in the presence of $In(OTf)_3$ (10 mol%) as a catalyst by simple stirring in dry $CHCl_3$ (**Scheme 1**).




**Scheme 1**: Synthesis of pyrido[2,3-*d*]pyrimidine (**5**)

To the best of our knowledge, there are very few examples in which the use of (**4**) as aza-diene have been described.[21] In 2000, Yoon *et al* reported the aza-DAR of 5-iodo-6-(dimethylaminomethylene)amino-1,3-dimethyluracil with acetylenes in presence of palladium-catalyst resulting the pyrido[2,3-*d*]pyrimidines.[22] Pyrazolyl-2-azadiene having similar aza-diene unit like 6-[(dimethylamino)methylene]amino-1,3-dimethyluracil, reacted with nitroalkenes to form aza-DA products.[23]

The reaction might proceed in two steps, firstly formation of conjugated Schiff base (**3**) by reaction between cinnamaldehyde (**1**) and amino moieties (**2**), then secondly the reaction between 6-[(dimethylamino)methylene]amino uracil (**4**) and conjugated Schiff base (**3**) to form aza-DA product pyrido[2,3-*d*]pyrimidines (**5**). The structure of product (**5**) as pyrido[2,3-*d*]pyrimidine derivatives were confirmed on the basis of NMR ($^1$H and $^{13}$C) spectroscopy, FT-IR spectroscopy, mass spectrometry and elemental analyses.

In the $^1$H NMR spectuum of (**5a,** R= Ph), peaks at $\delta$ = 3.21 (3H) and 3.83 (3H) ppm are due to the two *N*-methyl groups, the peaks within $\delta$ = 6.68–7.85 ppm are due to ten aromatic protons and the peak at $\delta$ = 8.01 & 9.42 ppm are due to the imine proton.

**Table 1:** Synthesis of pyrido[2,3-*d*]pyrimidine derivatives (**5**)

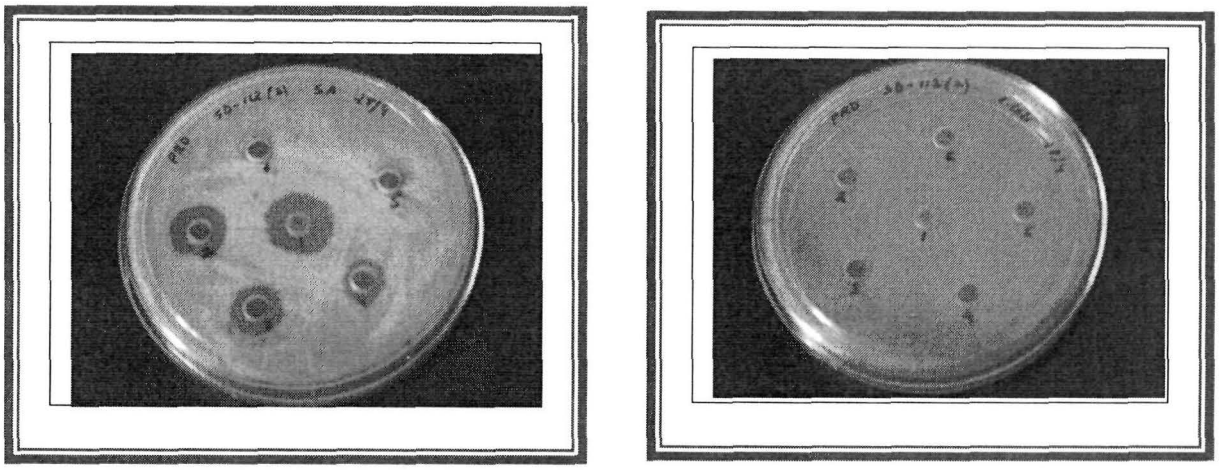
| Entry | Amino Compounds **2** | Time/h | m.p. (°C) | Yield (%)[a] |
|---|---|---|---|---|
| a | $C_6H_4NH_2$ | 8 | 243-244 | 71 |
| b | *p*-Cl-$C_6H_4NH_2$ | 8 | 243-245.3 | 73 |
| c | *p*-$OCH_3$-$C_6H_4NH_2$ | 9 | 265-267 | 67 |
| d | *p*-$CH_3$-$C_6H_4NH_2$ | 8 | 199-200 | 68 |
| e | *m*-$NO_2$-$C_6H_4NH_2$ | 8 | 140-145 | 63 |
| f | $CH_3NH_2$ | 9 | 182.7-185.8 | 66 |
| g | $CH_2$-$C_6H_4NH_2$ | 9 | 160.9-165 | 73 |
| h | $CH_2$-$CH_2$-$CH_2$-$CH_3NH_2$ | 9 | 144.5-146.1 | 61 |

[a] Isolated yield

Encouraged by this result, to study the scope and limitations of the reaction further, we extended the reaction to other differently substituted aromatic and aliphatic amines (**2a–h**) under the same optimized reaction conditions. The results are summarized in **Table 1** (entries **a–h**). All the amines (entries **a–h**, **Table 1**) reacted with equal ease within short times to furnish the pyrido[2,3-*d*]pyrimidines (**5a–h**) in good yields (61–73%).

**Bioactivity tests:**

The pyrido[2,3-*d*]pyrimidine derivatives (**5a-h**) were sereened against two non-pathogenic bacterial strains viz. *S. aureus* (gram +ve) and *E. coli* (gram -ve). Compounds possess inhibitory activities against *S. aureus* bacteria, whereas no inhibitory activity was found against *E. coli* bacteria. The main reason for this may be the difference in the cell wall structure of the gram +ve and gram -ve bacteria. The zone of inhibition for the compound (**5c**) is shown in **Fig. 1**. Further, the MIC values of the pyrido[2,3-*d*]pyrimidine derivatives (**5a-h**) were calculated by using MTT assay at different concentrations. The detail result of antibacterial activity tests and MIC values of pyrido[2,3-*d*]pyrimidine derivatives (**5a-h**) are shown in **Table 2** and **Table 3** respectively.



*S. aureus* (gram +ve) *E. coli* (gram –ve)

**Fig. 1:** Zone of inhibition of compound **(5c)**

**Table 2:** Result of antibacterial activity tests of pyrido[2,3-*d*]pyrimidine derivatives **(5)**

| Entry | R | Solubility | Antimicrobial activity against | |
|---|---|---|---|---|
| | | | *S. aureus* | *E. coli* |
| a | $C_6H_4$ | DMSO | ++ | - |
| b | *p*-Cl-$C_6H_4$ | DMSO | + | - |
| c | *p*-$OCH_3$-$C_6H_4$ | DMSO | +++ | - |
| d | *p*-$CH_3$-$C_6H_4$ | DMSO | ++ | - |
| e | *m*-$NO_2$-$C_6H_4$ | DMSO | + | - |
| f | $CH_3$ | DMSO | ++ | - |
| g | $CH_2$-$C_6H_4$ | DMSO | ++ | - |
| h | $CH_2$-$CH_2$-$CH_2$-$CH_3$ | DMSO | ++ | - |

Sign: - (Inactive); + (Poor); ++ (Good); +++ (Very good)

**Table 3:** MIC values of pyrido[2,3-*d*]pyrimidine derivatives (**5**)

| Conc. (mg/ml) | Entry 5a | Entry 5b | Entry 5c | Entry 5d | Entry 5e | Entry 5f | Entry 5g | Entry 5h |
|---|---|---|---|---|---|---|---|---|
| 1 | 12 | 12 | 14 | 12 | 5 | 6 | 5 | 6 |
| 0.5 | 8 | 8 | 12 | 8 | 2 | 5 | 4 | 4 |
| 0.2 | 5 | 2 | 10 | 4 | - | 3 | 3 | 2 |
| 0.1 | 3 | - | 8 | 2 | - | 2 | 2 | - |
| 0.05 | 1 | - | 5 | 1 | - | 1 | 1 | - |
| 0.025 | - | - | 1 | - | - | - | - | - |

The cell viability for different concentration of pyrido[2,3-*d*]pyrimidine derivatives (**5a-h)** showed that the cell viability was maximum at lowest concentration (10 μg/ml) and on increasing the concentration the cell viability decreased. The detail results for the compound (**5c**) are shown graphically in **Fig. 2**.

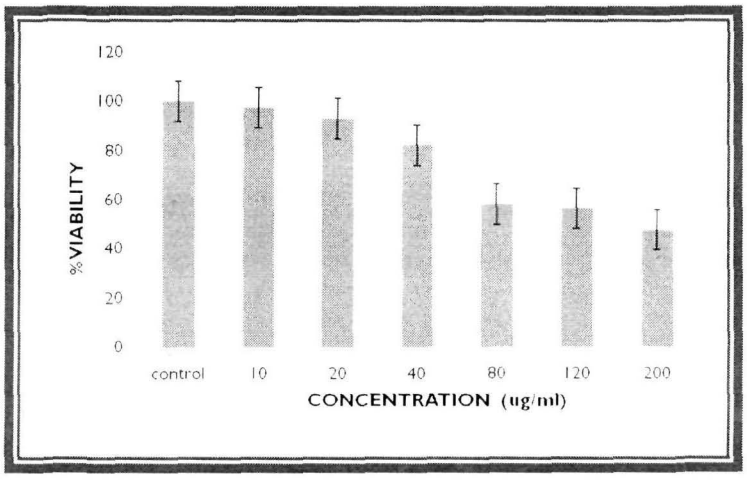



**Fig. 2:** The percentage of cell viability for (**5c)**

We also studied the induction of cytochrome P450 (CYP450) by the *p*-methoxy substituted pyrido[2,3-*d*]pyrimidine derivatives (**5c)**, which has the lowest MIC value (0.025 μg/ml) and the CYP450 exhibited maximum absorbance at wavelength 420 nm rather than 450 nm. This may be due to the denaturation of CYP450 during its isolation

from the hepatocytes and also due to the low viability (20%-30%) of the cells compared to the required viability of 85%. The results are shown in **Fig. 3** and **Fig. 4.**

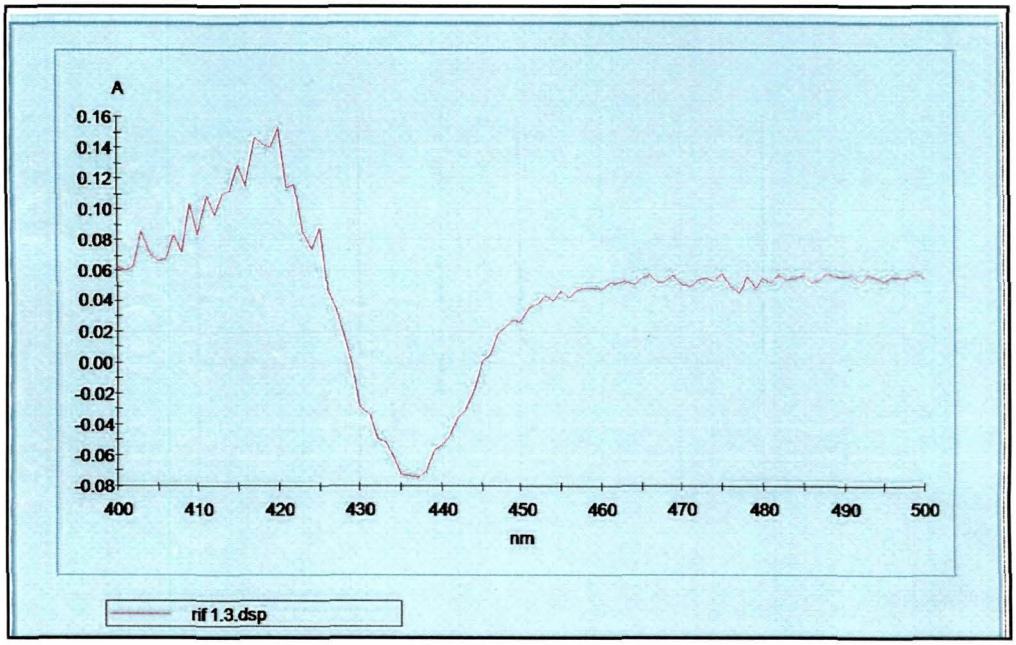



**Fig. 3:** CO diffference spectra of rifampicin

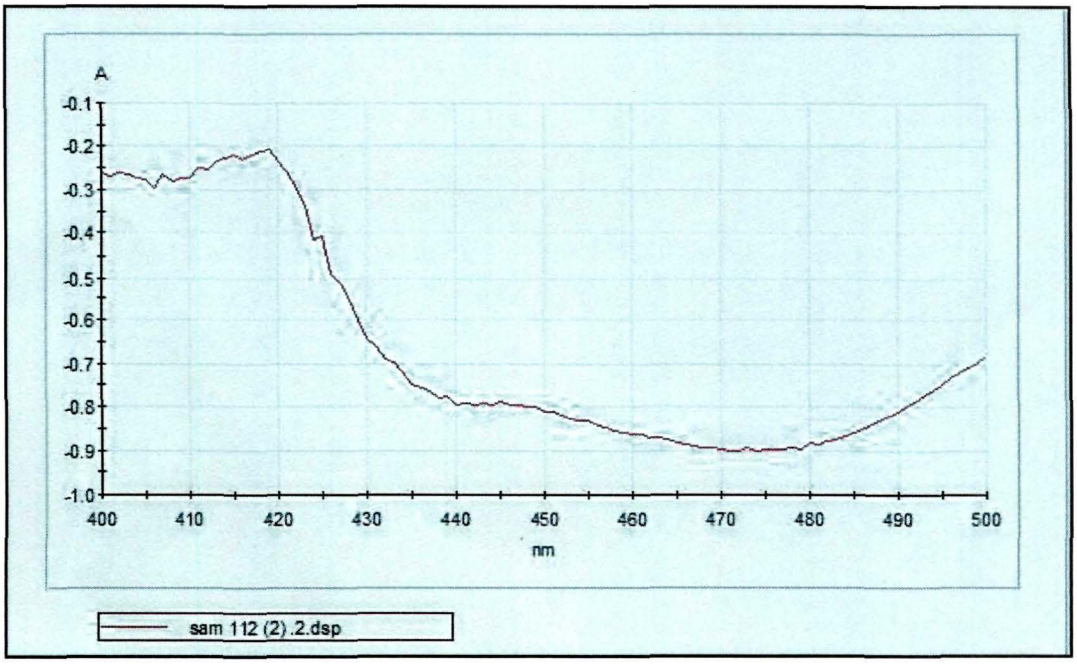



**Fig. 4:** CO difference spectra of **(5c)**

**Conclusion:**

Multi component reaction strategy utilising aza-DA methodology has been used successfully for the construction of fused heterocyclic systems like pyrido[2,3-*d*]pyrimidine-2,4-dione derivatives.

Anti-bacterial activity of 1,3-dimethyl-5-phenylpyrido[2,3-*d*]pyrimidine-2,4-dione derivatives, cell-viability test of 1,3-dimethyl-5-phenylpyrido [2,3-*d*] pyrimidine-2,4-dione derivatives were done and all the compounds showed anti-microbial properties. Cytotoxicity test of 1,3-dimethyl-5-phenylpyrido[2,3-*d*]pyrimidine-2,4-dione derivatives exhibit variability in survival percentage with different loading concentration and methoxy-substituted-pyrido[2,3-*d*]pyrimidine derimatives showed potency to induce cytochrome P450.

**Experimental:**

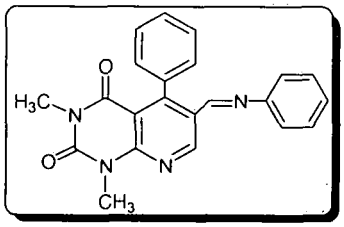
**Typical reaction procedure for the one-pot synthesis of pyrido[2,3-*d*]pyrimidine derivatives (5)**

Cinnamaldehyde (**1**, 0.13 ml, 1 mmol), aniline (**2a**, 0.091 ml, 1 mmol) and 6-[(dimethylamino)methylene]1,3-dimethylaminouracil (**4**, 209 mg, 1 mmol) were mixed in dry chloroform (20 mL) taken in a 50 mL round-bottomed flask and the mixture was stirred at room temperature for 10 min. Cooled the reaction mixture to 0 °C using ice bath, then added the $In(OTf)_3$ (5.6 mg, 10% of 1 mmol) with constant stirring. Gradually, the reaction mixture was allowed to come to room temperature. Then we refluxed the reaction mixture at 60 °C with constant stirring and after 6 h, we got the pure product (**5a**) in 71% yield. The product was separated through TLC, dissolved in distilled ethanol (98%) and then warmed, filtered, allowed to cool and evaporate the filtrate at room temperature, when square shaped yellow shining transparent crystals came out. The crystals were collected through filtration and dried; m.p. 243-244 °C.

The same procedure was followed for the other substrates.

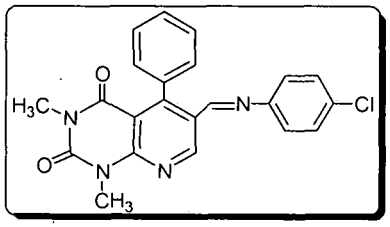
**Physical and spectral data:**

**1,3-dimethyl-5-phenyl-6-((phenylimino)methyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5a)**



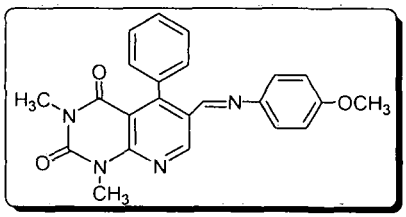
Colour- brown shining transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. m.p. 243-244 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3034.17, 2927.33, 1685.91, 1626.87, 1567.91, 1493.81; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.35 (s, 3H, $NCH_3$), 3.83 (s, 3H, $NCH_3$), 6.68-7.85 (m, 10H, arom), 8.01 (s, H, CH=N), 9.52 (s, H, CH=N) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.8, 29.1, 125.7, 126.6, 127.9, 128.7, 129.4, 129.9, 130.1, 132.5, 138.1, 149.1, 150.9, 154.4, 158.5, 164.8 ppm. MS, *m/z* 370 ($M^+$); Anal. Calcd (%) for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found C, 71.31; H, 4.95; N, 15.10.

**6-((*p*-chlorophenylimino)methyl)-1,3-dimethyl-5-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5b)**



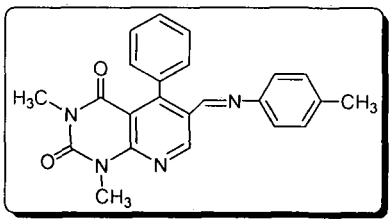
Colour- yellow shining transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. m.p. 243-245 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3034.21, 2926.31, 1696.19, 1627.17, 1589.19, 1493.10; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.37 (s, 3H, $NCH_3$), 3.44 (s, 3H, $NCH_3$), 6.89-7.58 (m, 9H, arom), 8.11 (s, H, CH=N), 9.25 (s, H, CH=N) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.6, 29.4, 124.8, 125.7, 127.8, 128.4, 129.4, 129.9, 130.4, 132.7, 138.1, 149.2, 151.0, 154.3, 158.5, 164.3 ppm. MS, *m/z* 405 ($M^+$); Anal. Calcd (%) for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.23; N, 13.84. Found C, 65.31; H, 4.26; N, 13.81.

**6-((*p*-methoxyphenylimino)methyl)-1,3-dimethyl-5-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5c)**



Colour- yellow shining transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. m.p. 265-267 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3032.11, 2927.31, 1697.61, 1636.17, 1583.91, 1497.88; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.36 (s, 3H, $NCH_3$), 3.45 (s, 3H, $NCH_3$), 3.91 (s, 3H, $OCH_3$), 7.12-7.87 (m, 9H, arom), 8.15 (s, H, CH=N), 9.15 (s, H, CH=N) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.6, 29.4, 53.6, 124.8, 115.8, 125.5, 128.5, 129.2, 129.2, 130.1, 132.7, 138.1, 148.9, 151.3, 153.8, 158.5, 163.2 ppm. MS, *m/z* 401 ($M^+$); Anal. Calcd (%) for $C_{23}H_{20}N_4O_3$: C, 68.99; H, 5.03; N, 13.99. Found C, 68.96; H, 5.06; N, 13.95.

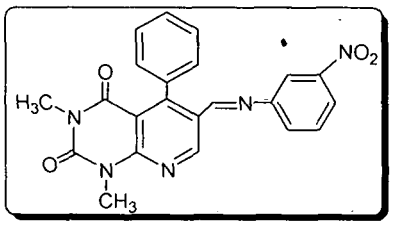
**6-((*p*-tolylimino)methyl)-1,3-dimethyl-5-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5d)**



Colour- brown shining transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. m.p. 199-200 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3033.10, 2927.13, 1695.96, 1626.70, 1587.29, 1493.08; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.13 (s, 3H, $CH_3$), 3.36 (s, 3H, $NCH_3$), 3.44 (s, 3H, $NCH_3$), 7.11-7.77 (m, 9H, arom), 8.10 (s, H, CH=N), 9.20 (s, H, CH=N) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 20.9, 30.6, 36.5, 123.6, 127.1, 128.3, 128.9, 129.3, 130.2, 131.0, 135.1, 136.2, 139.5, 142.0, 149.2, 153.1, 158.4, 163.2 ppm. MS, *m/z* 384 ($M^+$); Anal. Calcd (%) for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found C, 71.86; H, 5.26; N, 14.53.

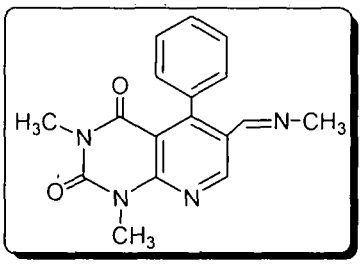
**6-((*m*-nitrophenylimino)methyl)-1,3-dimethyl-5-phenylpyrido[2,3-*d*]-pyrimidine-2,4-(1*H*,3*H*)-dione (5e)**

Colour- brown shining transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. m.p. 140-145 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3036.51, 2924.33, 1696.19, 1625.73, 1589.31, 1491.81; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.35 (s, 3H, $NCH_3$),



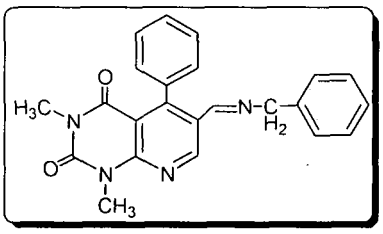
3.47 (s, 3H, $NCH_3$), 7.07-7.90 (m, 9H, arom), 8.09 (s, H, CH=N), 8.99 (s, H, CH=N) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 29.9, 34.5, 119.2, 123.6, 127.1, 128.2, 128.9, 129.0, 129.2, 130.4, 131.3, 136.2, 149.7, 154.4, 158.7, 164.3 ppm. MS, *m/z* 415 ($M^+$); Anal. Calcd (%) for $C_{22}H_{17}N_5O_4$: C, 63.61; H, 4.12; N, 16.86. Found C, 63.59; H, 4.16; N, 16.81.

**1,3-dimethyl-6-((methylimino)methyl)-5-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5f)**



Colour- brown shining transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. m.p 182.7-185.8 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3033.32, 2927.31, 1696.19, 1627.17, 1586.12, 1495.18; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.13 (s, 3H, $CH_3$), 3.33 (s, 3H, $NCH_3$), 3.35 (s, 3H, $NCH_3$), 7.23-7.58 (m, 5H, arom), 8.10 (s, H, CH=N), 9.18 (s, H, CH=N) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 20.7, 30.1, 33.5, 127.7, 128.0, 128.6, 128.6, 128.6, 143.5, 148.8, 154.3, 158.3, 163.2 ppm. MS, *m/z* 308 ($M^+$); Anal. Calcd (%) for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17. Found C, 66.23; H, 5.26; N, 18.15.

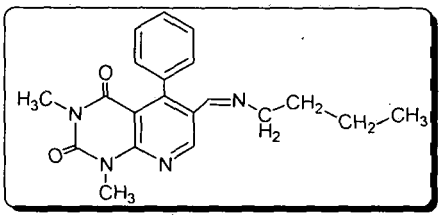
**6-((benzylimino)methyl)-1,3-dimethyl-5-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5g)**



Colour- brown shining transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. m.p. 160.9-165 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3033.21, 2923.31, 1694.19, 1625.27, 1588.12, 1493.18; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.33 (s, 3H, $NCH_3$), 3.75 (s, 3H, $NCH_3$), 4.15 (s, 2H, $CH_2$), 7.15-7.55 (m, 10H, arom), 8.11 (s, H, CH=N), 9.25 (s, H, CH=N) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 31.6, 36.5, 63.8, 127.3, 128.3, 128.7, 129.0, 129.2, 129.7, 129.9, 130.1, 135.0, 137.1, 140.2, 142.1, 146.7, 158.7, 163.2 ppm.

MS, *m/z* 384 ($M^+$); Anal. Calcd (%) for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found C, 71.83; H, 5.26; N, 14.56.

**6-((*Z*)-(butylimino)methyl)-1,3-dimethyl-5-phenylpyrido[2,3-*d*]pyrimidine-2,4 - (1*H*,3*H*)-dione (5h)**



Colour- brown shining transparent crystalline solid. Solubility- insoluble in water, soluble in common organic solvents. m.p. 144.5-146.1 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3032.91, 2928.01, 1696.15, 1626.70, 1586.42, 1493.45; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 0.84-0.88 (m, 3H, $CH_3$), 1.26-1.30 (m, 4H, $CH_2$-$CH_2$), 2.05-2.21 (m, 2H, $CH_2$), 3.33 (s, 3H, $NCH_3$), 3.37 (s, 3H, $NCH_3$), 7.18-7.54 (m, 5H, arom), 8.11 (s, H, CH=N), 9.24 (s, H, CH=N) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 13.3, 21.8, 27.4, 27.9, 31.6, 35.5, 127.3, 128.3, 128.7, 129.0, 129.3. 137.1, 140.2, 142.1, 148.7, 158.7, 163.2 ppm. MS, *m/z* 350 ($M^+$); Anal. Calcd (%) for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.33; N, 15.99. Found C, 68.53; H, 6.36; N, 15.97.

# Section B

## Hetero-Diels-Alder methodology for the synthesis of novel bispyrimido-[4,5-*d*]pyrimidine derivatives and their biological activities

**Introduction:**

Pyrimido[4,5-*d*]pyrimidine derivatives are one of the major classes of fused uracil derivatives and have shown bioactivities such as antibacterial[24] and antifugal.[25] Recently, pyrimido[4,5-*d*]pyrimidine derivatives have shown good potentiality in *in vitro* HBV DNA replication inhibition and as nucleoside transport inhibitor.[26] In view of the biological significances of pyrimido[4,5-*d*]pyrimidine and other pyrimidine compounds (references 24, **Chapter-2**, page-107), we have been focussing on design and synthesis of such pyrimidine derivatives.

**Materials and Methods:**

Melting points were determined with a Büchi 504 apparatus. IR spectra were recorded as KBr pallets with a Nicolet (Impact 410) FT-IR spectrophotometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded with a JNM ECS 400 MHz NMR spectrophotometer (JEOL) using tetramethylsilane (TMS) as the internal standard. X-ray intensity data were collected with a Bruker SMART APEX CCD area-detector diffractometer with Mo-$K\alpha$ radiation ($\lambda$ = 0.71073 Å). The structures were solved by SHELX97 and refined by full-matrix least-squares on $F^2$ (SHELX97). Reactions were monitored by thin-layer chromatography using aluminium sheets with silica gel $60F_{254}$ (Merck). Elemental analyses were carried out with a Perkin–Elmer CHN analyser (2400 series II). Mass spectra were recorded with a Waters Q-TOF Premier and Aquity UPLC spectrometer. All the chemicals were used as received.

**Compounds:**

All the tested compounds were synthesized by our general reaction procedure. The structures of the products were confirmed on the basis of IR, $^{1}H$ and $^{13}C$ NMR spectroscopy, mass spectrometry, elemental analyses and single crystal X-ray analysis.

**Chemicals:**

All the chemicals used including the solvents, were of analytical grade. Bacterial strains were kindly provided by Dr. Tapash Medhi, Department of Molecular Biology & Biotechnology, Tezpur University, Assam, India and fungal strains were supplied by Department of Plant Pathology, Assam Agriculture University, Assam, India.

**Preparation of standards for bioactivity tests:**

Same as described in **Chapter-4** (Section-A, Page No. 137).

**Antibacterial and antifungal assay procedure:**

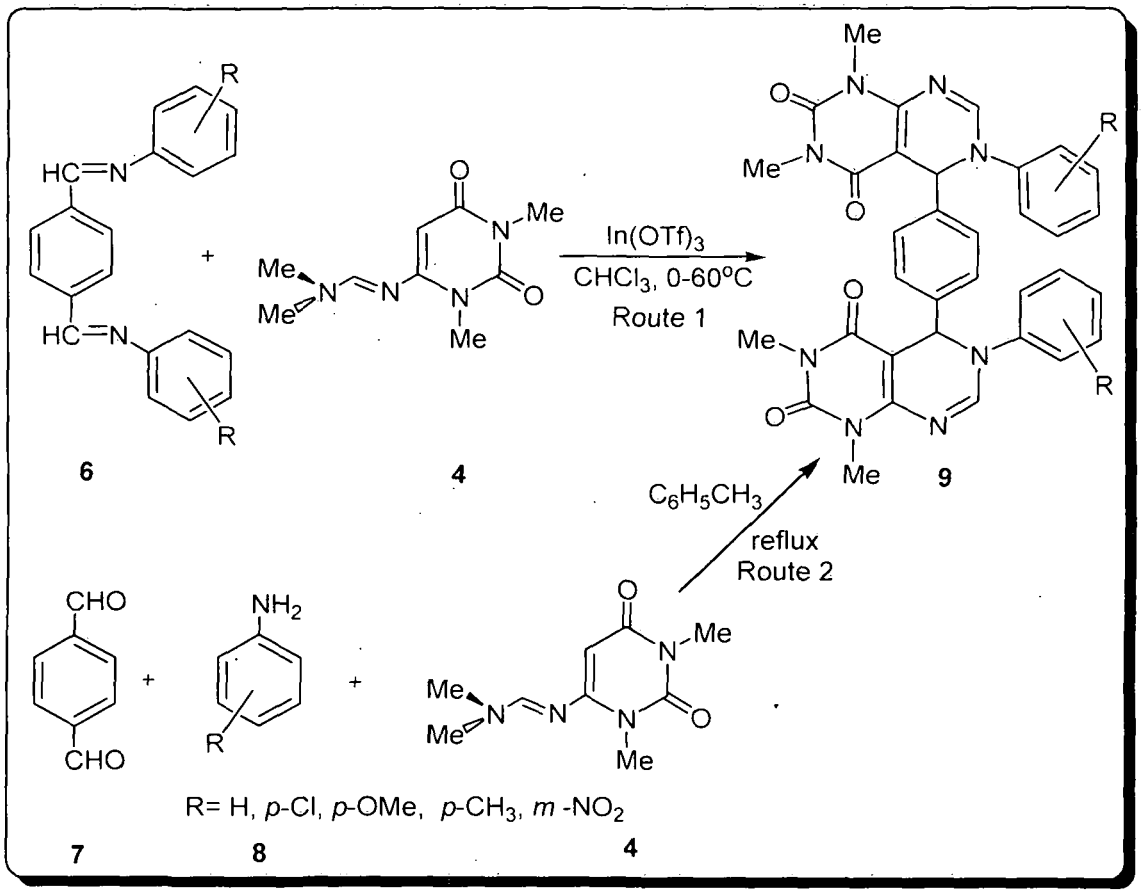
Same as described in **Chapter-4** (Section-A, Page No. 137).

**MIC assay procedure:**

Same as described in **Chapter-2** (Section-A, Page No. 55).

**Results and Discussion:**

In this section, we have reported the synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**9**, **13** & **16**) by the reaction between the amidine (**4**) and bis-imines (**6, 10** & **14**) (**Schemes 2**, **3** and **4**) respectively. For our convenience and as per demand of the situation, we have developed a new synthetic methodology for the synthesis of symmetrical bis-imines under microwave irradiation without using any solvent in presence of *p*-TSA as catalyst (**chapter 5**). The symmetrical bis-imines so synthesized will be used here.




**Scheme 2:** Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**9**)

Treatment of 2 equivalent of (**4**), with 1 equivalent of bis-imines (**6**) in presence of $In(OTf)_3$ (10 mol %) as a catalyst by simple stirring in dry $CHCl_3$ for 8-12 h afforded, after elimination of dimethylamine from the 1:1 cycloadduct, the corresponding bis-pyrimido[4,5-*d*]pyrimidine derivatives (**9**) as a major product in 55-85% yields (**Scheme 2**). Bis-imines (**6a-e**) were obtained from the reaction between aromatic dialdehyde (**7**) and aromatic amines (**8**).

For generalization of our work, we have synthesised three different types of bispyrimido[4,5-*d*]pyrimidine derivatives (**9**, **13** & **16**) by changing the bis-imine derivatives keeping the amidine (**4**) fixed. The results are summarised in **Table 4**. As a model reaction, firstly we carried out the reaction between amidine (**4**) and the imine *N*-(phenylimino)methyl)benzylidene)benzenamine (**6a**) (**Scheme 2**). The product (**9a**) was obtained in 91% yield (reaction time, 11 h).

**Table 4:** Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**9**)

| Entry | Amino Compounds **2** | Time/h | m.p. (°C) | Yield (%)[a] |
|---|---|---|---|---|
| a | $C_6H_4NH_2$ | 11 | 360 | 91 |
| b | *p*-Cl-$C_6H_4NH_2$ | 10 | 365 | 97 |
| c | *p*-$OCH_3$-$C_6H_4NH_2$ | 12 | 276 | 73 |
| d | *p*-$CH_3$-$C_6H_4NH_2$ | 11 | 193 | 81 |
| e | *m*-$NO_2$-$C_6H_4NH_2$ | 8 | 287 | 98 |

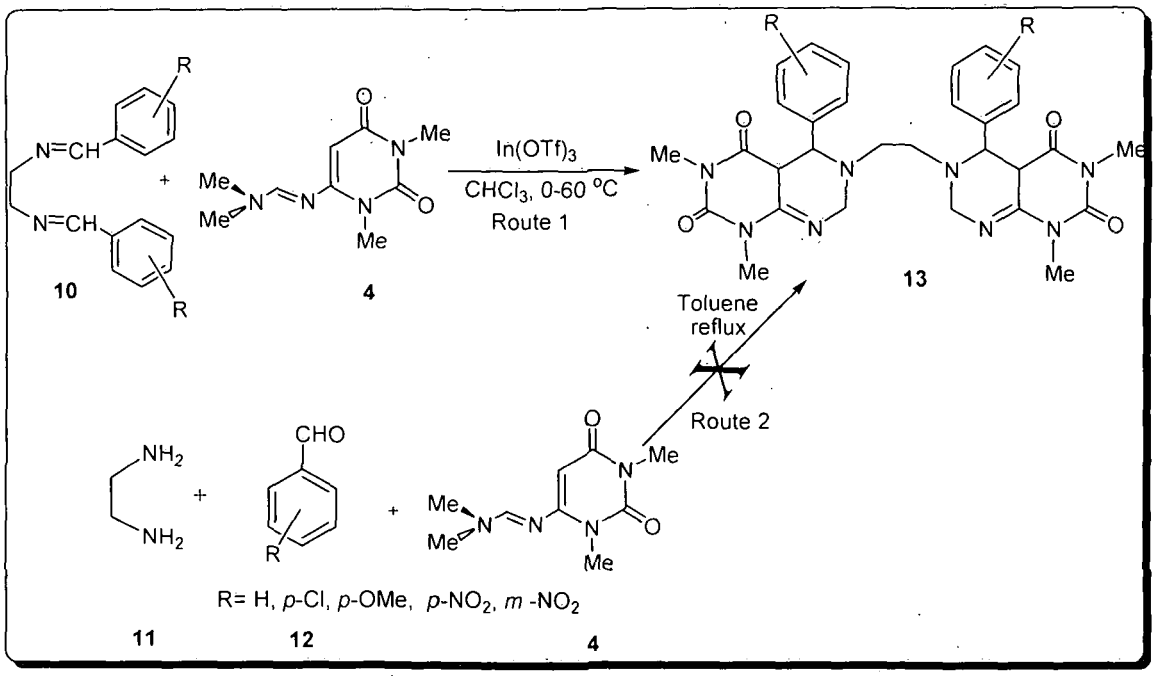
[a] Isolated yield

In the $^1$H NMR spectrum of (**9a,** R= Ph), peaks at δ = 3.25 (6H) and 3.31 (6H) ppm are due to the six *N*-methyl groups, at $\delta$ = 5.99 ppm is due to the >CH proton, within $\delta$ = 7.03-7.06 and $\delta$ = 7.27-7.38 ppm are due to five aromatic protons and at $\delta$ = 7.78 ppm is due to the imine proton.

Similarly, (**9b**) was obtained in 97% yield within 10 h. The structure was further confirmed through single crystal X-ray analysis. Suitable crystals were obtained by slow evaporation of (**9b**) from DMF solution. ORTEP diagram of the compound (**9b**) is shown in **Fig. 5**. Alternatively, a three componrnt reaction invilving dialdehyde (7), amine (**6a-e**) and amidine (**4**) in refluxing toluene for overnight afforded the product (**9a-e**) in comparable yields. No catalyst was used. However, when the three component reaction was done at 0 °C in dry $CHCl_3$ using $In(OTf_3)$ as catalyst, the result was not encouraging.

Using the same reaction procedure as mentioned above, we extended the reaction to bis-imines (**10**), keeping the amidine (**4**) fixed (**Scheme 3**). The results are summerised in **Table 5**). Bis-imines (**10a-e**) were obtained from the reaction between aliphatic diamine (**11**) and aromatic aldehydes (**12**).

Here, the yields of the products (**13**) are somewhat lower. The alternative strategy, three component reaction involving diamine (**11**), aldehyde (**12**) and amidine (**4**) in refluxing toluene didnot provide any product.



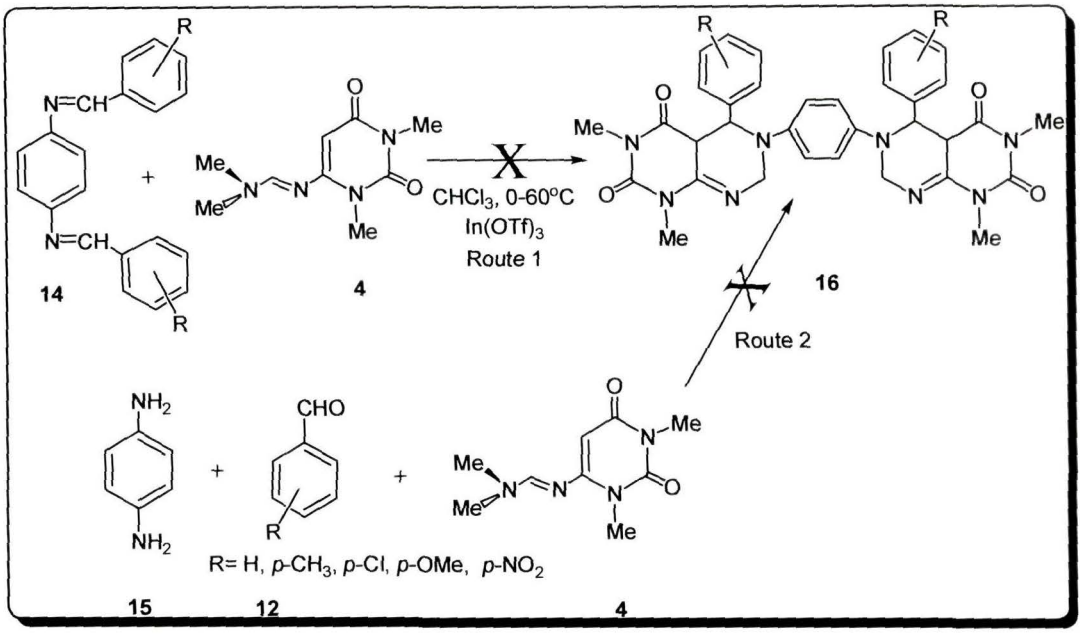

**Scheme 3:** Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**13**)

Going one step further, we next investigated the reaction between the imine (**14**) and amidine (**4**) (**Scheme 4**) under the same condition as defined in **Scheme 2 & 3**. Although from TLC, we could see something happening but we failed to isolate any product. The alterrnative three component strategy also failed in this case. Bis-imines (**14a-e**) were obtained from the reaction between aromatic diamine (**15**) and aromatic aldehydes (**12**).

**Table 5:** Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**13**)

| Entry | Aldehyde Compounds **12** | Time/h | m.p. (°C) | Yield (%)[a] |
|---|---|---|---|---|
| a | $C_6H_4NH_2$ | 8 | 189-191 | 63 |
| b | *p*-Cl-$C_6H_4NH_2$ | 8 | 197-198 | 71 |
| c | *p*-$OCH_3$-$C_6H_4NH_2$ | 9 | 201-203 | 56 |
| d | *p*-$NO_2$-$C_6H_4NH_2$ | 8 | 181-183 | 73 |
| e | *m*-$NO_2$-$C_6H_4NH_2$ | 8 | 180-184 | 73 |

[a] Isolated yield




**Scheme 4:** Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**16**)

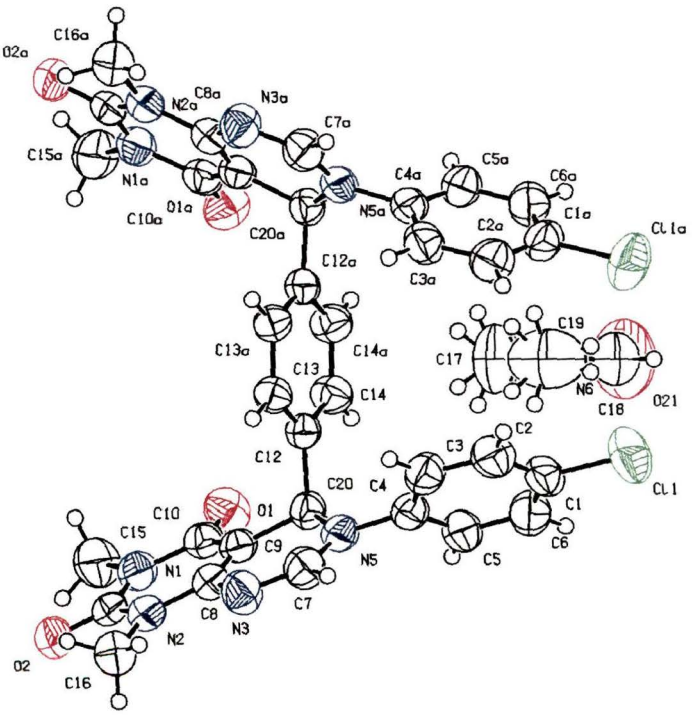



**Fig. 5:** ORTEP diagram of Compound (**9b**)

**Table 6**: Detail crystallographic data of **(9b)**

| | |
|---|---|
| Formula | $C_{37}H_{35}Cl_2N_9O_5$ |
| M | 760.64 |
| Crystal system | Orthorhombic |
| Temperature/K | 294 (2) |
| Space group | Pnma |
| *a*/ Å | 16.0317(17) |
| *b*/ Å | 22.181(2) |
| *c*/ Å | 10.2433(13) |
| α (°) | 90 |
| β (°) | 90 |
| γ (°) | 90 |
| *V*/ $Å^3$ | 3642.5(7) |
| *Z* | 4 |
| *Dc*/ $mg \cdot m^{-3}$ | 1.387 |
| Reflns. Collected | 31686 |
| Reflns. Unique | 3292 |
| R(*int*) | 0.1051 |
| Index ranges | -19<=h<=19, -26<=k<=29, -12<=h<=12 |
| Refinement method | Full-matrix, least squares on $F^2$ |
| $wR_2$ 0.2567(3292) | $R_1$ 0.0844(1970) *GoF* 1.396 |

**Bioactivity tests:**

The synthesised pyrimido[4,5-*d*]pyrimidine derivatives **(9a-e)** & **(13a-e)** were screened against four non-pathogenic bacterial strains viz. *S. aureus* (gram +ve), *B. subtilis* (gram +ve), *E. coli* (gram -ve), *P. diminuta* (gram -ve). Compounds possess inhibitory activities against *E. coli* and *P. diminuta*, whereas no inhibitory activity was observed against *S. aureus* and *B. subtilis* bacteria (**Table 7**). The main reason for this may be the difference in the cell wall structure of the gram positive and gram negative bacteria. No inhibitory

activity was also noticed against fungi (**Table 7).** The detail zones of inhibition are shown in **Table 7**. Further, we have calculated the MIC values of the pyrimido[4,5-*d*]pyrimidine derivatives **(9a-e) & (13a-e)** by using MTT assay at different concentration. The detail antibacterial activities and MIC values of pyrimido[4,5-*d*]pyrimidine derivatives **(9a-h) & (13a-e)** are shown in **Table 9**.

**Table 7:** Antibacterial activity test of bis-pyrimido[4,5-*d*]pyrimidine derivatives **(9a-e) & (13a-e)**

| Entry | Gram Negative Bacteria | | Gram Positive Bacteria | | Fungi | |
|---|---|---|---|---|---|---|
| | *E. coli* | *P. diminuta* | *B. subtilis* | *S. aureus* | *C. albicans* | *A. niger* |
| 9a | + | + | - | - | - | - |
| 9b | + | + | - | - | - | - |
| 9c | + | + | - | - | - | - |
| 9d | + | + | - | - | - | - |
| 9e | + | + | - | - | - | - |
| 13a | + | + | - | - | - | - |
| 13b | + | + | - | - | - | - |
| 13c | + | + | - | - | - | - |
| 13d | + | + | - | - | - | - |
| 13e | + | + | - | - | - | - |

Sign: - (Inactive); + (active)

**Table 8:** Zone of inhibitions of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**9a-e**) & (**13a-e**)

| Compound ID | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *E. coli* | | *P. diminuta* | |
| 9a | 11 | 10 | 11 | 11 |
| 9b | 13 | 12 | 11 | 11 |
| 9c | 12 | 11 | 13 | 13 |
| 9d | 11 | 11 | 12 | 11 |
| 9e | 11 | 11 | 13 | 13 |
| 13a | 11 | 11 | 13 | 12 |
| 13b | 12 | 13 | 13 | 13 |
| 13c | 11 | 11 | 11 | 11 |
| 13d | 12 | 12 | 10 | 10 |
| 13e | 13 | 12 | 11 | 11 |

**Table 9:** MIC Values of pyrimido[4,5-*d*]pyrimidine derivatives (**9a-e**) & (**13a-e**)

| Compound ID | MIC (mg/ml) | |
|---|---|---|
| | *E. coli* | *P. diminuta* |
| 9a | 0.5 | 0.25 |
| 9b | 0.125 | 0.25 |
| 9c | 0.25 | 0.25 |
| 9d | 0.25 | 0.25 |
| 9e | 0.25 | 0.25 |
| 13a | 0.25 | 0.5 |
| 13b | 0.125 | 0.25 |
| 13c | 0.25 | 0.5 |
| 13d | 0.25 | 0.25 |
| 13e | 0.125 | 0.25 |

**Conclusion:**

Diels-Alder methodology (aza-version) has been shown to be very useful for the construction of fused heterocyclic systems like bis-pyrimido[4,5-*d*]pyrimidine derivatives in good to excellent yields. Two different sets of bis-pyrimido[4,5-*d*]pyrimidine derivatives were synthesised by simply changing one of the reactant (bis-imines).

Anti-bacterial activity of bis-pyrimido[4,5-*d*]pyrimidine derivatives so synthesised were evaluated and found to possess good inhibitory activities against gram negative bacteria, whereas no inhibitory activity was found against gram positive bacteria. Bis-pyrimido[4,5-*d*]pyrimidine derivatives were found to be inactive against fungi.

**Experimental section:**

**Typical reaction procedure for the synthesis of pyrimido[4,5-*d*]pyrimidine derivatives (9) & (13):**

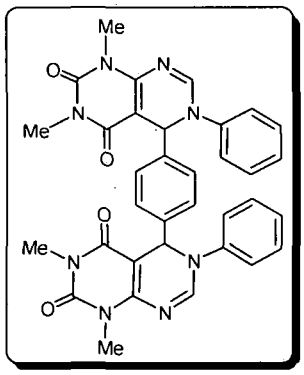
For route 1 in all the schemes, same procedure was followed as described in **Chapter-4** (Section-A, Page No. 144).

For route 2; A mixture of 6-[(dimethylamino)methylene]1,3-dimethylaminouracil (**4**) (209 mg, 1 mmol), terepthaldehyde (**7**, 134 mg, $^1/_2$ mmol) and aniline (**8a,** 0.091 ml, 1 mmol) was taken in a 50 ml round bottom flask, then added ~15 ml toluene and allowed to reflux for overnight. After completion of the reaction the product precipitated out from the reaction mixture which was then washed with ethylacetate to remove any unreacted reactant and other byproducts. The pure product was dried at high vaccum using a pump and solublised in DMF, recrystallized and we got the yellow shining pure product (**9a**) in 91 % yield; m.p. 360 °C.

The same reaction procedure was followed for the rest of the substrates.

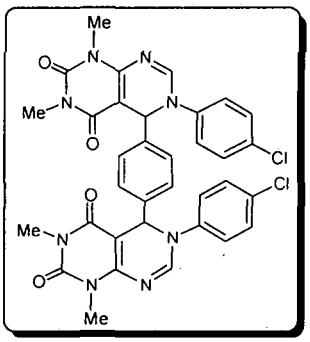
**Physical and spectral data:**

**5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxo-6-phenylpyrimido-[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-phenylpyrimido[4,5-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione (9a)**



Colour- white shining yellowish transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3073.78, 2941.61, 1698.17, 1640.16, 1532.89, 1471.29; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 3.25 (s, 6H, $NCH_3$), 3.31 (s, 6H, $NCH_3$), 5.99 (d, 2H, *J*=10.52 Hz, CH), 7.03-7.06 (m, 4H, arom), 7.27-7.38 (m, 8H, arom), 7.78 (d, 2H, *J*=7.8 Hz, CH=N) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.2 ($2NCH_3$), 29.7 ($2NCH_3$), 58.5 & 58.7 (C-5 & C′-5) 91.1 (C-10 & C′-10), 123.7 (arom), 123.8 (arom), 127.1 (arom), 127.2 (arom), 130.1 (arom), 130.2 (arom), 133.5 (arom), 140.1 (arom), 141.9 (arom), 142.0 (arom), 148.9 (C=N, C7 & C′-7), 152.0 & 152.1 (C=O, C-2 & C′-2), 161.1 (C=O, C-4 & C′-4) ppm. MS, *m/z* 615 ($M^+$); Anal. Calcd (%) for $C_{34}H_{30}N_8O_4$: C, 66.44; H, 4.92; N, 18.23. Found C, 66.43; H, 4.96; N, 18.21.

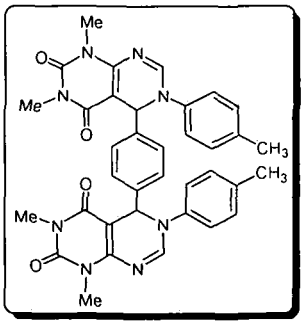
**6-(4-chlorophenyl)-5-(4-(6-(4-chlorophenyl)-1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxopyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-5,6-dihydro-1,3-dimethylpyrimido-[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9b)**



Colour- white shining yellowish transparent crystalline solid. Solubility- insoluble in water, common organic solvents, sparingly soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3071.98, 2943.13, 1698.27, 1641.06, 1533.19, 1471.27; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.27 (s, 6H, $NCH_3$), 3.54 (s, 6H, $NCH_3$), 5.96 (d, 2H, *J*=10.8 Hz, CH), 6.98-7.00 (m, 4H, arom), 7.28-7.37 (m, 8H, arom), 7.73 (d, 2H, *J*=7.8 Hz, CH=N) ppm; $^{13}$C NMR (100 MHz,

$CDCl_3$) $\delta_c$ 28.7 ($2NCH_3$), 29.9 ($2NCH_3$), 58.4 & 58.6 (C-5 & C′-5) 91.0 (C-10 & C′-10), 122.9 (arom), 123.4 (arom), 126.9 (arom), 127.0 (arom), 129.7 (arom), 129.8 (arom), 133.6 (arom), 140.1 (arom), 141.7 (arom), 142.2 (arom), 148.7 (C=N, C7 & C′-7), 152.7 & 153.1 (C=O, C-2 & C′-2), 161.7 (C=O, C-4 & C′-4) ppm. MS, *m/z* 684 ($M^+$); Anal. Calcd (%) for $C_{34}H_{28}Cl_2N_8O_4$: C, 59.74; H, 4.13; N, 16.39. Found C, 59.71; H, 4.21; N, 16.36.

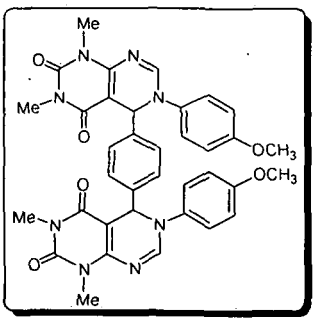
**5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxo-6-*p*-tolylpyrimido -[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-*p*-tolylpyrimido[4,5-*d*]pyrimidine-2,4 -(1*H*,3*H*)-dione (9c)**



Colour- white shining yellowish transparent crystalline solid. Solubility- insoluble in water, common organic solvents, sparingly soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3073.76, 2942.14, 1698.43, 1641.28, 1532.73, 1471.45; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.06 (s, 3H, $CH_3$), 3.38 (s, 6H, $NCH_3$), 3.35 (s, 6H, $NCH_3$), 6.42 (d, 2H, *J*=8.28 Hz, CH), 6.75 (d, 2H, *J*=8.24 Hz, arom), 7.20 (m, 2H, arom) 7.63-8.00 (m, 8H, arom), 8.07 (d, 2H, *J*=7.24 Hz, CH=N) ppm; 21.6 ($CH_3$), 28.9 ($2NCH_3$), 29.3 ($2NCH_3$), 57.9 & 58.1 (C-5 & C′-5) 89.8 (C-10 & C′-10), 122.7 (arom), 123.1 (arom), 126.9 (arom), 127.2 (arom), 129.6 (arom), 129.9 (arom), 132.8 (arom), 140.3 (arom), 141.2 (arom), 142.1 (arom), 149.1 (C=N, C7 & C′-7), 152.7 & 153.1 (C=O, C-2 & C′-2), 162.5 (C=O, C-4 & C′-4) ppm. MS, *m/z* 643 ($M^+$); Anal. Calcd (%) for $C_{36}H_{34}N_8O_4$: C, 67.28; H, 5.33; N, 17.43. Found C, 67.31; H, 5.36; N, 17.41.

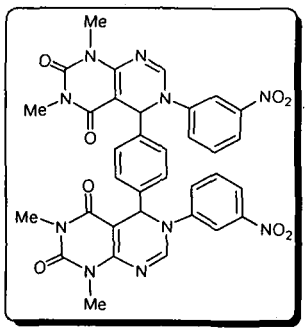
**5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-6-(4-methoxyphenyl)-1,3-dimethyl-2,4-dioxo -pyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-6-(4-methoxyphenyl)-1,3-dimethyl pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9d)**

Colour- Yellow shining transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3072.89, 2941.34, 1697.68, 1641.43,



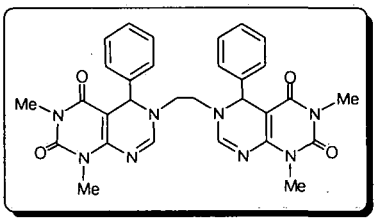
1532.71, 1470.91; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.36 (s, 6H, $NCH_3$), 3.39 (s, 6H, $NCH_3$), 3.48 (s, 3H, $OCH_3$), 6.43 (d, 2H, *J*=10.2 Hz, CH), 6.77 (m, 2H, arom), 7.21 (m, 2H, arom) 7.68-8.00 (m, 8H, arom), 8.10 (d, 2H, *J*=7.8 Hz, CH=N) ppm; 28.8 ($2NCH_3$), 29.1 ($2NCH_3$), 34.8 ($OCH_3$), 58.0 & 58.2 (C-5 & C′-5) 89.7 (C-10 & C′-10), 123.1 (arom), 123.6 (arom), 126.9 (arom), 127.1 (arom), 129.6 (arom), 130.1 (arom), 132.8 (arom), 140.4 (arom), 141.3 (arom), 142.2 (arom), 149.5 (C=N, C7 & C′-7), 152.7 & 153.1 (C=O, C-2 & C′-2), 163.2 (C=O, C-4 & C′-4) ppm. MS, *m/z* 675 ($M^+$); Anal. Calcd (%) for $C_{36}H_{34}N_8O_6$: C, 64.09; H, 5.08; N, 16.61. Found C, 64.07; H, 5.05; N, 16.65.

**5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-6-(3-nitrophenyl)-2,4-dioxo pyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-(3-nitrophenyl)pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9e)**



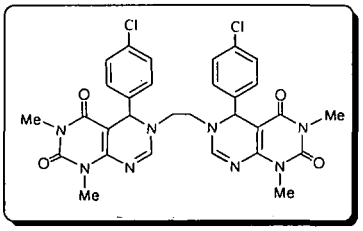
Colour- Yellow shining transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3073.56, 2941.63, 1698.31, 1640.17, 1532.78, 1471.41; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.28 (s, 6H, $NCH_3$), 3.37 (s, 6H, $NCH_3$), 5.86 (d, 2H, *J*=9.4 Hz, CH), 7.10-7.47 (m, 4H, arom), 7.63-7.74 (m, 8H, arom), 8.09 (d, 2H, *J*=7.78 Hz, CH=N) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 30.3 ($2NCH_3$), 309.8 ($2NCH_3$), 59.1 & 59.4 (C-5 & C′-5) 87.9 (C-10 & C′-10), 122.7 (arom), 123.7 (arom), 126.9 (arom), 127.2 (arom), 129.1 (arom), 130.0 (arom), 132.3 (arom), 140.1 (arom), 141.6 (arom), 142.7 (arom), 148.8 (C=N, C7 & C′-7), 152.5 & 153.1 (C=O, C-2 & C′-2), 162.7 (C=O, C-4 & C′-4) ppm. MS, *m/z* 705 ($M^+$); Anal. Calcd (%) for $C_{34}H_{28}N_{10}O_8$: C, 57.95; H, 4.01; N, 19.88. Found C, 57.93; H, 4.06; N, 19.87.

**5,6-dihydro-6-(2-(1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxo-5-phenylpyrimido[4,5-*d*]pyrimidin-6(5*H*)-yl)ethyl)-1,3-dimethyl-5-phenylpyrimido[4,5-*d*]pyrimidine-2,4 - (1*H*,3*H*)-dione (13a)**



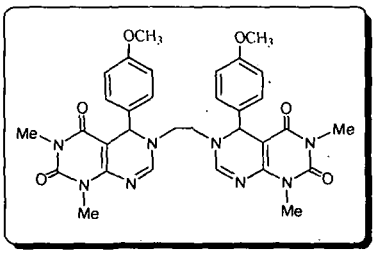
Colour- Yellow shining transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3073.78, 2972.31, 1697.13, 1643.12, 1533.81, 1470.91; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.60 (s, 2H, $CH_2$), 2.66 (s, 2H, $CH_2$), 3.12 (s, 3H, $NCH_3$), 3.27 (s, 3H, $NCH_3$), 3.30 (s, 3H, $NCH_3$), 3.31 (s, 3H, $NCH_3$), 5.06 (s, 1H, CH), 5.97 (s, 1H, CH), 7.05-7.31 (m, 10H, arom), 7.77 (s, H, CH=N), 7.67 (s, H, CH=N) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.3, 29.5, 31.1, 32.3, 34.1, 36.5, 89.9, 98.1, 127.2, 130.1, 131.1, 132.5, 142.7, 149.2, 150.1, 153.1, 154.3, 162.5 ppm. MS, *m/z* 567 ($M^+$); Anal. Calcd (%) for $C_{30}H_{30}N_8O_4$: C, 63.59; H, 5.34; N, 19.78. Found C, 63.61; H, 5.36; N, 19.74.

**5-(4-chlorophenyl)-6-(2-(5-(4-chlorophenyl)-1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxopyrimido[4,5-*d*]pyrimidin-6(5*H*)-yl)ethyl)-5,6-dihydro-1,3-dimethylpyrimido - [4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (13b)**



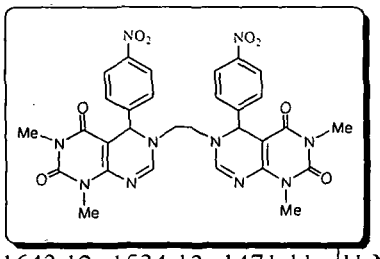
Colour- yellow shining transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3071.45, 2972.34, 1697.13, 1642.42, 1534.56, 1470.91; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.89 (s, 2H, $CH_2$), 3.05 (s, 2H, $CH_2$), 3.30 (s, 3H, $NCH_3$), 3.34 (s, 3H, $NCH_3$), 3.38 (s, 3H, $NCH_3$), 3.43 (s, 3H, $NCH_3$), 5.70 (s, 1H, CH), 6.17 (s, 1H, CH), 7.01-7.26 (m, 8H, arom), 7.50 (s, H, CH=N), 7.78 (s, H, CH=N) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.1, 29.6, 31.0, 33.3, 39.9, 41.5, 89.9, 98.8, 127.6, 130.3, 130.9, 143.4, 149.7, 152.0, 154.3, 157.3, 163.5 ppm. MS, *m/z* 636 ($M^+$); Anal. Calcd (%) for $C_{30}H_{28}Cl_2N_8O_4$: C, 56.70; H, 4.44; N, 17.63. Found C, 56.71; H, 4.46; N, 17.60.

**5,6-dihydro-6-(2-(1,2,3,4-tetrahydro-5-(4-methoxyphenyl)-1,3-dimethyl-2,4-dioxopyrimido[4,5-*d*]pyrimidin-6(5*H*)-yl)ethyl)-5-(4-methoxyphenyl)-1,3-dimethyl pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (13c)**



Colour- Yellow shining transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3072.48, 2973.31, 1697.19, 1643.21, 1533.78, 1471.15; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.80 (s, 2H, $CH_2$), 3.02 (s, 2H, $CH_2$), 3.29 (s, 3H, $NCH_3$), 3.32 (s, 3H, $NCH_3$), 3.34 (s, 3H, $NCH_3$), 3.39 (s, 3H, $NCH_3$), 3.76 (s, 3H, $OCH_3$), 5.61 (s, 1H, CH), 5.87 (s, 1H, CH), 6.75-7.15 (m, 8H, arom), 7.56 (s, H, CH=N), 7.68 (s, H, CH=N) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.7, 29.3, 31.8, 33.1, 34.2, 38.3, 40.8, 88.9, 97.3, 124.2, 127.8, 141.3, 144.7, 149.5, 150.5, 151.1, 153.1, 152.9, 162.1, 163.3 ppm. MS, *m/z* 627 ($M^+$); Anal. Calcd (%) for $C_{32}H_{43}N_8O_6$: C, 61.33; H, 5.47; N, 17.88. Found C, 61.31; H, 5.49; N, 17.81.

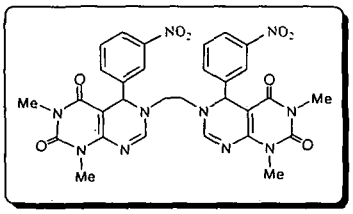
**5,6-dihydro-6-(2-(1,2,3,4-tetrahydro-1,3-dimethyl-5-(4-nitrophenyl)-2,4-dioxo pyrimido[4,5-*d*]pyrimidin-6(5*H*)-yl)ethyl)-1,3-dimethyl-5-(4-nitrophenyl)pyrimido [4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (13d)**



Colour- brown shining yellowish transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3073.78, 2971.71, 1696.61, 1643.12, 1534.13, 1471.11; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.92 (s, 2H, $CH_2$), 3.04 (s, 2H, $CH_2$), 3.26 (s, 3H, $NCH_3$), 3.33 (s, 3H, $NCH_3$), 3.37 (s, 3H, $NCH_3$), 3.45 (s, 3H, $NCH_3$), 5.83 (s, 1H, CH), 6.38 (s, 1H, CH), 7.15-7.24 (m, 8H, arom), 8.05 (s, H, CH=N), 8.07 (s, H, CH=N) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.5, 29.2, 31.7, 34.5, 38.0, 40.5, 88.8, 98.4, 123.5, 127.5, 146.0, 149.9, 151.2, 151.4, 153.2, 153.8, 161.1, 162.2,

165.4 ppm. MS, *m/z* 657 ($M^+$); Anal. Calcd (%) for $C_{30}H_{28}N_{10}O_8$: C, 54.88; H, 4.30; N, 21.33. Found C, 54.89; H, 4.36; N, 21.31.

**5,6-dihydro-6-(2-(1,2,3,4-tetrahydro-1,3-dimethyl-5-(3-nitrophenyl)-2,4-dioxo pyrimido[4,5-d]pyrimidin-6(5*H*)-yl)ethyl)-1,3-dimethyl-5-(3-nitrophenyl)pyrimido [4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (13e)**



Colour- brown shining transparent crystalline solid. Solubility- insoluble in water, sparingly soluble in common organic solvents, soluble in aprotic polar solvents like DMF, DMSO, DMAc etc. IR (KBr) ($\nu_{max}/cm^{-1}$) 3073.78, 2971.71, 1696.61, 1643.12, 1534.13, 1471.11; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.65 (s, 2H, $CH_2$), 2.73 (s, 2H, $CH_2$), 3.22 (s, 3H, $NCH_3$), 3.25 (s, 3H, $NCH_3$), 3.29 (s, 3H, $NCH_3$), 3.49 (s, 3H, $NCH_3$), 5.25 (s, 1H, CH), 5.71 (s, 1H, CH), 7.35-7.99 (m, 8H, arom), 8.17 (s, H, CH=N) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.3, 29.5, 31.2, 33.6, 38.0, 40.1, 88.9, 97.6, 123.9, 127.6, 146.1, 148.9, 151.1, 151.3, 154.1, 157.8, 161.4, 162.3 ppm. MS, *m/z* 657 ($M^+$); Anal. Calcd (%) for $C_{30}H_{28}N_{10}O_8$: C, 54.88; H, 4.30; N, 21.33. Found C, 54.89; H, 4.36; N, 21.31.

**References:**


1. a) Hughes, R.A.; Thompson, S.P.; Alcaraz, L.; Moody, C.J. Total synthesis of the thiopeptide antibiotic amythiamicin D, *J. Am. Chem. Soc.* **127**, 15644-15651 (2005). b) Koepler, O. *et al* Synthesis and DNA binding properties of novel benzo[*b*]isoquino[2,3-*h*]naphthyridines, *Org. Biomol. Chem.* **3**, 2848-2858 (2005). c) Goodall, G.W.; Hayes, W. Advances in cycloaddition polymerizations, *Chem. Soc. Rev.* **35**, 280-312 (2006).
2. a) Kobayashi, S. *et al* A novel and efficient stereo-controlled synthesis of hexahydroquinolinones via the diene-transmissive hetero-Diels-Alder reaction of cross-conjugated azatrienes with ketenes and electrophilic dienophiles, *Tetrahedron* **65**, 920-933 (2009). b) de la Hoz, A.; Diaz-Ortiz, A.; Moreno, A.; Langa, F. Cycloadditions under microwave irradiation conditions: Methods and applications, *Eur. J. Org. Chem.* 3659-3673 (2000). c) Hong, B.C. *et al* Unprecedented

microwave effects on the cycloaddition of fulvenes. A new approach to the construction of polycyclic ring systems, *Org. Lett.* **6**, 3453-3456 (2004).

3. a) Oikawa, H.; Tokiwano, T. Enzymatic catalysis of the Diels–Alder reaction in the biosynthesis of natural products, *Nat. Prod. Rep.* **21**, 321-352 (2004). b) Nicolaou, K.C.; Snyder, S.A.; Montagnon, T.; Vassilikogiannakis, G. The Diels-Alder reaction in total synthesis, *Angew. Chem. Int. Ed. Eng.* **41**, 1668-1698 (2002). c) Miller, K.A.; Tsukamoto, S.; Williams, R.M. Asymmetric total syntheses of (+)- and (-)-versicolamide B and biosynthetic implications, *Nature Chem.* **1**, 63-68 (2009).
4. a) Boger, D. L. Heterodiene Additions; In *Comprehensive Organic Synthesis* (Ed. Paquette, L.A. Pergamon: New York, **5**, 451-512, 1991). b) Jung, M.E.; Shapiro, J.J. Synthesis and structure determination of 2-azabicyclo[2.2.1]hept-2-enes and their derivatives. W effect on chemical shifts, *J. Am. Chem. Soc.* **102**, 7862-7866 (1980). c) Rao, C.N.R.; Basu, P.R.; Hedge, M.S. Systematic organic UV photoelectron spectroscopy, *Appl. Spectrosc. Rev.* **15**, 1-139 (1979). d) Jordan, K.D.; Burrow, P.D. Studies of the temporary anion states of unsaturated hydrocarbons by electron transmission spectroscopy, *Acc. Chem. Res.* **11**, 341-348 (1978).
5. a) Prajapati, D.; Thakur, A.J. Studies on 6-[(dimethylamino)methylene]aminouracil: A facile one-pot synthesis of novel pyrimido[4,5-*d*]pyrimidine derivatives, *Tetrahedron Lett.* **46**, 1433-1436 (2005). b) Prajapati, D.; Gohain, M.; Thakur, A.J. Regiospecific one-pot synthesis of pyrimido[4,5-*d*]pyrimidine derivatives in the solid state under microwave irradiations, *Bioorg. Med. Chem. Lett.* **16**, 3537-3540 (2006).
6. a) Jadidi, K.; Ghahremanzadeh, R.; Bazgir, A. Efficient synthesis of spiro[chromeno[2,3-*d*]pyrimidine-5,3'-indoline]tetraones by a one-pot and three-component reaction, *J. Comb. Chem.* **11**, 341-344 (2009). b) Trost, B.M. Atom economy - A challenge for organic synthesis: Homogeneous catalysis leads the way, *Angew. Chem., Int. Ed. Eng.* **34**, 259 (1995). c) Zhu, J., Bienaymé, H., Eds. *Multicomponent Reactions* (Wiley-VCH: Weinheim, 2005). d) Armstrong, R.W. *et*

*al* Multiple-component condensation strategies for combinatorial library synthesis, *Acc. Chem. Res.* **29**, 123-131 (1996).

7. a) A. Dömling, Recent developments in isocyanide based multicomponent reactions in applied chemistry, *Chem. Rev.* **106**, 17-89 (2006). b) M. Lancaster, *Green Chemistry: An Introductory Text* (Royal Society of Chemistry, Cambridge, 2002).
8. Valderrama, J.A. *et al* Studies on quinones. Part 44: Novel angucyclinone *N*-heterocyclic analogues endowed with antitumoral activity, *Bioorg. Med. Chem.* **16**, 10172-10181 (2008).
9. Nair, V. *et al* A heterocyclic molecule with significant activity against dengue virus, *Bioorg. Med. Chem. Lett.* **19**, 1425-1427 (2009).
10. a) Shaabani, A. *et al* New one-pot four-component synthesis of disubstituted pyrido[2,3-*d*]pyrimidine-6-carboxamide derivatives, *J. Comb. Chem.* **11**, 375-377 (2009). b) Kanth, S.R. *et al* Convenient synthesis of novel 4-substitutedamino-5-trifluoromethyl-2,7-disubstituted pyrido[2,3-*d*]pyrimidines and their antibacterial activity, *Eur. J. Med. Chem.* **41**, 1011-1016 (2006). c) Bazgira, A.; Khanaposhtani, M.M.; Soorki, A.A. One-pot synthesis and antibacterial activities of pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyrimidinedione derivatives, *Bioorg. Med. Chem. Lett.* **18**, 5800-5803 (2008).
11. El-Gazzar, A.B.A. *et al* Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines as anti-oxidant, anti-inflammatory and analgesic activities, *Eur. J. Med. Chem.* **44**, 609-624 (2009).
12. Valderrama, J.A. *et al* Studies on quinones. Part 44: Novel angucyclinone *N*-heterocyclic analogues endowed with antitumoral activity, *Bioorg. Med. Chem.* **16**, 10172-10181 (2008).
13. Agarwal, A. *et al* Dihydropyrido[2,3-*d*]pyrimidines as a new class of antileishmanial agents, *Bioorg. Med. Chem.* **13**, 6678-6684 (2005).
14. El-Gazzar, A.B.A.; Hafez, H.N. Synthesis of 4-substituted pyrido[2,3-*d*]pyrimidin-4(1*H*)-one as analgesic and anti-inflammatory agents, *Bioorg. Med. Chem. Lett.* **19**, 3392-3397 (2009).
15. Nam, G. *et al* Syntheses and evaluation of pyrido[2,3-*d*]pyrimidine-2,4-diones as PDE 4 inhibitors, *Bioorg. Med. Chem. Lett.* **11**, 611-614 (2001).

16. a) Colotta, V. *et al* 1,2,4-Triazolo[4,3-*a*]quinoxalin-1-one: A versatile tool for the synthesis of potent and selective adenosine receptor antagonists, *J. Med. Chem.* **52**, 2407-2419 (2009). b) Bulicz, J. *et al* Synthesis and pharmacology of pyrido[2,3-*d*]pyrimidinediones bearing polar substituents as adenosine receptor antagonists, *Bioorg. Med. Chem.* **14**, 2837-2849 (2006).

17. a) Thakur, A.J.; Saikia, P.; Prajapati, D.; Sandhu, J.S. Studies on 6-[(dimethylamino)methylene]aminouracils: A facile one-pot synthesis of pyrimido -[4,5-d]pyrimidines and pyrido[2,3-*d*]pyrimidines, *Synlett.* **8**, 1299-1301 (2001). b) Das, S.; Saikia, B.K.; Sridhar, B.; Thakur, A.J. 6-[(Dimethylamino)-methyleneamino]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione dihydrate, *Acta Cryst.* **E64**, o1662 (2008). c) Thakur, A.J.; Das, S.; Phukan, A.K. Replay of amide type resonance in 6-[(dimethylamino)methylene]1,3-dimethylaminouracil: A dynamic NMR and density functional theory study, *J. Mol. Struc.* **929**, 134-140 (2009). d) Das, S.; Saikia, B.K.; Das, B.; Saikia, L.; Thakur, A.J. 6,6'-Diamino-1,1',3,3'-tetramethyl-5,5'-(4-chlorobenzylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione], *Acta Cryst.* **65E**, o2416-o2417 (2009). e) Das, S.; Thakur, A.J. A clean, highly efficient and one-pot green synthesis of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimethyluracil-5-yl)methanes in water, *Eur. J. Org. Chem.* 2301-2308 (2011).

18. Boakye-Yiadom, I.K. Antimicrobial activity of two flavonone isolates from the Cameroonian plant *Enythiana sigmoides, Planta Medicia.* **54**, 126-212 (1979).

19. Banso, A.; Adeyemo, S.O. Phytochemical and antimicrobial evaluation of ethanolic extracts *Draclena mannii* bark, *Nig. J. Biotech.* **18,** 27-32 (2007).

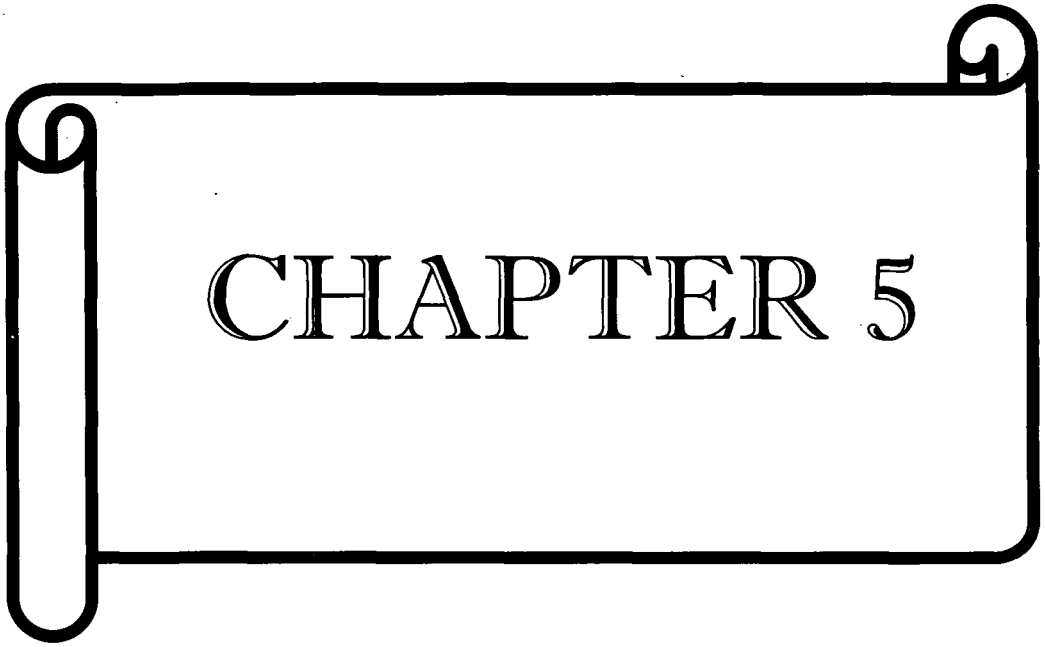
20. Radhika, P.; Sastry, B.S.; Harica, B.M. Antimicrobial screening of *Andrographis paniculata* (Acanthaceae) root extracts. *Res. J. Biotech.* **3**, 62-63 (2008)

21. Bae, J.W. *et al* Synthesis of pyrido[2,3-*d*]pyrimidines via palladium-catalyzed reaction of iodouracil with acetylenes, *Tetrahedron Lett.* **41**, 5899-5902 (2000).

22. Diaz-Ortiz, A. *et al* First Diels-Alder reaction of pyrazolyl imines under microwave irradiation, *Synlett.* **10**, 1069-1070 (1998).

23. Sharma, P.; Rane, N.; Gurram, V.K. Synthesis and QSAR studies of pyrimido[4,5-*d*]pyrimidine-2,5-dione derivatives as potential antimicrobial agents, *Bioorg. Med. Chem. Lett.* **14**, 4185-4190 (2004).

24. Sharma, P.; Kumar, A.; Rane, N.; Gurram, V. Hetero Diels–Alder reaction: A novel strategy to regioselective synthesis of pyrimido[4,5-*d*]pyrimidine analogues from Biginelli derivative, *Tetrahedron*, **61**, 4237-4248 (2005).
25. Yang, H.Z.; Pan, M.Y.; Jiang D.W.; He, Y. Synthesis of Janus type nucleoside analogues and their preliminary bioactivity, *Org. Biomol. Chem.* **9**, 1516-1522 (2011).
26. Saravanan, K. *et al* Nucleoside transport inhibitors: Structure-activity relationships for pyrimido[5,4-*d*]pyrimidine derivatives that potentiate pemetrexed cytotoxicity in the presence of $\alpha_1$-acid glycoprotein, *J. Med. Chem.* **54**, 1847-1859 (2011).


CHAPTER 5

Organic chemistry is one of the most rapidly ever developing areas of chemistry. Everday, useful and superior new reagents, chiral and achiral catalysts, reaction conditions and reactions are reported in the chemical literature. Generally, most basic reactions of organic chemistry are the transformations of one functional group to another. These are mainly reduction, oxidation, elimination, substitutation, rearrangement, dehydration etc. Great practitioners in this area still devote their research efforts for development of new selective highly efficient and selective procedures such that the procedure should be generally used for the wide range of organic structures and wide variety of conditions, yield of product should be high, preparation should be relatively uncomplicated and should be able to be carried out in most of the laboratories, the laboratory operations should be safe and free from danger of explosion, environment friendly and high optical yields should be possible in chiral systems.

The concepts of atom economy, selective (both stereo- and regio-) transformations and catalytic processes have become primary requirements for the development of synthetic organic chemistry to be one of the leading scientific disciplines. During the last fifteen years, synthetic organic chemistry has seen enormous growth, not only in terms of development of new methodologies in organic transformations by performing the reactions under microwave irradiation (MWI), using green solvents like water, ionic liquids, polyethylene glycol, grinding process etc. but also in terms of new reagents, catalysts, strategies, transformations and technologies often involving the concepts of atom economy and selectivity.

Literature data for more than 2 million organic chemical reactions show that approximately 70% of them have been carried out below 100 °C and that the majority of reaction times are 1-4 h.[1] Traditional methods for organic synthesis mostly involve glass reaction vessels operated at atmospheric pressure or below. Routine use of such equipment for the past one and a half centuries accounts for the bias toward modest temperatures and lengthy times for reactions.

If reactions can be performed at significantly higher temperatures than normal, considerable savings in time and energy can be realized. With traditional laboratory glassware, however, when a temperature in the order of 200 °C is desired, high boiling solvents are employed. This limits the choice of solvents and subsequent removal and

recycling can create problems. Continuous and batch microwave reactors enable the use of low-boiling solvents under pressurized conditions to speed up reactions and facilitate product isolation. With these systems, processes that are notoriously sluggish by traditional methods have been performed faster and in higher yield. Elevated temperatures also can be an attractive alternative to the use of aggressive reagents at lower temperatures. Some reactions proceed in the presence of less amount of catalyst, with a milder catalyst or without the use of a catalyst. These and other advances in hardware for synthesis have revolutionised approaches in organic chemistry over the past few years.

Some selected recent state-of-art in organic synthesis is mentioned below:

1. Supported catalysts and reagents
2. Ionic liquids-new solution for transition metal catalysis
3. Biotransformations
4. Solvent free organic synthesis
5. Multi component reactions
6. Supercritical fluid
7. Nano catalysis

In this chapter, we have described the development of some synthetic methods by performing the reaction under microwave irradiation (MWI) and using ionic liquid as a green solvent. It is subdivided into two **Sections- A & B**; **Section-A** describes "A green development of Bernthsen 9-substituted acridine synthesis in the absence of solvent catalyzed by *p*-toluenesulphonic acid (*p*-TSA)" and in **Section-B**, author describes "Environment-friendly and solventfree synthesis of symmetrical bis-imines under microwave Irradiation".

# Section A

## A green development of Bernthsen 9-substituted acridine synthesis in the absence of solvent catalyzed by *p*-toluenesulphonic acid (*p*-TSA)

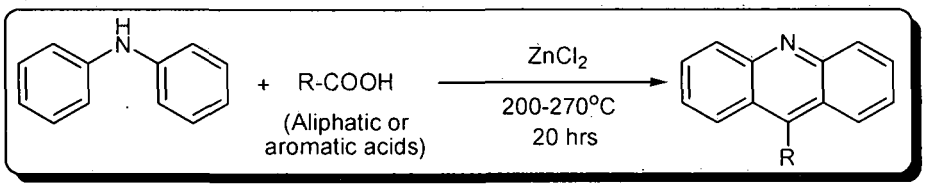
**Introduction:**

Acridines represent an important class of nitrogen heterocycles[2] having several significant properties such as pigment and dye properties,[3] photochemical / physical properties,[4] electrochemical properties,[5] potent antimalarial activity,[6] anticancer activity,[7] antifungal activity,[8] etc. Natural and synthetic acridines and their derivatives are effective DNA and RNA-binding compounds[9] owing to their intercalation abilities as well as being a lipophilic carrier molecule. It is the acridine chromophore that renders to the molecules a planar structure allowing them to bind DNA by stacking between base pairs. Recently, structure-activity relationship of acridine analogs as hairpin and DYRK2 kinase inhibitors has been studied.[10]

In one of our ongoing synthetic programs on bioactive molecules and organic synthesis involving microwave energy, which provides greener reaction condition coupled with increased yield and time economy, we came across Bernthsen reaction. Owing to the bioactivity of acridines, we became interested therein. Use of microwave energy for the enhancement of organic reactions, i.e. microwave organic reactions enhancement (MORE) is well known.[11] That is why, we decided to explore the same in our ongoing program on bioactive molecules.

Acridine family members can be prepared by classical Bernthsen reaction by coupling a carboxylic acid (aromatic or aliphatic) and diphenylamine (DPA) in the presence of zinc chloride (**Scheme 1**) at a temperature of 200-210 °C for about 20 h.[12] The conditions for this reaction tend to be quite vigorous coupled with very low yield. In addition, more than stoichiometric amounts of $ZnCl_2$ is required [1:5:1 (DPA: $ZnCl_2$:

carboxylic acid)]. In 1962, Popp reported a modified Bernthsen reaction procedure by replacing the Lewis acid $ZnCl_2$ with polyphosphoric acid (PPA),[13] which was more convenient in regard to reaction times despite the poorer yields. Toma *et al*[14] and Seijas *et al*[15] have reported an improved synthesis of acridine derivatives assisted by microwave and catalyzed by $ZnCl_2$. But, as compared to synthetic methodologies for other classes of compounds, this reaction has not been explored adequately as evidenced by reports of only two catalysts ($ZnCl_2$ and PPA) are being employed.



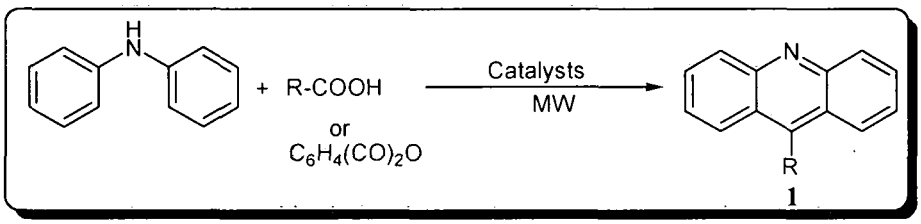

**Scheme 1**: Classical Bernthsen reaction for the synthesis of acridine

But none of these procedures were satisfactory in terms of yield and time economy. In this regard, hence, there is still need for looking at this reaction in order to have improved reaction conditions/superior catalysts so that better yields could be achieved. At the same time, we should not forget the cost, environment, etc. Accordingly, our main aim of this present investigation is to find alternative conditions/catalysts for Bernthsen reaction with the following objectives: (a) to search for milder reaction condition, (b) to reduce the reaction time, (c) to increase the yield, (d) to promote economical and environmentally friendly experimental procedures (green chemistry) by performing the reaction under microwave and solventless condition, and (e) to use cost effective, easily available, mild, water tolerant, and economical catalysts alternative to $ZnCl_2$ and polyphosphoric acid.

In an effort to amend the situation above, in this communication, we report a simple and general solventless reaction for the synthesis of 9-substituted acridines, by a modification of classical Bernthsen reaction (**Scheme 2**), using *p*-TSA (*p*-

toluenesulphonic acid) as the catalyst under MWI. This paves the way for an environmentally benign condition without compromising viability and speed. Our method is equally applicable to both aliphatic and aromatic carboxylic acids, thereby providing more generality and flexibility.

An inspection of the literatures revealed that in Bernthsen reaction using $ZnCl_2$ and PPA, the stoichiometry used for DPA and carboxylic acid was not 1:1. The acid was taken always in excess. The catalyst used was also in stoichiometric amount or higher. In our green chemistry approach, we searched for the conditions where the substrates would be used in stoichiometric ratios and the catalyst used would be in minimum amount, e.g. (10 mol% or lower than that).




R= $-C_6H_5$, *p*-$ClC_6H_4$, *o*-$ClC_6H_4$, *p*-$NO_2C_6H_4$, *p*-$NH_2C_6H_4$, *p*-$OHC_6H_4$, $-(CH_2)_4$-COOH, $-(CH_2)_2$-COOH, $-CH_2$-COOH, *o*-$C_6H_4COOH$

Catalysts = *p*-TSA, Basic alumina, CAN, Zirconium oxychloride octahydrate, Potassium dichromate, Anhydrous aluminium chloride

**Scheme 2**: Our development for the synthesis of 9-substituted acridine (**1**)

**Materials and Methods:**

Melting points were determined on a Büchi 504 apparatus and are uncorrected. IR spectra were recorded in KBr pallets on a Nicolet (Impact 410) FT-IR spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were recorded on a Varian Mercury Plus 400 MHz NMR spectrophotometer using tetramethylsilane (TMS) as internal standard. Coupling constants are expressed in hertz. Microwave synthesis system used was (model CAT-2R) from Catalyst$^{TM}$ systems (**Fig. 1**). The progress of the reaction was monitored by thin

layer chromatography (TLC) run on silica gel G (Merck). All the chemicals were used as received. Elemental analyses were done in a Perkin Elmer CHN analyzer (2400 series II).

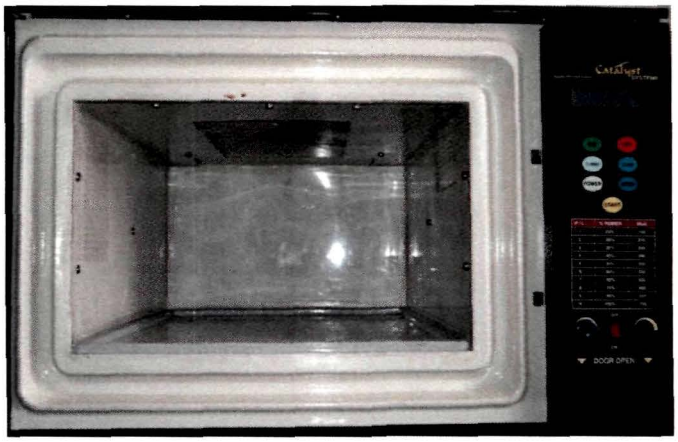


**Fig. 1:** Microwave synthesis system, model CAT-2R from Catalyst$^{TM}$ systems

**Results and Discussion:**

Other than *p*-TSA, we studied the effects of several compounds as catalyst, e.g. basic $Al_2O_3$, cerric ammonium nitrate (CAN), $ZrOCl_2 \cdot 8H_2O$, $K_2Cr_2O_7$, anhydrous $AlCl_3$ in Bernthsen reaction (reaction of DPA and benzoic acid was chosen as the model reaction) under thermal as well as MWI in the absence of solvent. However, the best results in terms of yields and reaction time were obtained with *p*-TSA (**Table 1**) under MWI.

CAN and basic alumina also afforded the products but yields were poorer. In order to demonstrate the scope of this reaction, differently substituted benzoic acids, aliphatic mono/dicarboxylic acids were taken up for the systematic study which is depicted in **Table 2**. However, in the conditions we explored, it was found that in the ratio of 1:1 (DPA and carboxylic acid), only entries (**1a**) and (**1k**) (**Table 2**) worked well; in all other cases the yields were not satisfactory. Too many products were observed in TLC and also we faced difficulty in separation by column chromatography. The best results were obtained when we used the acids in two folds with respect to DPA. To our delight, we were successful in reducing the amount of catalyst from stoichiometric ratios

to 10 mol%, a significant outcome in comparison to the classical methods. Aromatic anhydride also responded and showed reactivity under this condition (entry **1k**, **Table 2**). *p*-Nitro (entry **1k**, **Table 2**) and *p*-Amino benzoic acids (entry **1e**, **Table 2**) also afforded acridine under the reaction condition. Overheating and increasing the reaction time did not increase the reaction yield; rather some polymeric products were obtained.

**Table 1:** Screening of catalysts for the synthesis of 9-(phenyl)acridine (**1a, R=Ph**) via **Scheme 2**

| Entry | Catalysts | Under MWI | | Thermal heating | |
|---|---|---|---|---|---|
| | | Power/ Reaction time (min) | Yield (%)[a,b] | Reaction time (h) | Yield (%)[a,b] |
| 1 | *p*-Toluenesulfonic Acid | 450/4 | 80 | 12 | 75 |
| 2 | Ceric Ammonium Nitrate | 600/12 | ~31 | 20 | ~30 |
| 3 | Basic alumina | 600/12 | ~25 | 20 | ~27 |
| 4 | Zirconium oxychloride octahydrate | 600/12 | --- | 20 | --- |
| 5 | Potassium dichromate | 600/12 | --- | 20 | --- |
| 6 | Anhydrous aluminium chloride | 600/12 | --- | 20 | --- |

[a] Isolated yield; [ b] All the products were characterised by IR, $^{1}$H NMR and $^{13}$C NMR spectroscopy and Mass spectrometry, elemental analysis and m.p.

We also studied the effect of ionic liquid in the synthesis of 9-(phenyl)acridine (**1a**) and for that 1-Pentyl-3-methylimidazolium bromide[pmIm]Br (**Fig. 2**) was used as an ionic liquid. We observed that at room temp. with constant stirring, we got very low yields but when we heated at ~130 °C for 7 h we got the product in 75% yield. We extended the reaction to other acids also. But, all these reactions required very long time

at higher temp. and the yields were also not improved, hence we did not study the reaction in detail.

**Table 2:** *p*-TSA catalysed synthesis of acridine derivatives (**1**) via **Scheme 2**

| Entry | Acid/ Acid Anhydride | Stoichiometry (DPA: Acid moiety) | Under MWI | | Thermal heating | |
|---|---|---|---|---|---|---|
| | | | Power/Reaction time (min) | Yield (%)[a,b] | Reaction time (h) | Yield (%)[a,b] |
| a | R=-$C_6H_5$ | 1:1 | 450/4 | 80 | 12 | 75 |
| b | R=*p*-$ClC_6H_4$ | 1:2 | 600/2 | 84 | 10 | 73 |
| c | R=*o*-$ClC_6H_4$ | 1:2 | 600/2.5 | 82 | 10 | 73 |
| d | R=*p*-$NO_2C_6H_4$ | 1:2 | 600/7 | 74 | 15 | 42 |
| e | R=*p*-$NH_2C_6H_4$ | 1:2 | 600/9 | 66 | 15 | 38 |
| f | R=*p*-$OHC_6H_4$ | 1:2 | 450/3 | 49 | 12 | 20 |
| g | R=*o*-$C_6H_4COOH$ | 1:2 | 450/3.5 | 84 | 10 | 64 |
| h | R=-$CH_2COOH$ | 1:2 | 600/3 | 48 | 10 | 31 |
| i | R=-$(CH_2)_2COOH$ | 1:2 | 600/3 | 78 | 10 | 67 |
| j | R=-$(CH_2)_4COOH$ | 1:2 | 300/2.5 | 88 | 10 | 71 |
| k | $C_6H_4(CO)_2O$ | 1:1 | 450/3 | 79 | 10 | 67 |

[a] Isolated yield; [b] All the products were characterised by IR, $^1$H NMR, $^{13}$C NMR spectroscopy and Mass spectrometry, elemental analysis and m.p.

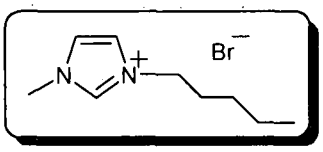



**Fig. 2:** The ionic liquid, 1-pentyl-3-methylimidazolium bromide, [pmIm]Br

**Conclusion:**

In conclusion, Bernthsen acridine synthesis is achieved in a simple, clean, fast, and solventless reaction catalyzed by *p*-TSA utilising non traditional MORE technique. Good

time economy and better yields of the products as compared to conventional Bernthsen reaction was observed. The reaction is equally applicable to aromatic as well as aliphatic acids (mono- and di-). Considering the environmental issues that require the substitution of toxic catalysts by more friendly catalysts, our methodology is consistent with green chemistry philosophy.[16] Moreover, *p*-TSA is easily available and can be easily removed from the reaction mixture being water-soluble. This is a good development over existing methods.

**Experimental:**

***General procedure for the synthesis of 9-substituted acridines (1) under MWI:***

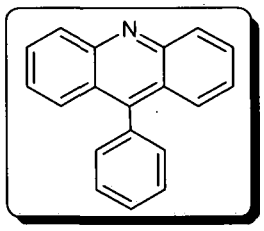
A mixture of *N,N*-DPA (169 mg, 1 mmol), benzoic acid (122 mg, 1 mmol), and pure *p*-TSA (19 mg, 10 mol%) was irradiated in a microwave oven (450 W) for 5 mins. The reaction was monitored by TLC. The crude product was extracted with chloroform (20ml×2) and washed with 10% NaOH followed by distilled water. The organic layer was dried over $Na_2SO_4$, evaporated and the residue was subjected to column chromatography to give 9-phenylacridine (**1a**) (244.80 mg, 80% yield). The pure products were characterized by IR, $^1$H-NMR, $^{13}$C-NMR spectroscopy, mass spectrometry, elemental analysis and m.p.

**General procedure for the synthesis of 9- substituted acridines (1a) using ionic liquid:**

A mixture of *N,N*-DPA (169 mg, 1 mmol), benzoic acid (122 mg, 1 mmol) and ionic liquid (IL) [pmIm]Br (231 mg, 1 mmol) was subjected to thermal heating (≈130 °C) for 7 h with constant stirring. The reaction was monitored by TLC. The crude product was extracted with diethyl ether (10×10ml). Diethyl ether was evaporated in a rotary evaporator and the residue was subjected to column chromatography to give 9-phenylacridine (**1a**) (191.25 mg, 75% yield). The pure product was characterized by IR, $^1$H and $^{13}$C-NMR spectroscopy, mass spectrometry, elemental analysis and m.p.

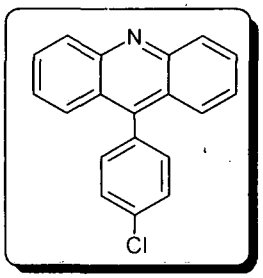
**Physical and spectral data:**

**9-(phenyl)acridine (1a)**



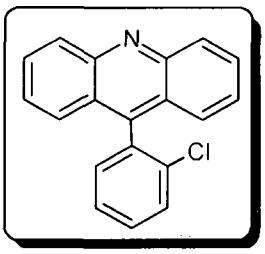
Yield 75%. m.p. 183-185 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1652.64 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.42-7.46 (m, 4H, C-2 & C7 & Ar-H), 7.59-7.61 (m, 3H, Ar-H), 7.71 (d, 2H, *J*=4.4 Hz, Ar-H), 7.76-7.80 (m, 2H, Ar-H), 8.28 (d, 2H, *J*=4.4 Hz, Ar-H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 125.2, 125.6, 126.9, 128.4, 128.5, 129.7, 130.0, 130.5, 135.9, 147.1, 148.7 ppm. MS, *m/z* 255 ($M^+$); Anal. Calcd (%) for $C_{19}H_{13}N$: C, 89.38; H, 5.13; N, 5.49. Found: C, 89.37; H, 5.16; N, 5.51.

**9-(4-chlorophenyl)acridine (1b)**



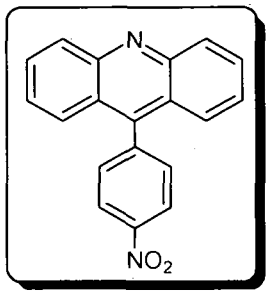
Yield 73%. m.p. 245-248 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1650.41 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.13 (d, 2H, *J*=3.25 Hz, Ar-H), 7.17 (t, 2H, *J*=3.05 Hz, Ar-H), 7.26 (d, 2H, *J*=3.28 Hz, Ar-H), 7.40-8.06 (m, 6H, Ar-H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 125.5, 126.2, 126.5, 127.4, 128.5, 129.6, 130.0, 133.4, 135.2, 142.6, 168.4 ppm. MS, *m/z* 289 ($M^+$); Anal. Calcd (%) for $C_{19}H_{12}ClN$: C, 78.76; H, 4.17; N, 4.83. Found: C, 78.77; H, 4.15; N, 4.86.

**9-(2-chlorophenyl)acridine (1c)**



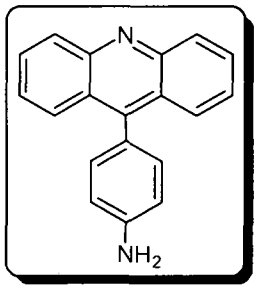
Yield 73%. m.p. 269-271 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1651.01 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.33-7.42 (m, 4H, Ar-H), 7.59-7.63 (m, 4H, Ar-H), 7.77-8.17 (m, 4H, Ar-H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 125.3, 126.3, 126.5, 127.2, 128.7, 129.8, 130.3, 132.2, 135.1, 144.1, 160.3 ppm. MS, *m/z* 289 ($M^+$). Anal. Calcd (%) for $C_{19}H_{12}ClN$: C, 78.76; H, 4.17; N, 4.83. Found: C, 78.77; H, 4.20; N, 4.84.

**9-(4-nitrophenyl)acridine (1d)**



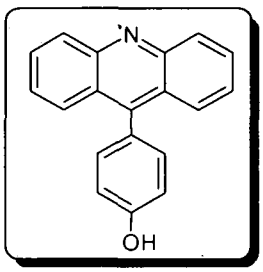
Yield 42%. m.p. 258-260 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1646.91 (C=N), 1523 (-$NO_2$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.46 (t, 2H, *J*=3.09 Hz, Ar-H), 7.61-7.64 (m, 6H, Ar-H), 8.16 (d, 2H, *J*=3.17 Hz, Ar-H), 8.21 (d, 2H, *J*=3.17 Hz, Ar-H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 124.7, 125.1, 126.4, 128.1, 128.5, 129.7, 130.1, 135.8, 139.4, 146.9, 162.6 ppm. MS, *m/z* 300 ($M^+$). Anal. Calcd (%) for $C_{19}H_{12}N_2O_2$: C, 75.99; H, 4.03; N, 9.33. Found: C, 75.98; H, 4.01; N, 9.36.

**9-(4-aminophenyl)acridine (1e)**



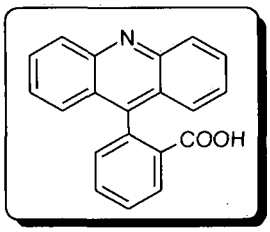
Yield 38%. m.p. 270-272 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3334 (-$NH_2$), 1650.46 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 4.07 (br, s, 2H, $NH_2$), 7.26 (t, 2H, *J*=3.18 Hz, Ar-H), 7.61-7.64 (m, 6H, Ar-H), 8.14 (d, 2H, *J*=3.17 Hz, Ar-H), 8.23 (d, 2H, *J*=3.17 Hz, Ar-H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 124.9, 125.4, 126.7, 128.2, 128.6, 129.9, 130.2, 135.8, 137.7, 147.1, 148.5 ppm. MS, *m/z* 270 ($M^+$). Anal. Calcd (%) for $C_{19}H_{14}N_2$: C, 84.42; H, 5.22; N, 10.36. Found: C, 84.47; H, 5.31; N, 10.38.

**9-(4-hydroxyphenyl)acridine (1f)**



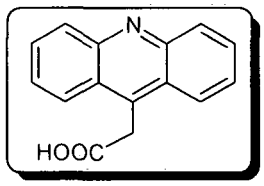
Yield 20%. m.p. >300 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3632 (-OH), 1650.46 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.05-7.35 (m, 6H, Ar-H), 7.56-7.97 (m, 6H, Ar-H), 8.68 (br, s, $^1$H, -OH) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 124.9, 125.4, 126.7, 128.2, 128.6, 129.9, 130.2, 135.8, 137.7, 147.1, 148.5 ppm. MS, *m/z* 271 ($M^+$). Anal. Calcd (%) for $C_{19}H_{13}NO$: C, 84.11; H, 4.83; N, 5.16. Found: C, 84.10; H, 4.85; N, 5.17.

**2-(acridin-9-yl)benzoic acid (1g & 1k)**



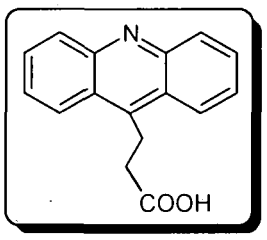

Yield 64%. m.p. 265-266 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3456 (OH), 1737.41 (C=O), 1652.64 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.55 (t, 2H, *J*=3.01 Hz, ArH), 7.70 (t, 2H, *J*=3.01 Hz, ArH), 8.14-8.28 (m, 4H, ArH) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 119.3, 125.9, 126.8, 127.4, 128.1, 128.3, 128.7, 132.8, 138.1, 141.3, 147.9, 191.9 ppm. MS, *m/z* 299 ($M^+$). Anal. Calcd (%) for $C_{20}H_{13}NO_2$: C, 80.25; H, 4.38; N, 4.68. Found: C, 80.21; H, 4.34; N, 4.65.

**2-(acridin-9-yl)acetic acid (1h)**



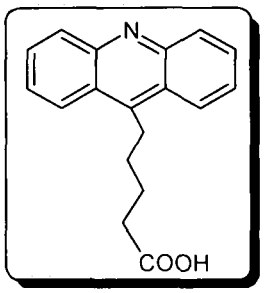

Yield 31%. m.p. Decomp. IR (KBr) ($\nu_{max}/cm^{-1}$) 3446 (OH), 1731.83 (C=O), 1637.76 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.85 (s, 2H, $CH_2$), 7.51 (t, 2H, *J*=2.98 Hz, ArH), 7.67 (t, 2H, *J*=2.99 Hz, ArH), 8.10-8.23 (m, 4H, ArH) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 54.0, 124.3, 125.5, 127.5, 128.5, 129.3, 135.4, 147.5, 200.3 ppm. MS, *m/z* 237 ($M^+$); Anal. Calcd (%) for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.96; H, 4.68; N, 5.87.

**3-(acridin-9-yl)propanoic acid (1i)**




Yield 67%. m.p. 305-3078 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3440 (OH), 1732.96 (C=O), 1638.91 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.50 (t, 2H, *J*=3.01 Hz, $CH_2$), 2.71 (t, 2H, *J*=3.02 Hz, $CH_2COOH$), 7.54 (t, 2H, *J*=2.93 Hz, ArH), 7.66 (t, 2H, *J*=2.93 Hz, ArH), 8.00-8.17 (m, 4H, ArH) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 27.0, 35.6, 124.9, 125.8, 127.6, 128.8, 129.3, 135.3, 147.1, 200.7 ppm. MS, *m/z* 251 ($M^+$). Anal. Calcd (%) for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.43; H, 5.23; N, 5.61.

**5-(acridin-9-yl)pentanoic acid (1j)**




Yield 71%. m.p. 265-69 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3442 (OH), 1731.61 (C=O), 1637.13 (C=N); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.24-1.20 (m, 4H, $2CH_2$), 1.98 (t, 2H, *J*=2.99 Hz, $CH_2COOH$), 2.11 (t, 2H, *J*=2.97 Hz, $ArCH_2$), 7.58 (t, 2H, *J*=2.97 Hz, ArH), 7.69 (t, 2H, *J*=2.96 Hz, ArH), 8.05-8.07 (m, 4H, ArH) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 22.8, 27.0, 35.6, 54.0, 124.9, 125.8, 127.4, 128.6, 129.5, 135.3, 147.1, 200.3 ppm. MS, *m/z* 279 ($M^+$). Anal. Calcd (%) for $C_{19}H_{21}NO_2$: C, 77.40; H, 6.13; N, 5.0. Found: C, 77.45; H, 6.12; N, 4.99.

# Section B

## Environment-friendly and solventfree synthesis of symmetrical bis-imines under microwave irradiation

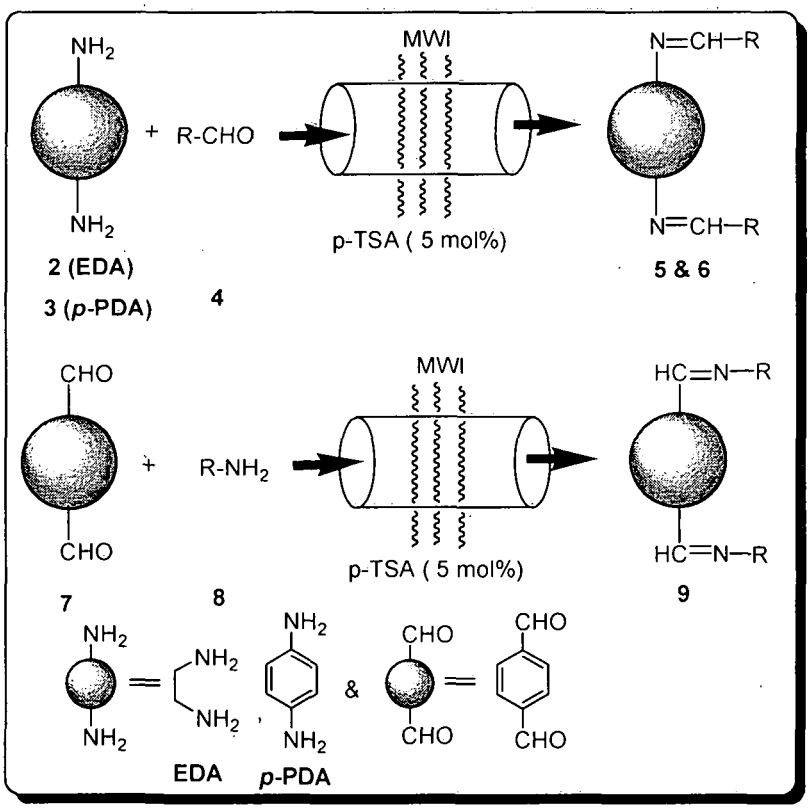
**Introduction:**

Imines are valuable functional groups or chemical compounds containing a carbon nitrogen double (C=N) bond. Condensation reaction of aldehydes and primary amines resulting imines, commonly called Schiff bases (first discovered by Schiff in 1864) or azomethines are well known in organic synthesis. In inorganic chemistry, these versatile Schiff bases are used as chelate for metal ion complexation.[17] Some of these metal complexes are used as catalysts in various organic reactions.[18] Biologically, imines show anti-convulsant, antidepressant, analgesic, anti-inflammatory, antiplatelet, antimalarial, antimicrobial, antibacterial, and antiviral activity.[19] Similar to imines, bis-imines (bis-Schiff bases) also finds uses as analytical, medicinal, polymer and liquid crystalline materials,[20] because of their model character. Most of the biologically active nitrogen containing heterocyclic compounds and biologically active inorganic metal complexes are synthesized using bis-imines as starting materials and as synthetic intermediates.[21]

The C=N bond in imines often suffer exchange reaction[22] as well as hydrolysis. However, such reversible C=N bond formation[23] is sometimes very useful to synthesize the most thermodynamically stable macrocyclic[24] and interlocked compounds.[25] Also, there are literatures available on the heterocyclic substituted bis-imines and some of the fused bis-imines showing interesting bio-activity,[26] e.g. bis-imines of isatin and their derivatives are known to possess a wide spectrum of pharmacological properties including antibacterial, antifungal, anti-HIV and antiviral activity.[27]

Different types of bis-imines were required in one of our proposed programmes. The conventional synthesis of such compounds involves longer reaction time using volatile organic solvents followed by extensive separation and/or purification.[28] As a part of our green chemistry programme and practice,[29] in embracing the principles of green chemistry,[30] herein we want to divulge a simple and general approach for the synthesis of

bis-imines, by a modification of classical methods using 5 mol% *p*-Toluenesulphonic acid (TSA) as a cheap catalyst (also being water soluble, can be easily removed from the reaction mixture) under microwave irradiation (MWI) without using any solvent (**Scheme 1**), thereby paving the way for an environmentally benign condition.




**Scheme 3:** General reaction scheme for the synthesis of bis-imines (**5**), (**6**) and (**9**)

Use of microwave energy for the enhancement of organic reactions, i.e. MORE (Microwave Organic Reactions Enhancement) provides greener reaction condition coupled with clean product, increase yield and better time economy.[31] The present investigation is aimed at looking alternative conditions for the synthesis of bis-imines with the following objectives: – (a) search for milder reaction condition (b) to reduce the reaction time (c) to increase the yield (d) to promote economical and environmental friendly experimental (green procedures) by performing the reaction under microwave and solvent less condition (e) to use cost-effective, mild and economical catalysts.

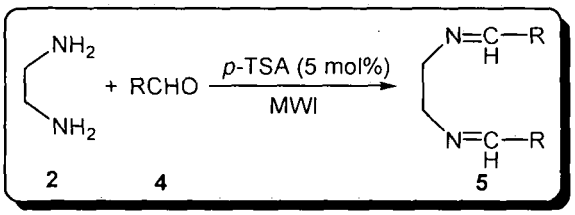
**Materials and Methods:**

Melting points were determined in open capillary tubes on a Büchi 504 apparatus and are uncorrected. IR spectra were recorded in KBr pallets on a Nicolet (Impact 410) FT-IR spectrophotometer. $^{1}H$ NMR & $^{13}C$ NMR spectra were recorded on a JNM ECS 400 MHz NMR spectrophotometer (JEOL) using Tetramethylsilane (TMS) as the internal standard. Microwave synthesis system used was (model CAT-2R) from Catalyst$^{TM}$ systems. The reactions were monitored by thin layer chromatography using aluminium sheets with silica gel $60F_{254}$ (Merck). All the chemicals used as received.

**Results and Discussion:**

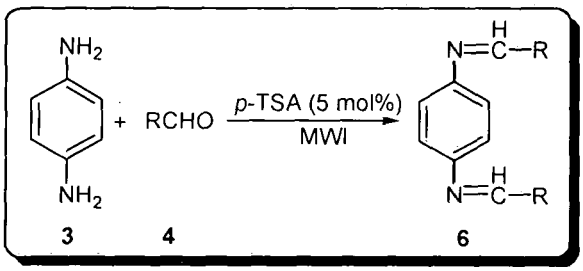
An inspection of the literatures revealed that synthesis of bis-imine was accomplished by using a mixture of aldehyde derivatives and aryl/alkyl diamine (or amine derivatives and aryl/alkyl dialdehyde) in the presence of anhydrous $MgSO_4$ in dry dichloromethane at room temperature for about 15 hours[32] or refluxing the reactants in ethanolic solution[33] and toxic solvent like benzene.[34] In recent years, environmentally benign synthetic methods have received a considerable attention and some solvent-free protocols have been developed. Varma *et al* reported clay catalyzed synthesis of monoimines and enamines under solvent-free conditions using microwave irradiation.[35] The condensation reaction of aldehyde and amines in water suspension medium was also possible as shown by Tanaka *et al.*[36] Ancker *et al*[37] reported synthesis of bis-imines by (i) a solvent-free method, grinding and (ii) using PEG (polyethylene glycol) as a benign reaction medium. However, the reported procedures have some drawbacks in terms of yield, reaction time, wide spectrum of generality, harsh reaction condition, use of costly and environmentally harmful catalysts etc. In this regard, hence, there is still a demand for developments by looking at alternative methodologies. In our green chemistry approach, we wanted to search for the conditions where the substrates would be used in stoichiometric ratios and the catalyst used would be in minimum amount. We studied the effects of *p*-TSA, under thermal as well as MWI in the absence of solvent to optimise the reaction between

ethylene diamine (EDA) and benzaldehyde. Under MWI, within few min we got the products with excellent to very good yields as compared to several hours under thermal heating condition, so we continued our generalization under MWI and those results have been discussed here.




**Scheme 4:** Synthesis of bis-imines (**5**) from EDA (**2**) and aldehyde derivatives (**4**)

In order to extend the scope, generality and to have a short library of bis-imines we varied both the amine and aldehyde part. Synthesis of bis-imines (**5**) from EDA (**2**) and aldehyde derivatives (**4**) (**Scheme 4**) has been elaborated in **Table 4** (entries **a-m**). Good to excellent yields (61-99%) of the products were obtained. As compared to aromatic ones (entries **a-j**, **Table 4**), aliphatic aldehydes provided somewhat lower yields (61-63%) of the products (entries **j-m**, **Table 4**). **Table 5** (entries **a-m**) summarises the results of the reaction between *p*-phenylenediamine (*p*-PDA) (**3**) and aldehyde derivatives (**4**) (**Scheme 5**) providing 69-99% yield of the products. Similar to **Scheme 3**, here also aliphatic aldehydes (entries **j-m**, **Table 5**) provided somewhat lower yields (69-72%) as compared to aromatic ones.




**Scheme 5:** Synthesis of bis-imines (**6**) from *p*-PDA (**3**) and aldehyde derivatives (**4**)

**Table 4:** Synthesis of bis-imines (**5**) from EDA (**2**) and aldehyde derivatives (**4**)

| Entry | R | MW power (watt)/ time (min) | Yield (%)[a] |
|---|---|---|---|
| a | Ph | 600/10 | 75 |
| b | Ph-CH=CH- | 600/13 | 99 |
| c | *p*-OMe-$C_6H_4$ | 600/8 | 98 |
| d | *p*-Cl-$C_6H_4$ | 600/7 | 99 |
| e | *o*-Cl-$C_6H_4$ | 600/7 | 98 |
| f | *p*-OH-$C_6H_4$ | 600/3 | 95 |
| g | *o*-OH-$C_6H_4$ | 600/2 | 99 |
| h | *p*-Me-$C_6H_4$ | 600/6 | 98 |
| i | *p*-$NO_2$-$C_6H_4$ | 600/9 | 83 |
| j | *m*-$NO_2$-$C_6H_4$ | 600/2 | 98 |
| k | Me | 600/10 | 61 |
| l | Me-$(CH_2)_2$- | 600/10 | 63 |
| m | Me-$(CH_2)_3$- | 600/9.5 | 63 |

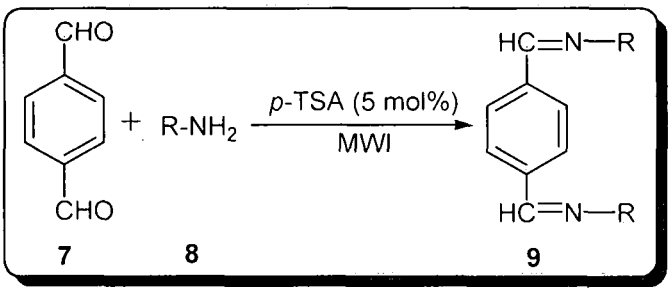
[a] Isolated yield

**Table 5:** Synthesis of bis-imines (**6**) from *p*-PDA (**3**) and aldehyde derivatives (**4**)

| Entry | R | MW power (watt)/ time (min) | Yield (%)[a] |
|---|---|---|---|
| a | Ph | 450/5 | 80 |
| b | Ph-CH=CH | 450/2.5 | 99 |
| c | *p*-OMe-$C_6H_4$ | 450/3 | 96 |
| d | *p*-Cl-$C_6H_4$ | 450/3 | 99 |
| e | *o*-Cl-$C_6H_4$ | 450/3 | 98 |
| f | *p*-OH-$C_6H_4$ | 450/3 | 92 |
| g | *o*-OH-$C_6H_4$ | 450/3 | 93 |
| h | *p*-Me-$C_6H_4$ | 450/6 | 98 |
| i | *p*-$NO_2$-$C_6H_4$ | 450/7 | 87 |
| j | *m*-$NO_2$-$C_6H_4$ | 450/3 | 98 |
| k | Me | 600/10 | 69 |
| l | Me-$(CH_2)_2$ | 600/10 | 69 |
| m | Me-$(CH_2)_3$ | 600/10 | 72 |

[a] Isolated yield

We also checked the reaction of terephthaldehyde (**7**) with amine derivatives (**8**) to provide bis-imines (**9**) (**Scheme 6**) in 71-92% yields. Summary of the synthesis of (**9**) has been described in **Table 6** (entries **a-k**).




**Scheme 6:** Synthesis of bis-imines (**9**) from terephthaldehyde (**7**) and amine derivatives (**8**)

**Table 6**: Synthesis of bis-imines (**9**) from terephthaldehyde (**7**) and amine derivatives (**8**)

| Entry | R | MW power (watt)/ time (min) | Yield (%)[a] |
|---|---|---|---|
| a | Ph | 450/9 | 85 |
| b | *p*-Cl-$C_6H_4$ | 450/7 | 90 |
| c | *o*-Cl-$C_6H_4$ | 450/6.5 | 91 |
| d | *m*-Cl-$C_6H_4$ | 450/9 | 89 |
| e | *p*-$NO_2$-$C_6H_4$ | 450/8 | 78 |
| f | *m*-$NO_2$-$C_6H_4$ | 450/5 | 88 |
| g | *p*-Me-$C_6H_4$ | 450/7 | 93 |
| h | *o*-Me-$C_6H_4$ | 450/7 | 92 |
| i | *p*-OMe-$C_6H_4$ | 450/8 | 82 |
| j | Me-$(CH_2)_3$ | 600/12 | 71 |
| k | Ph-$CH_2$ | 600/10 | 75 |

[a] Isolated yield

As can be seen from **Table 4, 5** and **6**, the reaction appears to be quite general and diverse types of bis-imines can be obtained by varying the amine part or the aldehydic part. Several pharmacologically important moieties can be introduced into the imines,

e.g. -Cl, -$NO_2$, -Me, -OMe, -OH, double bond etc. showing their tolerance to the reaction condition. Aliphatic and aromatic aldehydes and amines responded equally to the reaction. As reported by Fujioka *et al*[38] and Gogoi *et al*[39] where reaction between EDA and aldehyde derivatives afforded 2-dihydroimidazole derivatives, however, in our case we did not observe such product. Notably, present method of synthesis of bis-imines reduces the reaction time from several hours to few minutes. Using *p*-TSA as catalyst, we received excellent to good yields of the products. Being water soluble, the catalyst can be easily removed from the reaction mixture making the purification easier-an important point in the context of green chemistry. Finally, recrystallization from ethanol furnished the pure products. Overheating and increase in the reaction time didn't increase the yield; rather we got some insoluble waste materials.

We have also studied the effect of amount of *p*-TSA in all the reaction schemes. For this, the reaction between EDA (**2**) & *p*-chlorobenzaldehyde (**4d**), *p*-PDA (**3**) & *p*-chlorobenzaldehyde (**4d**), terephthaldehyde (**7**) & *p*-chloroaniline (**8b**) were chosen as the model reactions in **Schemes 4, 5** and **6** respectively. Different amounts of catalyst loading were tested. The results are shown in **Table 7**. In the absence of catalyst, the reaction proceeded to give poor yields. Using catalyst the yields were improved. Consequently, 5 mol% of *p*-TSA was found to be the optimum one.

**Table 7**: Effect of catalyst (*p*-TSA) loading

| Entry | Catalyst loading | Yield (%)[a] | | |
|---|---|---|---|---|
| | | Table 4, entry d | Table 5, entry d | Table 6, entry b |
| 1 | No catalyst | 56 | 45 | 53 |
| 2 | 3 mol % | 78 | 74 | 76 |
| 3 | 5 mol% | 99 | 99 | 90 |
| 4 | 7 mol% | 98 | 99 | 91 |
| 5 | 10 mol% | 81 | 84 | 81 |

[a] Isolated yield

Similarly, the optimum power of MW in each scheme was evaluated.

The products were supported by the absence of the carbonyl and primary amine bands of the reactants in IR spectra, and the presence of an imine $\nu_{(C=N)}$ band within 1590-1680 $cm^{-1}$ region. In the $^1H$ NMR spectra, the azomethine (CH=N) protons appear within $\delta$= 8.00–9.00 ppm. In the $^{13}C$ NMR spectra, the imine carbon appears within 150.00-170.00 ppm. Progress of the reaction can be easily monitored by IR and $^1H$ NMR spectroscopy in addition to TLC. In IR spectra, appearance of C=N peak and disappearance of >C=O peak and in $^1H$ NMR spectra, appearance of azomethine (CH=N) protons are the focusing point.

**Conclusion:**

The procedure described above has several advantages over existing methods for the synthesis of imines. The use of any drying agents or apparatus to remove the water formed during the reaction is not necessary. Time economy observed is amazing and also *p*-TSA as catalyst is very cheap and effective, which can be easily removed from the reaction mixture. Moreover, use of 'MWI' considerably improves the yield of the products as compared to the 'classical heating'.

Different steps (reaction, separation, and purification) in a synthesis contribute to the environmental footprint of the process. Since, our method reduces the amount of solvent used, hence the methodology is environmentally benign. Owing to the important precursors for the synthesis of several biologically versatile heterocyclic rings, our finding may be helpful.

In conclusion, a short library of symmetrical bis-imines has been constructed from the reaction between amines and aldehydes under MWI.

**Experimental Section:**

**General experimental procedure for the synthesis of bis-imines (5), (6) and (9):**

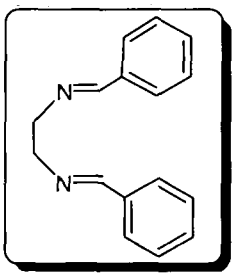
Typically, a mixture of EDA (**2**, 0.134 ml, 2 mmol), benzaldehyde (**4a**, 0.406 ml, 4 mmol) and pure *p*-TSA (19 mg, 5 mol%) were irradiated in a microwave synthesis

system (600 W) for 10 min. The reaction was monitored by TLC. After completion of the reaction, the crude product (**5a**) was dissolved in minimum amount of ethyl acetate and upon addition of hexane, bis-imine precipitated out. The precipitate was filtered and then dried in vacuum to afford the bis-imine (0.178 g, 75% yield). m.p. 53.6-54.3 °C.

Applying this reaction procedure we have synthesised and characterized all the bis-imine compounds. The pure product was characterized by IR, $^{1}$H-NMR, $^{13}$C-NMR spectroscopy, mass spectrometry, elemental analysis and comparison of melting point with reported one (which are available in the literature).

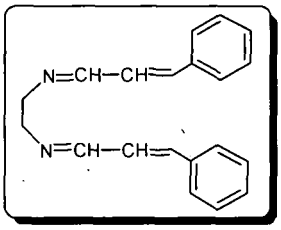
**Physical and spectral data:**

***N*$^{1}$,*N*$^{2}$-dibenzylideneethane-1,2-diamine (5a)**



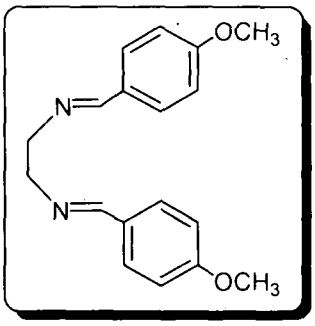
Yield 75%. m.p. 53.6-54.3 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1637.09 (-CH=N-); $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.94 (s, 4H, $2CH_2$), 7.34-7.36 (m, 6H, arom), 7.65-7.67 (m, 4H, arom), 8.25 (s, 2H, 2N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 61.8, 128.4, 128.7, 130.8, 136.3, 162.9; MS, *m/z* 237.13 ($M^+$); Anal. Calcd (%) for $C_{16}H_{16}N_2$: C, 81.32; H, 6.82; N, 11.85. Found C, 81.33; H, 6.85; N, 11.86.

***N*$^{1}$,*N*$^{2}$-bis(3-phenylallylidene)ethane-1,2-diamine (5b)**



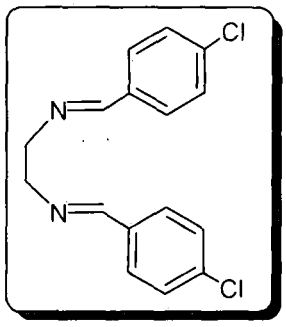
Yield 99%. m.p. 101.7-103.6 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1638.12 (-CH=N-); $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.84 (s, 4H, $2CH_2$), 6.85-6.96 (m, 4H, 2CH=CH), 7.29-7.37 (m, 6H, arom), 7.44-7.47 (m, 4H, arom), 8.04 (d, 2H, *J*=7.32 Hz, 2N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 61.5, 127.2, 127.6, 128.5, 12.78, 135.6, 142.0, 164.31; MS, *m/z* 288.16 ($M^+$); Anal. Calcd (%) for $C_{20}H_{20}N_2$: C, 83.30; H, 6.99; N, 9.71. Found C, 83.34; H, 7.03; N, 9.69.

**$N^1$,$N^2$-bis(4-methoxybenzylidene)ethane-1,2-diamine (5c)**



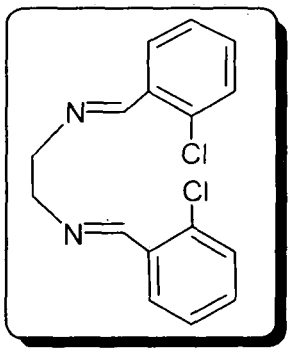

Yield 98%. m.p. 107.5-109.6 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1641.12 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.76 (s, 6H, $2CH_3$), 3.91 (s, 4H, $2CH_2$), 6.89 (d, 4H, *J*=8 Hz, arom), 7.64 (d, 4H, *J*=8.8 Hz, arom), 8.20 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 55.4, 61.8, 128.3, 128.7, 130.8, 136.3, 162.9; MS, *m/z* 296.15 ($M^+$); Anal. Calcd (%) for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found C, 72.99; H, 6.84; N, 9.46.

**$N^1$,$N^2$-bis(4-chlorobenzylidene)ethane-1,2-diamine (5d)**



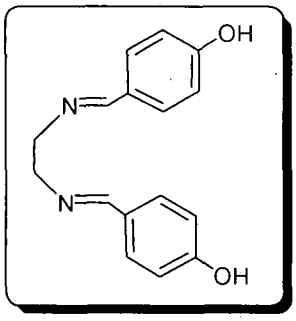

Yield 99%. m.p. 149.1-150.3 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1637.09 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.97 (s, 4H, $2CH_2$), 7.36 (d, 4H, *J*=8.24 Hz, arom), 7.64 (d, 4H, *J*=8.24 Hz, arom), 8.24 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 61.5, 128.5, 128.9, 134.6, 136.6, 161.4; MS, *m/z* 304.05 ($M^+$); Anal. Calcd (%) for $C_{16}H_{14}Cl_2N_2$: C, 62.97; H, 4.62; N, 9.18. Found C, 62.99; H, 4.66; N, 9.16.

**$N^1$,$N^2$-bis(2-chlorobenzylidene)ethane-1,2-diamine (5e)**



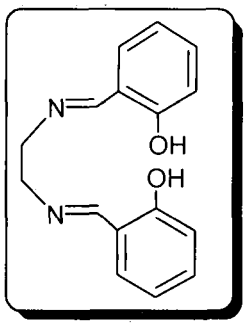

Yield 98%. m.p. 148.4-149.7 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1637.21 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.98 (s, 4H, $2CH_2$), 7.37-7.65 (m, 8H, arom), 8.24 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 61.6, 128.3, 128.8, 131.3, 132.6, 133.9, 136.5, 162.0; MS, *m/z* 304.05 ($M^+$); Anal. Calcd (%) for $C_{16}H_{14}Cl_2N_2$: C, 62.97; H, 4.62; N, 9.18. Found C, 63.00; H, 4.66; N, 9.18.

**$N^1$,$N^2$-bis(4-hydroxybenzylidene)ethane-1,2-diamine (5f)**



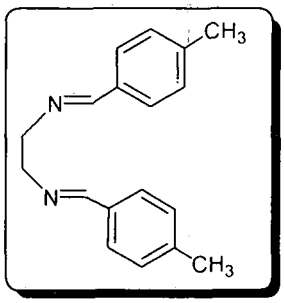

Yield 95%. m.p. 126.7-127.4 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3479.21 (-OH), 1637.18 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.93 (s, 4H, $2CH_2$), 6.89 (d, 4H, *J*=8.24 Hz, arom), 7.31 (d, 4H, *J*=8.72 Hz, arom), 8.31 (s, 2H, N=CH), 9.37 (s, 2H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 61.7, 128.1, 128.4, 131.1, 135.9, 162.7; MS, *m/z* 268.12 ($M^+$); Anal. Calcd (%) for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found C, 71.61; H, 6.04; N, 10.42.

**$N^1$,$N^2$-bis(2-hydroxybenzylidene)ethane-1,2-diamine (5g)**



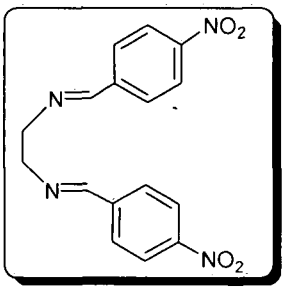

Yield 100%. m.p. 125.9-127.3 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3477.16 (-OH), 1667.89 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.93 (s, 4H, $2CH_2$), 6.83-6.94 (m, 4H, arom), 7.23-7.30 (m, 4H, arom), 8.35 (s, 2H, N=CH), 13.21 (s, 2H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 61.6, 128.3, 128.7, 129.9, 130.8, 132.5, 136.3, 162.9; MS, *m/z* 268.12 ($M^+$); Anal. Calcd (%) for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found C, 71.61; H, 6.04; N, 10.41.

**$N^1$,$N^2$-bis(4-methylbenzylidene)ethane-1,2-diamine (5h)**



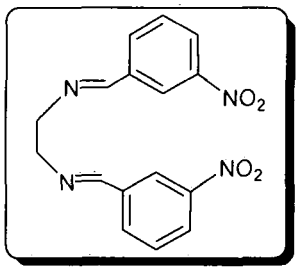

Yield 98%. m.p. 159.7-160.2 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1636.87 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.35 (s, 6H, $2CH_3$), 3.92 (s, 4H, $2CH_2$), 7.19 (d, 4H, *J*= 6.88 Hz, arom), 7.55 (d, 4H, *J*=6.88 Hz, arom), 8.23 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 28.8, 61.6, 128.0, 129.2, 133.5, 140.7, 162.5; MS, *m/z* 264.16 ($M^+$); Anal. Calcd (%) for $C_{18}H_{20}N_2$: C, 81.78; H, 7.63; N, 10.60. Found C, 81.79; H, 7.66; N, 10.57.

**$N^1$,$N^2$-bis(4-nitrobenzylidene)ethane-1,2-diamine (5i)**



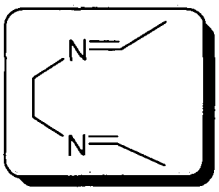
Yield 83%. m.p. 161.7-162.9 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1636.89 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.94 (s, 4H, $2CH_2$), 7.87 (d, 4H, *J*=7.32 Hz, arom), 8.21 (d, 4H, *J*=7.32 Hz, arom), 8.45 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 61.5, 127.7, 128.3, 132.8, 147.9, 161.3; MS, *m/z* 326.10 ($M^+$); Anal. Calcd (%) for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17. Found C, 58.91; H, 4.35; N, 17.14.

**$N^1$,$N^2$-bis(3-nitrobenzylidene)ethane-1,2-diamine (5j)**



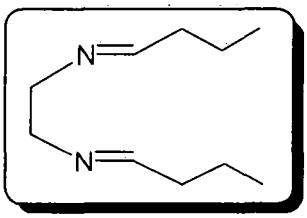
Yield 98%. m.p. 162.5-164.1 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1637.06 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 4.03 (s, 4H, $2CH_2$), 7.56-8.38 (m, 8H, arom), 8.53 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 61.44, 123.00, 125.32, 129.85, 133.84, 137.86, 148.79, 160.27; MS, *m/z* 326.10 ($M^+$); Anal. Calcd (%) for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17. Found C, 58.91; H, 4.36; N, 17.15.

**$N^1$,$N^2$-diethylideneethane-1,2-diamine (5k)**



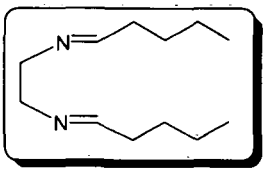
Yield 27%. m.p. 103.5-104.8 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1643.06 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.20-1.21 (m, 6H, $CH_3$), 3.93 (s, 4H, $2CH_2$), 8.23 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 16.1, 62.0, 158.6; MS, *m/z* 113.10 ($M^+$); Anal. Calcd (%) for $C_6H_{12}N_2$: C, 64.24; H, 10.78; N, 24.97. Found C, 64.28; H, 10.76; N, 24.95.

**$N^1$,$N^2$-dibutylideneethane-1,2-diamine (5l)**



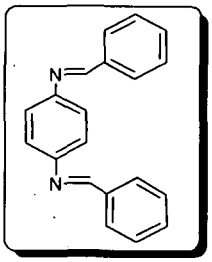
Yield 31%. m.p. 98.6-101.3 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1643.06 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.17-1.21 (m, 6H, $CH_3$), 1.67-2.24 (m, 8H, $CH_2$), 3.97 (s, 4H, $2CH_2$), 8.24 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 16.2, 27.1, 28.7, 61.5, 159.2; MS, *m/z* 169.17 ($M^+$); Anal. Calcd (%) for $C_{10}H_{20}N_2$: C, 71.37; H, 11.98; N, 16.65. Found C, 71.39; H, 11.95; N, 16.65.

**$N^1$,$N^2$-dipentylideneethane-1,2-diamine (5m)**



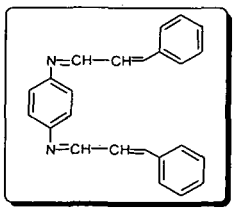
Yield 31%. m.p. 87.7-89.1 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1637.91 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.03-1.09 (m, 6H, $CH_3$), 1.55-2.14 (m, 8H, $CH_2$), 2.27-2.31 (m, 4H, $CH_2$), 3.97 (s, 4H, $2CH_2$), 8.24 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 16.1, 27.2, 27.4, 28.7, 61.5, 159.5; MS, *m/z* 197.2 ($M^+$); Anal. Calcd (%) for $C_{12}H_{24}N_2$: C, 73.41; H, 12.32; N, 14.27. Found C, 73.43; H, 12.31; N, 14.24.

**$N^1$,$N^4$-dibenzylidenebenzene-1,4-diamine (6a)**



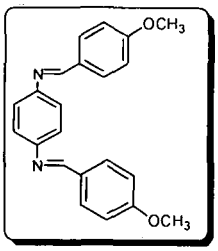
Yield 80%. m.p. 141-141.7 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1613.80 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.28 (s, 4H, arom), 7.47-7.49 (m, 6H, arom), 7.90-7.92 (m, 4H, arom), 8.50 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 121.8, 128.7, 131.3, 136.2, 149.9, 159.7; MS, *m/z* 285.13 ($M^+$); Anal. Calcd (%) for $C_{20}H_{16}N_2$: C, 84.48; H, 5.67; N, 9.85. Found C, 84.47; H, 5.76; N, 9.86.

**$N^1$,$N^4$-bis(3-phenylallylidene)benzene-1,4-diamine (6b)**



Yield 99%. m.p. 226.9-228.3 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1615.77 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.13-7.16 (m, 4H, 2CH=CH), 7.25 (s, 4H, arom), 7.36-7.42 (m, 6H, arom), 7.56-7.54 (m, 4H, arom), 8.32 (d, 2H, *J*=7.32 Hz, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 122.01, 127.61, 128.70, 129.03, 129.70, 135.69, 144.11, 149.82, 161.09; MS, *m/z* 336.16 ($M^+$); Anal. Calcd (%) for $C_{24}H_{20}N_2$: C, 85.68; H, 5.99; N, 8.33. Found C, 85.67; H, 6.01; N, 8.34.

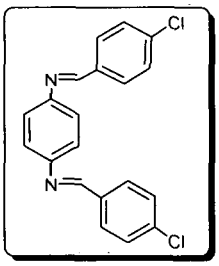
**$N^1$,$N^4$-bis(4-methoxybenzylidene)benzene-1,4-diamine (6c)**



Yield 96%. m.p. 206.7-207.9 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1613.80 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.85 (s, 6H, $OCH_3$), 6.96 (d, 4H, *J*=8.24 Hz, arom), 7.21 (d, 4H, *J*=8 Hz, arom), 7.83 (s, 4H, arom), 8.39 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 55.1,

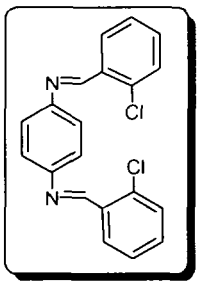
114.1, 121.4, 129.0, 129.7, 149.6, 158.6, 161.9; MS, *m/z* 344.15 ($M^+$); Anal. Calcd (%) for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found C, 76.75; H, 5.86; N, 8.14.

**$N^1$,$N^4$-bis(4-chlorobenzylidene)benzene-1,4-diamine (6d)**



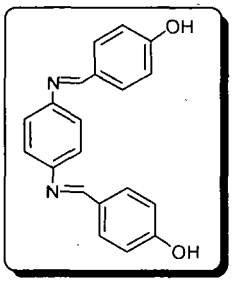
Yield 99%. m.p. 202.3-202.9 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1637.42 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.27 (s, 4H, arom), 7.44 (d, 4H, *J*=8.72 Hz, arom), 7.84 (d, 4H, *J*=8.24 Hz, arom), 8.46 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 121.9, 129.1, 130.0, 134.8, 137.4, 149.8, 158.2; MS, *m/z* 353.05 ($M^+$); Anal. Calcd (%) for $C_{20}H_{14}Cl_2N_2$: C, 68.00; H, 3.99; N, 7.93. Found C, 68.01; H, 4.01; N, 7.91.

**$N^1$,$N^4$-bis(2-chlorobenzylidene)benzene-1,4-diamine (6e)**



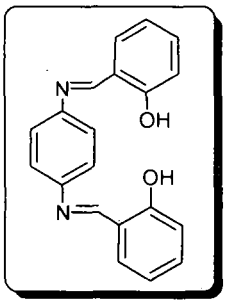
Yield 98%. m.p. 203-204.1 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1637.42 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.28 (s, 4H, arom) 7.47-7.85 (m, 8H, arom), 8.46 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 121.9, 128.8, 129.3, 129.7, 130.2, 134.8, 137.4, 149.7, 158.4; MS, *m/z* 353.05 ($M^+$); Anal. Calcd (%) for $C_{20}H_{14}Cl_2N_2$: C, 68.00; H, 3.99; N, 7.93. Found C, 68.03; H, 4.01; N, 7.96.

**$N^1$,$N^4$-bis(4-hydroxybenzylidene)benzene-1,4-diamine (6f)**



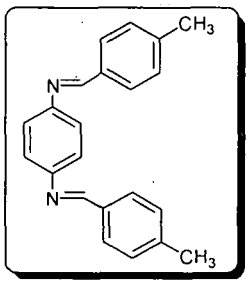
Yield 92%. m.p. 259.9-261 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 3533.76 (-OH), 1643.45 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 6.65-7.06 (m, 8H, arom), 7.69-7.76 (m, 4H, arom), 8.41 (s, 2H, N=CH), 9.78 (s, 2H, OH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 115.0, 115.6, 121.5, 121.8, 130.4, 131.9, 158.9; MS, *m/z* 316 ($M^+$); Anal. Calcd (%) for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86. Found C, 75.92; H, 5.13; N, 8.89.

**$N^1$,$N^4$-bis(2-hydroxybenzylidene)benzene-1,4-diamine (6g)**



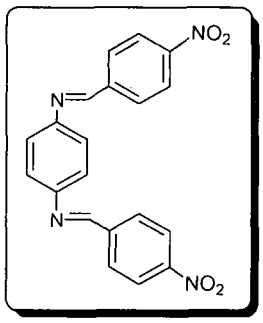
Yield 93%. m.p. 257-258.3 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3533.76 (-OH), 1643.45 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 6.68-7.12 (m, 8H, arom), 7.41-7.49 (m, 4H, arom), 8.41 (s, 2H, N=CH), 9.78 (s, 2H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 115.1, 115.6, 115.7, 121.7, 121.9, 129.8, 130.4, 131.9, 158.9; MS, *m/z* 316 ($M^+$); Anal. Calcd (%) for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86. Found C, 75.92; H, 5.13; N, 8.89.

**$N^1$,$N^4$-bis(4-methylbenzylidene)benzene-1,4-diamine (6h)**



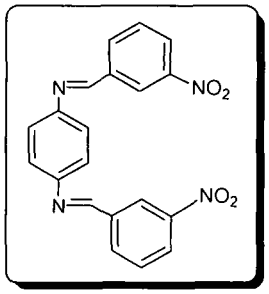
Yield 98%. m.p. 227-228.6 °C. (KBr) ($\nu_{max}$/cm$^{-1}$) 1546.24 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.31 (s, 6H, $CH_3$), 7.17-7.21 (m, 8H, arom), 7.93 (s, 4H, arom), 8.41 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 21.1, 121.1, 128.7, 129.4, 135.5, 138.6, 149.2, 159.1; MS, *m/z* 312.12 ($M^+$); Anal. Calcd (%) for $C_{22}H_{20}N_2$: C, 84.58; H, 6.45; N, 8.97. Found C, 84.60; H, 6.47; N, 8.96.

**$N^1$,$N^4$-bis(4-nitrobenzylidene)benzene-1,4-diamine (6i)**



Yield 87%. m.p. 259.1-259.9 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1607.67 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.36 (s, 4H, arom), 7.41 - 8.03 (m, 8H, arom), 8.42 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 117.3, 123.5, 129.2, 129.7, 149.9, 158.6, 162.3 MS, *m/z* 374.09 ($M^+$); Anal. Calcd (%) for $C_{20}H_{14}N_4O_4$: C, 64.17; H, 3.77; N, 14.97. Found C, 64.15; H, 3.79; N, 14.96.

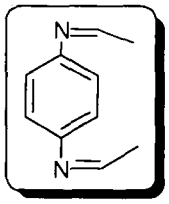
**$N^1$,$N^4$-bis(3-nitrobenzylidene)benzene-1,4-diamine (6j)**



Yield 98%. m.p. 259-260.9 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1607.67 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.37 (s, 4H, arom), 7.67-7.73 (m, 6H, arom), 8.01-8.12 (m, 4H, arom), 8.45 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 121.7, 127.9, 128.7, 129.3, 130.5,

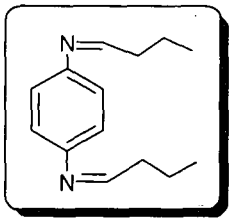
131.4, 136.2, 149.7, 160.6; MS, *m/z* 374.09 ($M^+$); Anal. Calcd (%) for $C_{20}H_{14}N_4O_4$: C, 64.17; H, 3.77; N, 14.97. Found C, 64.19; H, 3.76; N, 14.96.

**$N^1$,$N^4$-diethylidenebenzene-1,4-diamine (6k)**



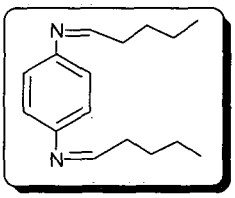
Yield 87%. m.p. 187.1-188.9 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1643.28 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.12-1.14 (m, 6H, $CH_3$), 7.28 (s, 4H, arom), 8.43 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 16.2, 127.8, 128.7, 149.7, 160.3; MS, *m/z* 161.10 ($M^+$); Anal. Calcd (%) for $C_{10}H_{12}N_2$: C, 74.97; H, 7.55; N, 17.48. Found C, 74.96; H, 7.56; N, 17.46.

**$N^1$,$N^4$-dibutylidenebenzene-1,4-diamine (6l)**



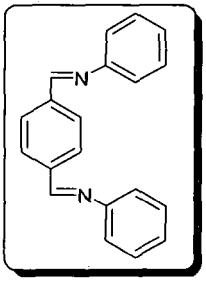
Yield 88%. m.p. 183.7-185.1 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1637.91 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.13-1.17 (m, 6H, $CH_3$), 1.25-2.04 (m, 8H, $CH_2$), 7.32 (s, 4H, arom), 8.41 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 16.1, 27.5, 28.7, 127.6, 128.3, 160.3; MS, *m/z* 217.2 ($M^+$); Anal. Calcd (%) for $C_{14}H_{20}N_2$: C, 77.73; H, 9.32; N, 12.95. Found C, 77.76; H, 9.33; N, 12.93.

**$N^1$,$N^4$-dipentylidenebenzene-1,4-diamine (6m)**



Yield 88%. m.p. 156.9-158.3 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1637.91 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.03-1.11 (m, 6H, $CH_3$), 1.22-1.94 (m, 8H, $CH_2$), 2.21-2.37 (m, 4H, $CH_2$), 7.29 (s, 4H, arom), 8.46 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 16.1. 27.1, 27.3, 28.8, 127.6, 128.1, 160.0; MS, *m/z* 245.2 ($M^+$); Anal. Calcd (%) for $C_{16}H_{24}N_2$: C, 78.64; H, 9.90; N, 11.46. Found C, 78.66; H, 9.93; N, 11.43.

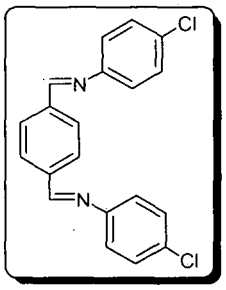
**N-(phenylimino)methyl)benzylidene)benzenamine (9a)**



Yield 85%. m.p. 161.7-163.5 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1577.71 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.22-7.25 (m, 6H, arom), 7.37-7.43 (m, 4H, arom), 7.98-8.00 (m, 4H, arom), 8.49 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 120.6, 126.0, 128.8, 128.9, 138.3,

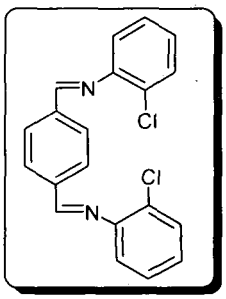
151.4, 159.1; MS, *m/z* 285.35 ($M^+$); Anal. Calcd (%) for $C_{20}H_{16}N_2$: C, 84.48; H, 5.67; N, 9.85. Found C, 84.47; H, 5.69; N, 9.86.

**N-(4-chlorophenylimino)methyl)benzylidene)-4-chlorobenzenamine (9b)**



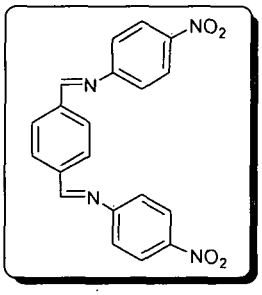

Yield 90%. m.p. 175.9-178.3 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1579.84 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.19 (d, 4H, *J*=8.72 Hz, arom), 7.36 (d, 4H, *J*=8.72 Hz, arom), 7.98-8.01 (m, 4H, arom), 8.48 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 121.7, 129.2, 129.5, 132.3, 138.3, 150.3, 159.4; MS, *m/z* 352.05 ($M^+$); Anal. Calcd (%) for $C_{20}H_{14}Cl_2N_2$: C, 68.00; H, 3.99; N, 7.93. Found C, 68.01; H, 4.01; N, 7.95.

**N-(2-chlorophenylimino)methyl)benzylidene)-2-chlorobenzenamine (9c)**



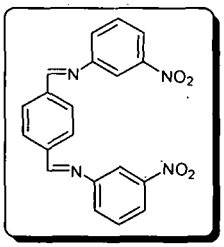

Yield 91%. m.p. 174.3-175.9 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1579.84 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.21-7.41 (m, 8H, arom), 7.99-8.01 (m, 4H, arom), 8.49 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 121.7, 128.5, 128.9, 129.2, 129.5, 132.3, 138.2, 150.1, 159.4; MS, *m/z* 352.05 ($M^+$); Anal. Calcd (%) for $C_{20}H_{14}Cl_2N_2$: C, 68.00; H, 3.99; N, 7.93. Found C, 68.02; H, 3.97; N, 7.95.

**N-(4-nitrophenylimino)methyl)benzylidene)-4-nitrobenzenamine (9d)**



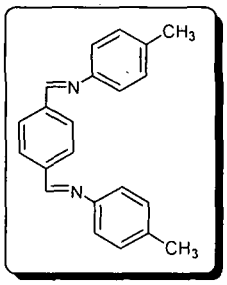

Yield 78%. m.p. 225-226.7 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1569.21 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.41 (d, 4H, *J*=8.24 Hz, arom), 7.49 (d, 4H, *J*=8.72 Hz, arom), 8.00 (s, 4H, arom), 8.48 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 122.3, 129.2, 129.7, 131.9, 139.1, 151.3, 159.4; MS, *m/z* 374.09 ($M^+$); Anal. Calcd (%) for $C_{20}H_{14}N_4O_4$: C, 64.17; H, 3.77; N, 14.97. Found C, 64.15; H, 3.76; N, 14.95.

***N*-(3-nitrophenylimino)methyl)benzylidene)-3-nitrobenzenamine (9e)**



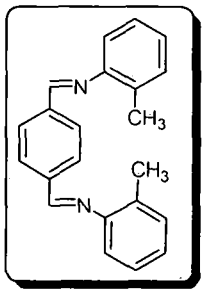
Yield 88%. m.p. 224.5-226.1 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1569.21 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.46-7.53 (m, 8H, arom), 7.98 (s, 4H, arom), 8.49 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 122.3, 128.6, 128.9, 129.2, 129.7, 131.9, 139.2, 151.3, 159.1; MS, *m/z* 374.09 ($M^+$); Anal. Calcd (%) for $C_{20}H_{14}N_4O_4$: C, 64.17; H, 3.77; N, 14.97. Found C, 64.15; H, 3.76; N, 14.98.

***N*-(*p*-tolylimino)methyl)benzylidene)-4-methylbenzenamine (9f)**



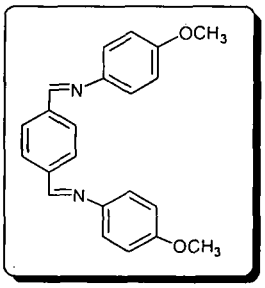
Yield 93%. m.p. 187-189.4 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1576.27 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.35 (s, 6H, $CH_3$), 7.13-7.17 (m, 8H, arom), 7.96 (s, 4H, arom), 8.48 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 21.1, 121.0, 129.1, 129.9, 136.3, 138.7, 149.2, 158.6; MS, *m/z* 312.12 ($M^+$); Anal. Calcd (%) for $C_{22}H_{20}N_2$: C, 84.58; H, 6.45; N, 8.97. Found C, 84.54; H, 6.47; N, 8.98.

***N*-(*o*-tolylimino)methyl)benzylidene)-2-methylbenzenamine (9g)**



Yield 85%. m.p. 186.7-187.5 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1576.27 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.35 (s, 6H, $CH_3$), 7.13-7.19 (m, 8H, arom), 7.97 (s, 4H, arom), 8.48 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 21.1, 121.1, 128.7, 129.1, 129.9, 130.3, 136.3, 138.7, 149.2, 158.6; MS, *m/z* 312.12 ($M^+$); Anal. Calcd (%) for $C_{22}H_{20}N_2$: C, 84.58; H, 6.45; N, 8.97. Found C, 84.55; H, 6.47; N, 8.96.

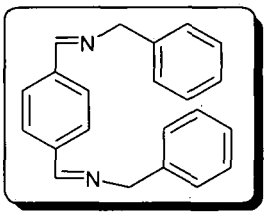
***N*-(4-methoxyphenylimino)methyl)benzylidene)-4-methoxybenzenamine (9h)**



Yield 85%. m.p. 223.5-224.3 °C. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 1576.98 (-CH=N-); $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 3.84 (s, 6H, $CH_3$), 6.95 (d, 4H, *J*=9.16 Hz, arom), 7.28 (d, 4H, *J*=8.68 Hz, arom), 7.98 (m, 4H, arom), 8.56 (s, 2H, N=CH); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_c$ 55.5, 114.5, 122.4, 128.9, 138.6, 144.6, 157.4, 158.6; MS, *m/z* 344.15 ($M^+$); Anal. Calcd (%) for $C_{22}H_{20}N_2O_2$: C,

76.72; H, 5.85; N, 8.13. Found C, 77.70; H, 5.86; N, 8.10.

**N-(benzylimino)methyl)benzylidene)(phenyl)methanamine (9i)**



Yield 99%. m.p. 87.8-90.4 °C. IR (KBr) ($\nu_{max}/cm^{-1}$) 1577.71 (-CH=N-); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$ 4.83 (s, 4H, $CH_2$), 7.24-7.35 (m, 10H, arom), 7.82 (s, 4H, arom), 8.40 (s, 2H, N=CH); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_c$ 65.2, 127.1, 128.1, 128.5, 128.6, 138.2, 139.1, 161.4; MS, *m/z* 313.16 ($M^+$); Anal. Calcd (%) for $C_{22}H_{20}N_2$: C, 84.58; H, 6.45; N, 8.97. Found C, 84.57; H, 6.47; N, 8.9

**References:**


1. Search conducted 11/03 using Beilstein CrossFire.
2. a) Albert, A. *The Acridines*, Ed. Arnold, E. (London, 1966). b) Acheson, R.M., Ed. *Acridines. The Chemistry of Heterocyclic Compounds* (Interscience Publishers: New York, 1956).
3. a) Katritzky, A.R. *Handbook of Heterocyclic Chemistry* (Pergamon Press: Oxford, 1985). b) Shanmugasundaram, P.; Prabahar, K.J.; Ramakrishnan, V.T. A new class of laser dyes from acridinedione derivatives, *J. Heterocycl. Chem.* **30**, 1003-1007 (1993). c) Islam, A.; Murugan, P.; Hwang, K.C.; Cheng, C.-H. Blue light-emitting devices based on 1,8-acridinedione derivatives, *Synth. Metals*, **139**, 347-353 (2003).
4. a) Mohan, H.; Srividya, N.; Ramamurthy, P.; Mittal, J.P. One-electron reduction of acridine-1,8-dione in aqueous solution: A pulse radiolysis study, *J. Phys. Chem. A* **101**, 2931-2935 (1997). b) Srividya, N.; Ramamurthy, P.; Ramakrishnan, V.T. Photophysical studies of acridine-(1,8)-dione dyes: A new class of laser dyes, *Spectrochim. Acta Part A*, **54**, 245-253 (1998). c) Mohan, H.; Mittal, J.P.; Srividya, N.; Ramamurthy, P. One-electron reduction of 3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-(1,8)-(2*H*,5*H*)-acridinedione: A pulse radiolysis study, *J. Phys. Chem. A*, **102**, 4444-4449 (1998).

5. Srividya, N.; Ramamurthy, P.; Shanmugasundaram, P.; Ramakrishnan, V.T. Synthesis, characterization, and electrochemistry of some acridine-1,8-dione dyes, *J. Org. Chem.* **61**, 5083-5089 (1996).
6. Dominguez, J.N. *et al* Synthesis and antimalarial effects of phenothiazine inhibitors of a Plasmodium falciparum cysteine protease, *J. Med. Chem.* **40**, 2726-2732 (1997).
7. a) Demeunynck, M.; Charmantray, F.; Martelli, A. Interest of acridine derivatives in the anticancer chemotherapy, *Curr. Pharm. Des.* **7**, 1703-1724 (2001). b) Santelli-Rouvier, C. *et al* Synthesis of 9-acridinyl sulfur derivatives: Sulfides, sulfoxides and sulfones. Comparison of their activity on tumour cells, *Eur. J. Med. Chem.* **39**, 1029-1038 (2004). c) Denny, W.A. Chemotherapeutic effects of acridine derivatives, *Med. Chem. Rev. Online*, **1**, 257-266 (2004). d) Denny, W.A.; Baguley, B.C. Dual topoisomerase I / II inhibitors in cancer therapy, *Curr. Top. Med. Chem.* **3**, 339-353 (2003). e) Moloney, G.P.; Kelly, D.P.; Mack, P. Synthesis of acridine-based DNA bis-intercalating agents, *Molecules*, **6**, 230-243 (2001). f) Chilin, A. *et al* Synthesis and antitumor activity of novel amsacrine analogs: The critical role of the acridine moiety in determining their biological activity, *Bioorg. Med. Chem.* **17**, 523-529 (2009).
8. a) Wu, B.J.; Thompson, S.T. Selective medium for *Pseudomonas cepacia* containing 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan and polymyxin B sulfate, *Appl. Environ. Microbiol.* **48**, 743-746 (1984). b) Monte, W.; Stamm, J. The synthesis of new acridinium salt aquatic antifungals, *Tetrahedron Lett.* **34**, 7161-7162 (1993).
9. a) Sivaraman, J.; Subramanian, K.; Ganesan, S.; Ramakrishnan, V.T. Spectroscopic studies on the interaction of three partially hydrogenated acridine dyes with calf thymus DNA and their structural comparison, *J. Biomol. Struct. Dyn.* **13**, 119-134, (1995). b) Hopcroft, N.H.; Brogden, A.L.; Searcey, M.; Cardin, C. X-ray crystallographic study of DNA duplex cross-linking: simultaneous binding to two $d(CGTACG)_2$ molecules by a bis(9-aminoacridine-4-carboxamide) derivative, *Nucl. Acids Res.* **4**, 6663-6672 (2006). c) Lerman, L.S. Structural considerations in the interaction of DNA and acridines, *J. Mol. Biol.* **3**, 18-30

(1961). d) Adams, A. *et al* Acridinecarboxamide topoisomerase poisons: Structural and kinetic studies of the DNA complexes of 5-substituted 9-amino-(*N*-(2-dimethylamino)ethyl)acridine-4-carboxamides, *Mol. Pharmacol.* **58**, 649-658 (2000).

10. Cuny, G.D. *et al* Structure-activity relationship study of acridine analogs as haspin and DYRK2 kinase inhibitors, *Bioorg. Med. Chem. Lett.* **20** (12), 3491-3494 (2010).
11. a) Polshettiwar, V.; Varma, R. Microwave-assisted organic synthesis and transformations using benign reaction media, *Acc. Chem. Res.* **41**, 629-639 (2008). b) Caddick, S. Microwave assisted organic reactions, *Tetrahedron* **51**, 10403-10432 (1995). c) Loupy, A. *et al* New solvent-free organic synthesis using focused microwaves, *Synthesis* **9**, 1213-1234 (1998).
12. a) Bernthsen, A. Bernthsen reaction, *Justus Liebiğgs Ann. Chem.* **224**, 1-56 (1984). b) Buu-Hoi, N.P.; Royer, R.; Hubert-Habart, M. Carcinogenic nitrogen compounds. Part XVII. The synthesis of angular benzacridines, *J. Chem. Soc.* 1082-1084 (1955). c) Neidle, S.; Agbandje, M.; Jenkins, T.C.; McConnaughie, A.W. Structure and conformational features of 9-(4-diethylaminophenyl)acridine, *Acta Cryst.* **B46**, 860-862 (1990).
13. Popp, F.D. Polyphosphoric acid in the Bernthsen reaction, *J. Org. Chem.* **22**, 2658-2659 (1962).
14. Veverková, E.; Nosková, M.; Toma, S. Microwave synthesis of 9-substituted acridine derivatives, *Synth. Commun.* **32**, 729-733 (2002).
15. Seijas, J.A.; Vázquez-Tato, M.P.; Martínez, M.M.; Rodríguez-Parga, J. Microwave enhanced synthesis of acridines. A new aspect in the Bernthsen reaction, *Green Chem.* **4**, 390-391 (2002).
16. Anastas, P.T.; Warner, J.C. *Green Chemistry: Theory and Practice* (Oxford University Press: Oxford, 2000).
17. a) Layer, R.W. The chemistry of imines, *Chem. Rev.* **63**, 489-510 (1963). b) Alexander, V. Design and synthesis of macrocyclic ligands and their complexes of lanthanides and actinides, *Chem. Rev.* **95**, 273-432 (1995). c) Borisova, N.E.; Reshetova, M.D.; Ustynyuk, Y.A. Metal-free methods in the synthesis of

macrocyclic Schiff bases, *Chem. Rev.* **107**, 46-79 (2007). d) Roland S.; Mangeney, P. Addition of Grignard reagents to chiral 1,2-bisimines: A diasteroselective preparation of unsymmetrical 1,2-diamines, *Eur. J. Org. Chem.* **4**, 611-616 (2000). e) Cerchiaro, G.; Micke, G.A.; Tavares, M.F.M.; Ferriera, A.M.D.C. *Meso*-$Me_2Si(1\text{-indenyl})_2ZrCl_2$/methylalumoxane catalyzed polymerization of the ethylene to ethyl-branched polyethylene, *J. Mol. Catal. A: Chem.* **221**, 29-33 (2004). f) Juana, Z.; Lua, C.; Haihonga, W.; Jianguo, Y. Highly efficient procedure for the synthesis of Schiff bases using hydrotalcite-like materials as catalyst, *Chinese J. Chem.* **27**, 1868-1870 (2009).

18. a) Jacobsen, E.N. *Catalytic Asymmetric Synthesis*, Ed. Ojima, I. (VCH: New York, 1993). b) Katsuki, T. Catalytic asymmetric oxidations using optically active (salen)manganese(III) complexes as catalysts, *Coord. Chem. Rev.* **140**, 189-214 (1995).
19. a) Rollas, S.; Kucukguzel, S.G. Biological activities of hydrazone derivatives, *Mol.* **12**, 1910-1939 (2007). b) Piato, A.L. *et al* Effects of marapuama in the chronic mild stress model: Further indication of antidepressant properties, *J. Ethnopharmacol.* **118**, 300-304 (2008). c) Lidija, B.R. The antidepressant activity of *Hypericum perforatum L.* measured by two experimental methods on mice, *Acta. Pharm.* **54**, 157-162 (2004).
20. a) Higuchi, M.; Yamamoto, K. Novel cyclic molecules: Selective synthesis of cyclic phenylazomethines, *Org. Lett.* **1**, 1881-1883 (1999). b) Adams, J.P. Imines, enamines and oximes, *J. Chem. Soc., Perkin Trans.* **1**, 125-139 (2000). c) Naeimi, H.; Moradian, M. Synthesis and characterization of nitro-Schiff bases derived from 5-nitro-salicylaldehyde and various diamines and their complexes of Co(II), *J. Coord. Chem.* **63**, 156-162 (2010).
21. a) Silva, J.F.M.D.; Garden, S.J.; Pinto. A.C. The chemistry of isatins: A review from 1975 to 1999, *J. Braz. Chem. Soc.* **12**, 273-324 (2001). b) Shi, M.; Cui, S.C. Aza-Diels–Alder reaction catalyzed by perfluorinated metal salts in fluorous phase, *New J. Chem.* **28**, 1286-1288 (2004). c) Jia, X. *et al* Cation radical imino Diels-Alder reaction: A new approach for the synthesis of tetrahydroquinolines, *Synlett.* **11**, 1707-1709 (2003). d) Behforouz, M.; Ahmadian, M. Diels–Alder

reactions of 1-azadienes, *Tetrahedron* **56**, 5259-5288 (2000). e) Zulfiqar, F.; Kitazume, T. One-pot aza-Diels–Alder reactions in ionic liquids, *Green Chem.* **2**, 137-139 (2000).

22. Cordes, E.H.; Jencks, W.P. On the mechanism of Schiff base formation and hydrolysis, *J. Am. Chem. Soc.* **84**, 832-837 (1962).
23. For reviews, see: Rowan, S.J.; Cantrill, S.J.; Cousins, G.R.L.; Sanders, J.K.M.; Stoddart, J.F. Dynamic Covalent Chemistry, *Angew. Chem., Int. Ed.* **41**, 898-952 (2002).
24. a) Schmidt, S. *et al* Spontaneous assembly of a Schiff Base tetramacrocycle, *Angew. Chem., Int. Ed. Eng.* **39**, 913-916 (2000). b) Gawronski, J.; Kolbon, H.; Kwit, M.; Katrusiak, A. Designing large triangular chiral macrocycles: Efficient [3+3] diamine-dialdehyde condensations based on conformational bias, *J. Org. Chem.* **65**, 5768-5773 (2000). c) Akine, S.; Taniguchi, T.; Nabeshima, T. Synthesis and crystal structure of a novel triangular macrocyclic molecule, tris($H_2$saloph), and its water complex, *Tetrahedron Lett.* **42**, 8861-8864 (2001). d) Zhao, D.; Moore, J.S. Synthesis and self-association of an imine-containing *m*-phenylene ethynylene macrocycle, *J. Org. Chem.* **67**, 3548-3554 (2002). e) Akine, S.; Hashimoto, D.; Saiki, T.; Nabeshima, T. Synthesis and structure of polyhydroxyl rigid triangular nano-macrocyclic imine having multiple hydrogen-bonding sites, *Tetrahedron Lett.* **45**, 4225-4227 (2004).
25. a) Cantrill, S.J.; Rowan, S.J.; Stoddart, J.F. Rotaxane formation under thermodynamic control, *Org. Lett.* **1**, 1363-1366 (1999). b) Rowan, S.J.; Stoddart, J.F. Thermodynamic synthesis of rotaxanes by imine exchange, *Org. Lett.* **1**, 1913-1916 (1999).
26. Venugopala, K.N.; Rao, G.K.; Pai, P.N.S. Synthesis and pharmacological study of some novel schiff bases of 4-hydroxy 6-carboxhydrazino benzothiophene analogs, *J. Pharmacol. Toxicol.* **2**, 248-255 (2007).
27. a) Jarrahpour, A.; Khalili, D.; Clercq, E.D.; Salmi, C.; Brunel, J.M. Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-Schiff bases of isatin and their derivatives, *Molecules* **12**, 1720-1730 (2007). b) Pandeya, S.N.; Sriram, D. Synthesis and screening for antibacterial activity of Schiff's and

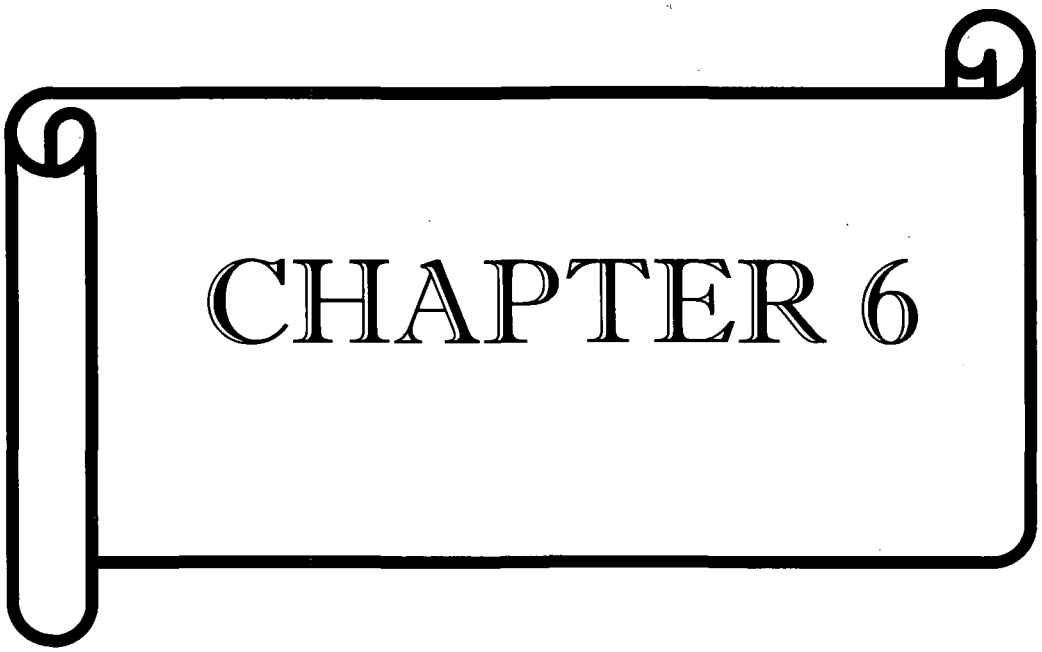
Mannich bases of isatin and its derivatives, *Acta Pharm. Turc.* **40**, 33-38 (1998). c) Sarangapani, M.; Reddy, V.M. Synthesis and antimicrobial activity of 1-[(*N*,*N*-disubstitutedamino)methyl]-3-[(2-phenyl-3,4-dihydro-4-oxoquinazoline-3-yl] - indole-2 one, *Ind. J. Heterocycl. Chem.* **3**, 257-260 (1994). d) Sridhar, S.K.; Pandeya, S.N.; Stables, J.P.; Ramesh, A. Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives, *Eur. J. Pharm. Sci.* **16**, 129-132 (2002). e) Varma, M.; Pandeya, S.N.; Singh, K.N.; Stables, J.P. Anticonvulsant activity of Schiff bases of isatin derivatives, *Acta Pharmaceut.* **54**, 49-56 (2004). f) Pandeya, S.N.; Sriram, D.; Nath, G.; Clercq, E.D. Synthesis, antibacterial, antifungal and anti-HIV activity of Schiff and Mannich bases of isatin with N-[6-chlorobenzthiazole-2-yl] thiosemicarbazide, *Ind. J. Pharm. Sci.* **61**, 358-361 (1983). g) Elmali, F.T.; Peksel, A.; Demirhan, N. Synthesis in solvent-free conditions, characterization and biological activity of a new Schiff-base ligand and its Cu(II) complex, *J. Coord. Chem.* **62**, 2548-2555 (2009).

28. a) Shakil, N.A. *et al* Synthetic, biocatalytic acetylation and anti-tuberculosis activity evaluation studies on (±)-4-alkyl-3,4-dihydro-3-ω-hydroxyalkyl-2*H*-1,3-benzoxazines, *Ind. J. Chem.* **42(B)**, 1958-1969 (2003). b) Tumerdem, G.T.; Turgut, Y. Asymmetric reduction of acetophenone using lithium aluminium hydride modified with some novel amino alcohol Schiff-bases, *Tetrahedron: Asymmetry* **16**, 865-868 (2005). c) Lewkowski, J.; Rzezniczak, M.; Skowronski, R. Synthesis of 1,4-phenylene-*bis*-aminomethanephosphonates: The stereochemical aspect, *Heteroatom Chem.* **11**, 144-151 (2000).
29. a) Das, S.; Thakur, A.J. A clean, highly efficient and one-pot green synthesis of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimethyluracil-5-yl)methanes in water, *Eur. J. Org. Chem.* 2301-2308 (2011). b) Das, S.; Thakur, A.J. A green development of Bernthsen 9-substituted acridine synthesis in the absence of solvent catalysed by *p*-toluenesulphonic acid (*p* -TSA), *Green Chem. Lett. Rev.* **4**, 131-135 (2011); (c) Saikia, L.; Das, S.; Thakur, A.J. A facile method for the conversion of oximes to carbonyl compounds by $ZrOCl_2 8H_2O$, *Synth. Commun.* **41**, 1071-1075, (2011).
30. a) Anastas, P.T.; Warner, J.C. in *Green Chemistry: Theory and Practice* (Oxford Publications, New York, 1998). b) Anastas, P.T.; Williamson, T. *Green*

*Chemistry, Frontiers in Benign Chemicals Synthesis and Processes* (Oxford Science publications, New York, 1998). c) Tanaka, K. *Solvent-free Organic Synthesis* (Wiley-VCH, Weinheim, 2003).

31. a) Prajapati, D.; Gohain, M.; Thakur, A.J. Regiospecific one-pot synthesis of pyrimido[4,5-*d*]pyrimidine derivatives in the solid state under microwave irradiations, *Bioorg. Med. Chem. Lett.* **16**, 3537-3540 (2006). b) Thakur, A.J.; Prajapati, D. Studies on 6-[(dimethylamino)methylene]aminouracil: A facile one-pot synthesis of novel pyrimido[4,5-*d*]pyrimidine derivatives, *Tetrahedron Lett.* **34**, 1433-1436 (2005). For review see: c) Caddick, S. Microwave assisted organic reactions, *Tetrahedron* **51**, 10403-10432 (1995). d) Loupy, A. *et al* New solvent-free organic synthesis using focused microwaves, *Synthesis* **9**, 1213-1234 (1998). e) Galema, S.A. Microwave chemistry, *Chem. Soc. Rev.* **26**, 233-238 (1997). f) Satyanarayana, V.S.V.; Sreevani, P.; Sivakumar, A.; Vijayakumar, V. Synthesis and antimicrobial activity of new Schiff-bases containing coumarin moiety and their spectral characterization, *Arkivoc* 221-233 (2008). g) Ali, P.; Ramakanth, P.; Meshram, J. Exploring microwave synthesis for co-ordination: Synthesis, spectral characterization and comparative study of transition metal complexes with binuclear core derived from 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, *J. Coord. Chem.* **63**, 323-329 (2010). h) Polshettiwar, V.; Varma, R.S. Microwave-assisted organic synthesis and transformations using benign reaction media, *Acc. Chem. Res.* **41**, 629-639 (2008).
32. Billman, J.H.; Chen Ho, J.Y.; Caswell, L.R. The formation of solid derivatives of aldehydes. I. 2-substituted-1,3-bis(*p*-methoxybenzyl)-tetrahydroimidazoles, *J. Org. Chem.* **17**, 1375-1378 (1952).
33. Ferguson, L.N.; Branch, G.E.K. Absorption spectra of some linear conjugated systems, *J. Am. Chem. Soc.* **66**,1467-1475 (1944).
34. Sharma, V.; Khan, M.S.Y. Synthesis of novel tetrahydroimidazole derivatives and studies for their biological properties, *Eur. J. Med. Chem.* **36**, 651-658 (2001).
35. Varma, R.S.; Dahiya, R.; Kumar, S. Clay catalyzed synthesis of imines and enamines under solvent-free conditions using microwave irradiation, *Tetrahedron Lett.* **38**, 2039-2042 (1997).

36. Tanaka, K.; Shiraishi, R. Clean and efficient condensation reactions of aldehydes and amines in a water suspension medium, *Green Chem.* **2**, 272-273 (2000).
37. van-den Ancker, T.R.; Caveb, G.W.V.; Rastonc, C.L. Benign approaches for the synthesis of bis-imine Schiff bases, *Green Chem.* **8**, 50-53 (2006).
38. Fujioka, H. *et al* A mild and efficient one-pot synthesis of 2-dihydroimidazoles from aldehydes. *Tetrahedron Lett.* **46**, 2197-2199 (2005).
39. Gogoi, P.; Konwar, D. An efficient and one-pot synthesis of imidazolines and benzimidazoles via anaerobic oxidation of carbon–nitrogen bonds in water, *Tetrahedron Lett.* **47**, 79–82 (2006).


CHAPTER 6

**Overall Conclusion:**

As mentioned in earlier chapters, purpose of this thesis is to explore synthesis, structure and bioactivity of pyrimidine derivatives and development of some synthetic methods.

The major findings of this thesis are described below:

1. Synthesis of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimethyluracil-5-yl)methanes in water was achieved with excellent yields just by simple stirring of 6-aminouracil and aldehydes (aromatic, aliphatic and heterocyclic), without applying heat, dehydrating agents, catalysts, other out driving forces or other volatile organic solvents. Separation of the compounds were done by simple filtration technique without using TLC and column chromatographic techniques, which reduces the capital and operating costs (60-80%) of the overall cost and environmental footprint of the process. Interestingly, the reaction between cinnamaldehyde and 6-aminouracil provided both the cis-/trans-bis-adducts, however, after recrystallization, only the *trans* isomer crystallized out. The reaction of salicyldehyde with 6-aminouracil provided the 5-(6-amino-1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxopyrimidin-5-yl)-1,3-dimethyl-pyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*,5*H*,10*H*)-dione tetrahydrate through the formation of bis-uracil adduct.

2. Nucleophilic addition of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione and 6-[(dimethylamino)methyleneamino]-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione with aldehydes (aromatic, aliphatic and heterocyclic) using 'green surfactant' isolated from *P. aeruginosa* OBP1 in water at room temperature is described. The reaction is highly efficient and environmentally friendly.

   To the best of our knowledge, for the first time we have introduced the use of bio-surfactant in organic transformation, we believe this methodology will open a new field in synthetic methodology.

   Some of the synthesized bis-uracil/tetrakis-uracil derivatives showed very good anti-oxidant properties, and moderate to good anti-microbial activities, but we did not observe anti-fungal activities.

3. C-N Rotational energy barrier for the dimethylamino group around C-N double bond in the exocyclic part of 6-[(dimethylamino)methylene]1,3-dimethylaminouracil was determined by VT-DNMR spectroscopy and it was observed that pyrimidine ring did not undergo any change in its conformation during the process, changes occur only in the exocyclic part as the dynamic process of fast exchange is induced by the application of heat energy.

   Theoretical calculation showed that the origin of the barrier lies in delocalization of the lone pair of electrons on the nitrogen atom into the anti-bonding orbital of the adjacent C-N double bond in the ground state. Experimentally, the C-N rotational barrier for 6-[(Dimethylamino)methylene]1,3-dimethylaminouracil in 1,1,2,2-tetrachloroethane-$d_2$ was found to be 18.97 Kcal/mol, which is in good agreement with the computed barrier of 19.8 Kcal/mol.

4. Diels-Alder methodology (aza-version) has been used for the construction of fused heterocyclic systems like pyrido[2,3-*d*]pyrimidine-2,4-dione derivatives and bis-pyrimido[4,5-*d*]pyrimidine derivatives.

   Anti-bacterial activity of pyrido[2,3-*d*]pyrimidine-2,4-dione derivatives and bis-pyrimido[4,5-*d*]pyrimidine derivatives were evaluated, cell-viability test of pyrido[2,3-*d*]pyrimidine-2,4-dione derivatives were done and some of the compounds showed anti-microbial properties.

   Cytotoxicity test of pyrido[2,3-*d*]pyrimidine-2,4-dione derivatives exhibit variability in survival percentage with different loading concentration and methoxy-substituted pyrido[2,3-*d*]pyrimidine derivatives showed potency to induce cytochrome P450.

5. A good development of Bernthsen reaction was achieved by carrying out the reaction under MWI in the solventless condition using *p*-TSA as a catalyst, that reduces the reaction time from several hours (conventional synthesis) to few minutes. Using ionic liquid and *p*-TSA we received good yield (comparable), whereas, CAN and basic alumina etc. also provided the product but yields were

poorer. In the original Bernthsen reaction (using $ZnCl_2$), *p*-Nitro and *p*-Amino benzoic acids did not lead to acridines. The same substrates lead to acridine derivatives in our reaction conditions. Both ionic liquid and *p*-TSA are easy to remove from reaction mixture (water soluble) making the purification easy- an important point in the context of green chemistry.

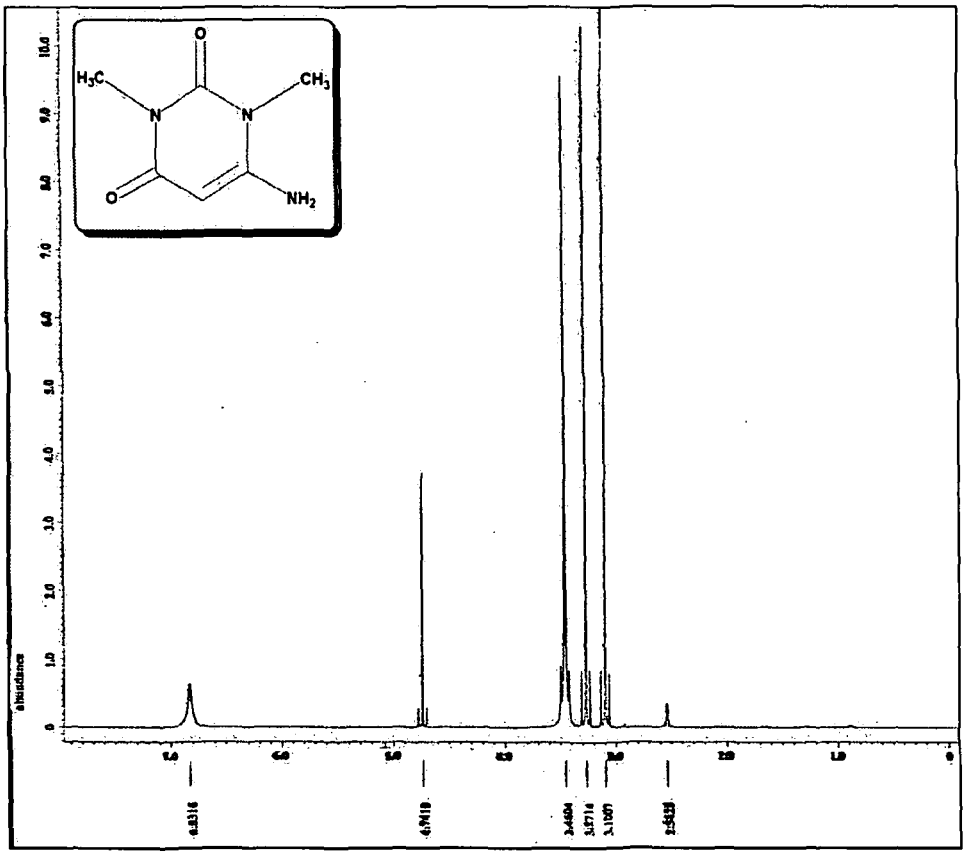
6. MWI has been shown to be a good way to optimise the synthesis of symmetrical bis-imines with good to excellent yields (61-99%). Time economy observed is amazing. Use of *p*-TSA as a catalyst is very effective, cheap and can be easily removed from the reaction mixture. The product separates by simple polar-nonpolar interaction, without applying any further separation techniques like column chromatography, TLC etc.

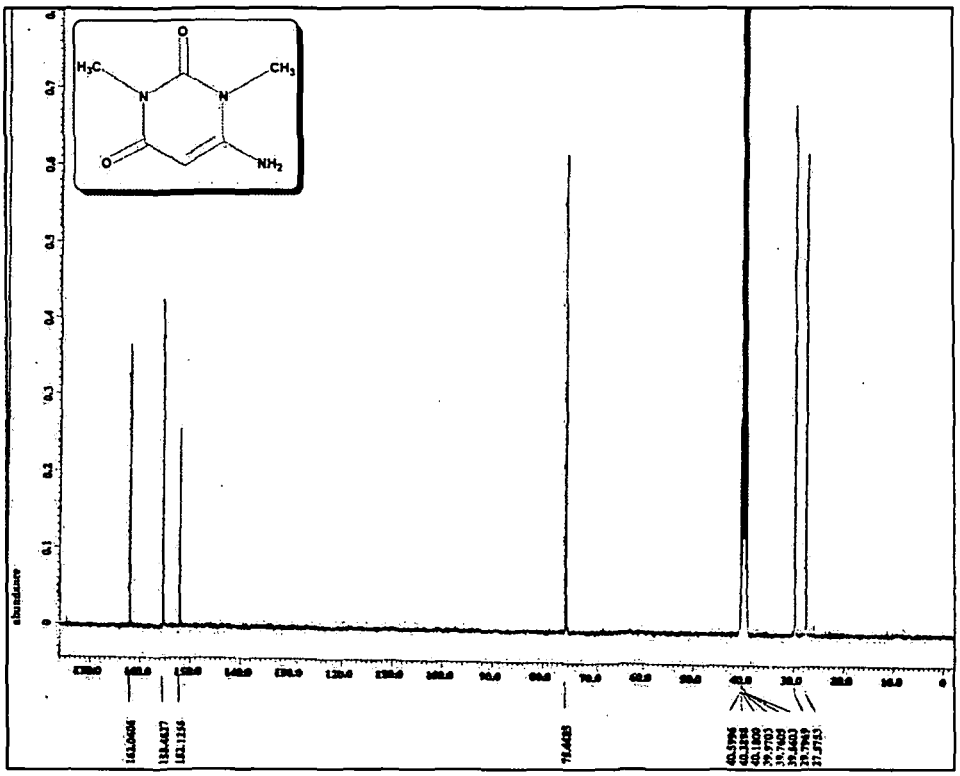
## Future Scopes:

- Construction of library of pyrimidine compounds with wide molecular diversity by varying the dienophiles
- Change in methodology of the approach, e.g. extend the works with 1,3-dipoles
- Attachment of pyrimidine derivatives to other bioactive molecules, natural alkaloids and hetero-polymer like materials
- Application of modern computational methods (e.g. DFT, ab initio etc.) to understand and decide the site of electrophilic or nucleophilic attack and differentiate between chemical reactivity behaviour of different pyrimidine compounds
- Extend the bioactivity tests to cell lines
- Molecular modelling and structure activity relationship studies will pave the way for looking into their bioactivities
- To study the supramolecular behaviour of pyrimidine compounds
- To search for new synthetic methods which will eliminate the associated drawbacks of previously reported reaction schemes with added advantage
- To accomplish the reaction with environmental friendly, economic and easily available reagents/catalysts

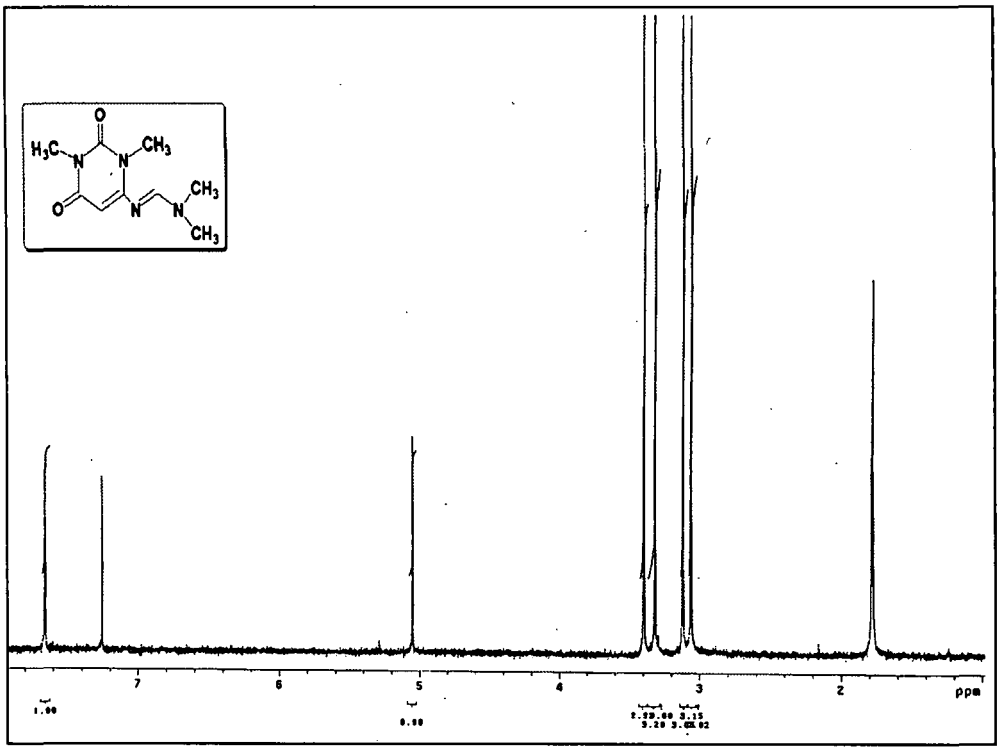
# APPENDIX 1

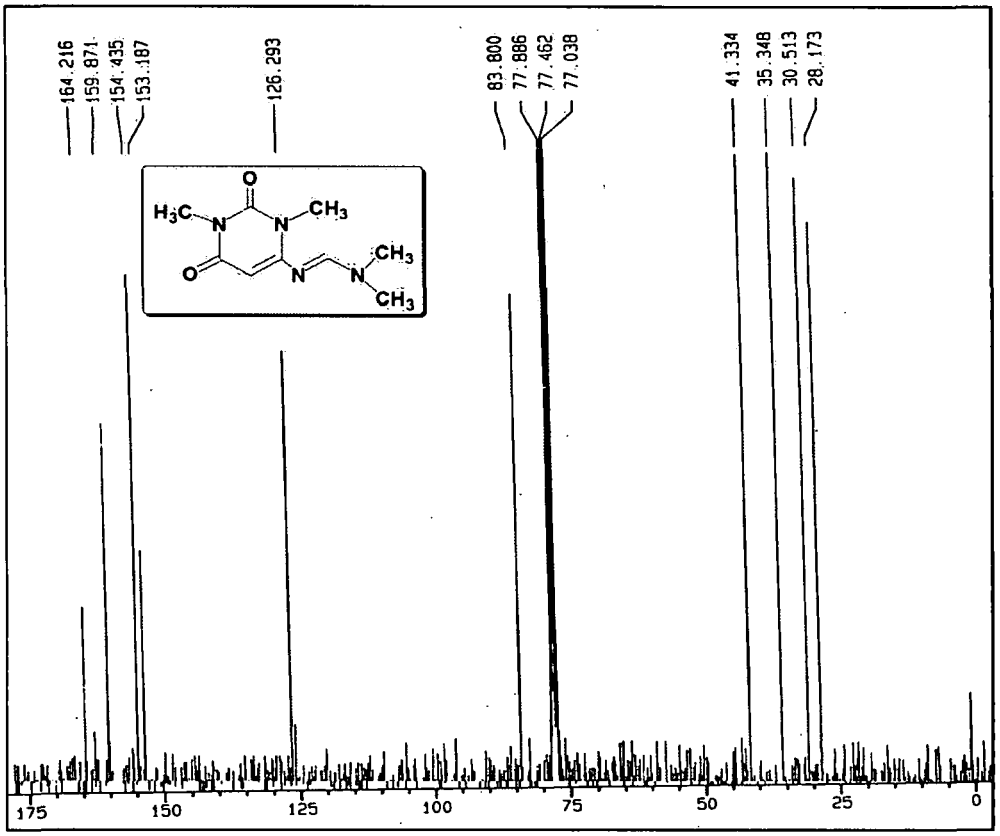
**$^{1}H$ and $^{13}C$ NMR spectra for 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione**



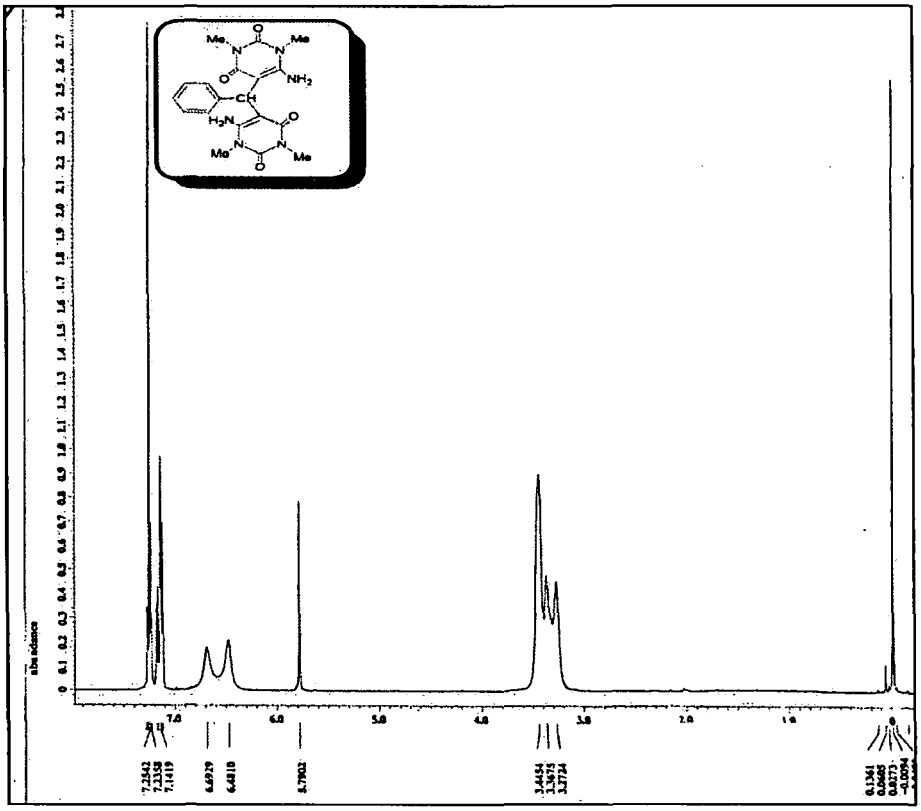



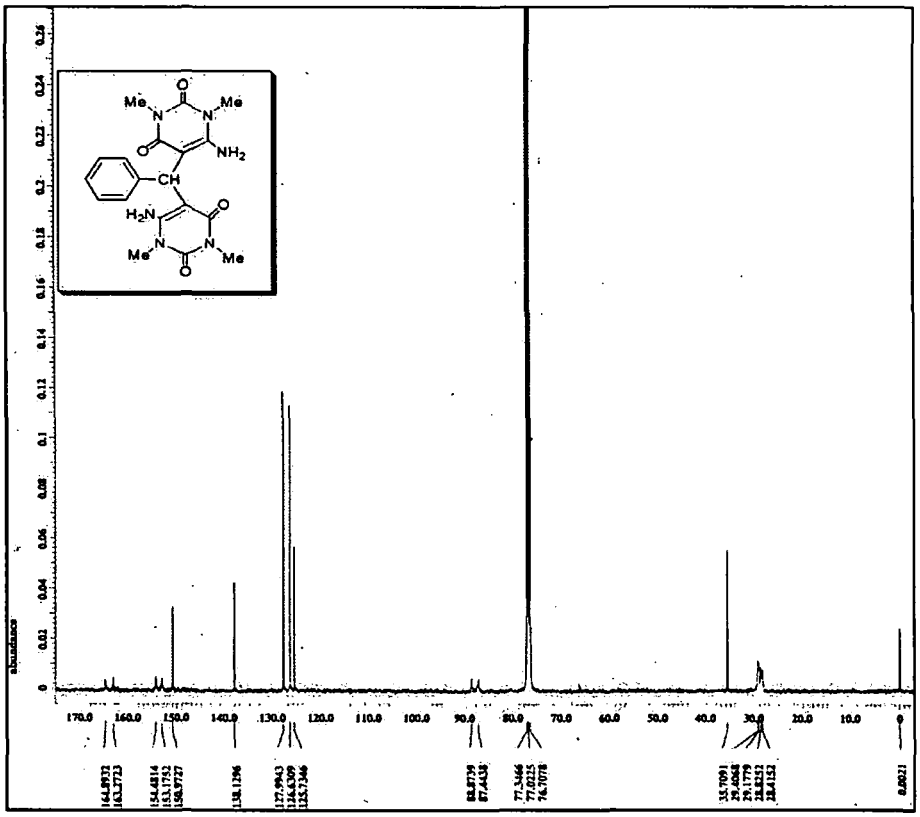
**$^{1}H$ and $^{13}C$ NMR spectra for 6-[(Dimethylamino)methylene]1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione**

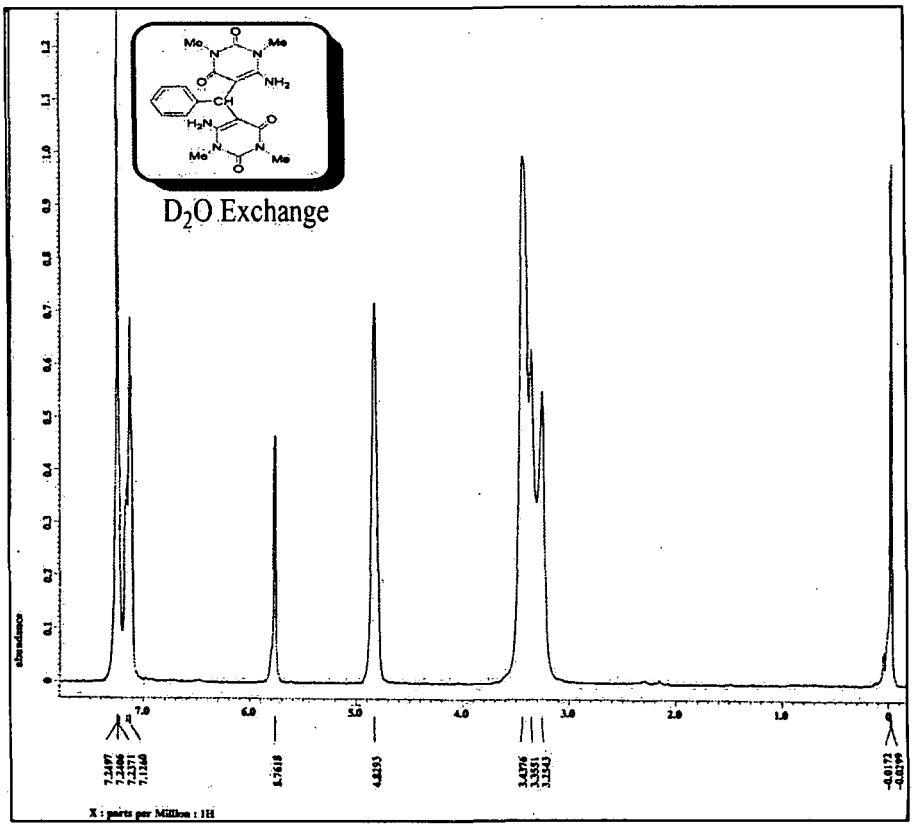


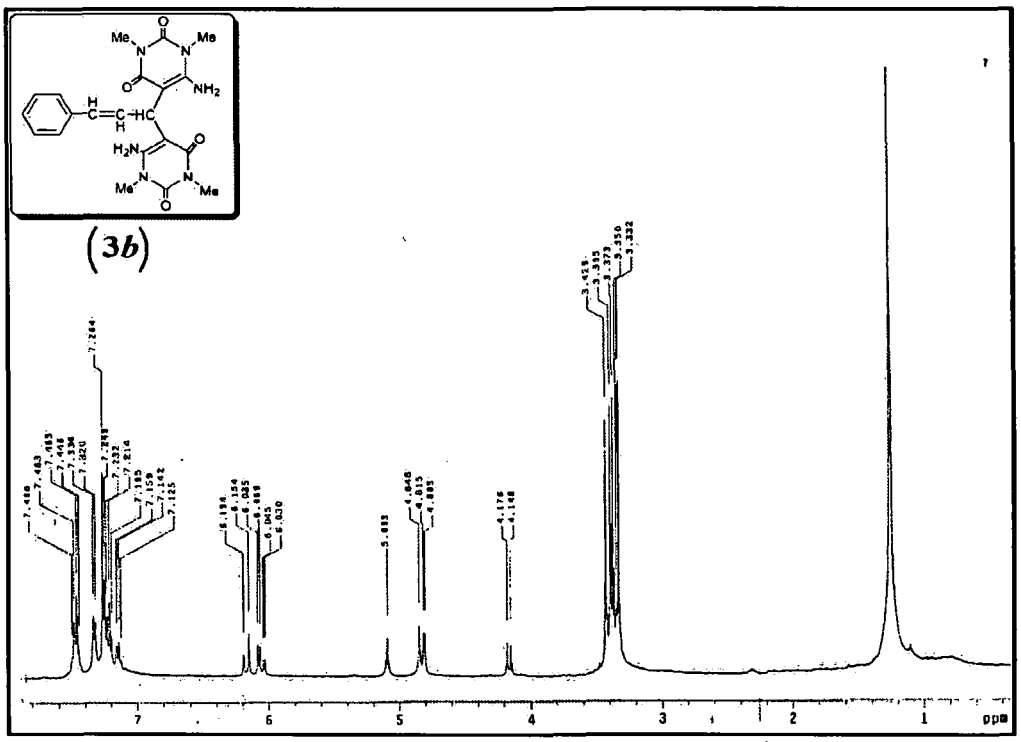
**$^{1}H$, $^{13}C$ NMR and $D_2O$ exchanged $^{1}H$ spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(benzylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**

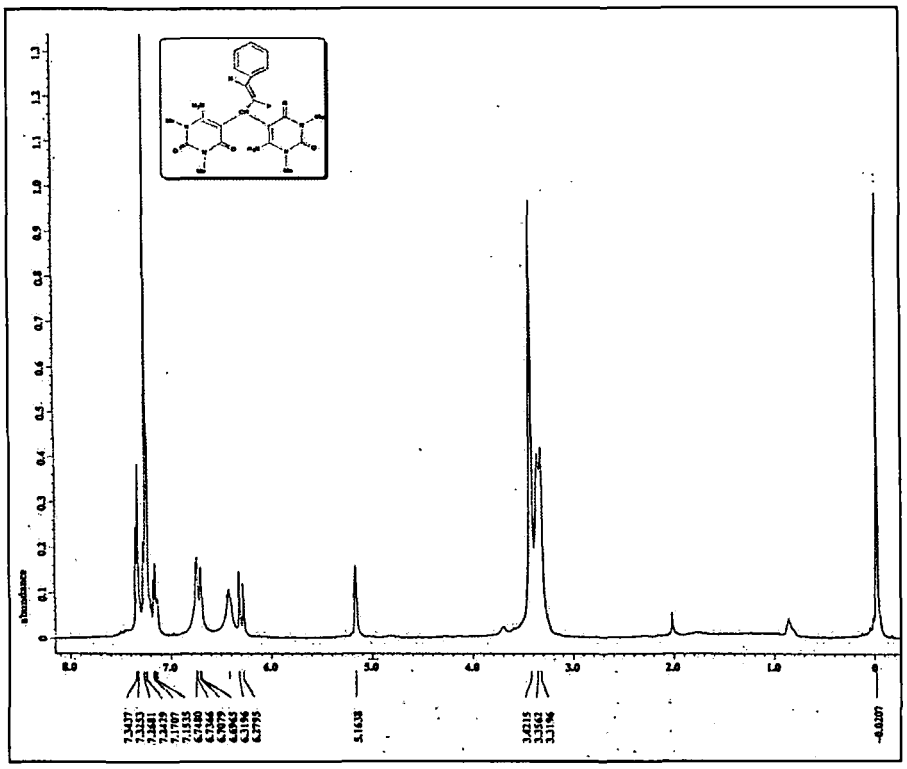




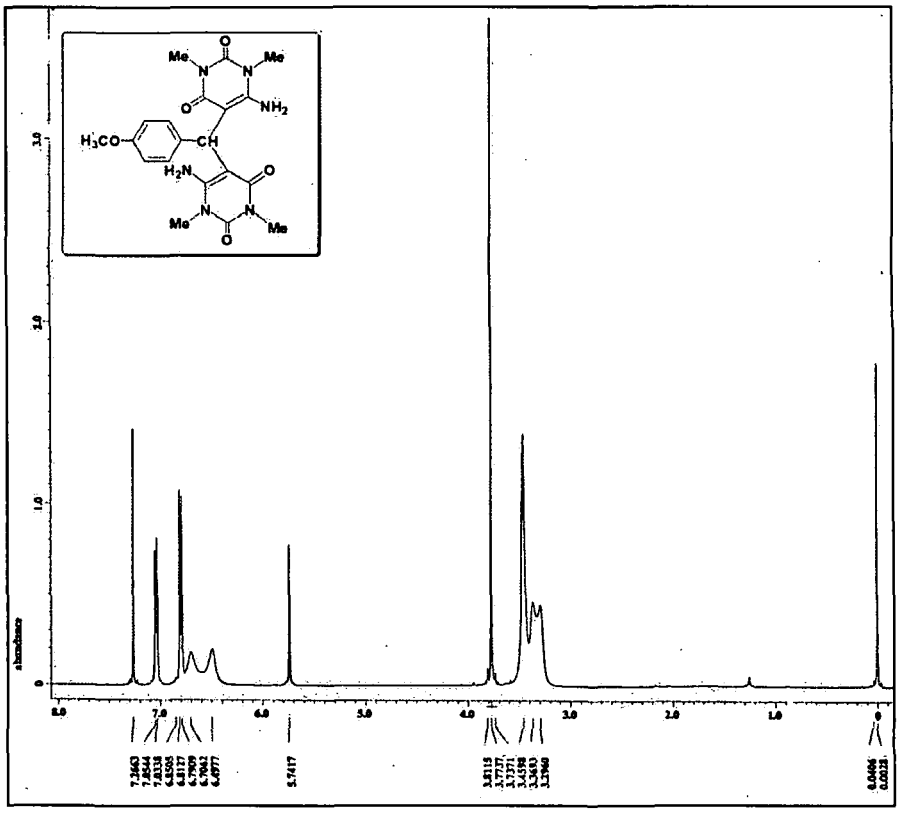


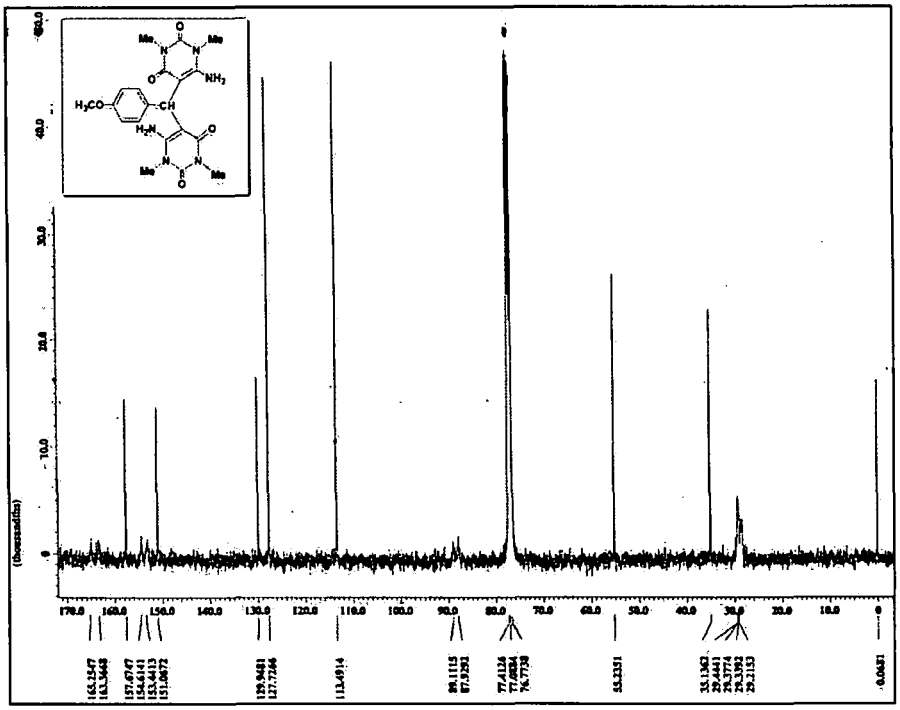

**$^1$H spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(cinamylidene)-bis[pyrimidine-2,4(1*H*,3*H*)-dione] (both cis/trans and trans isomer)**



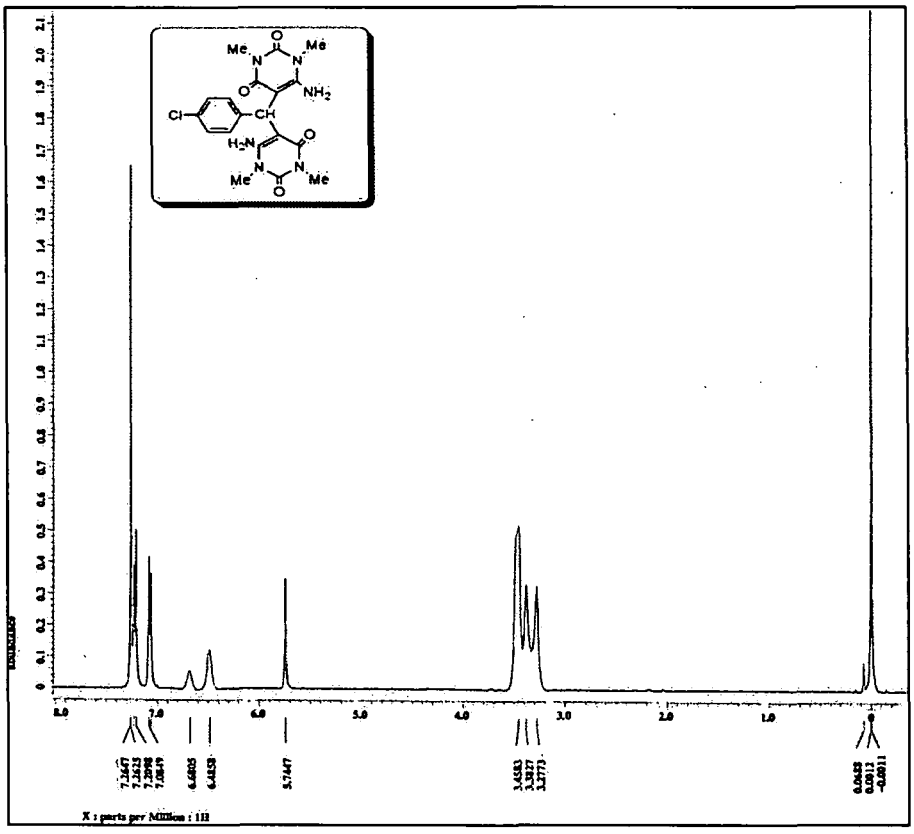


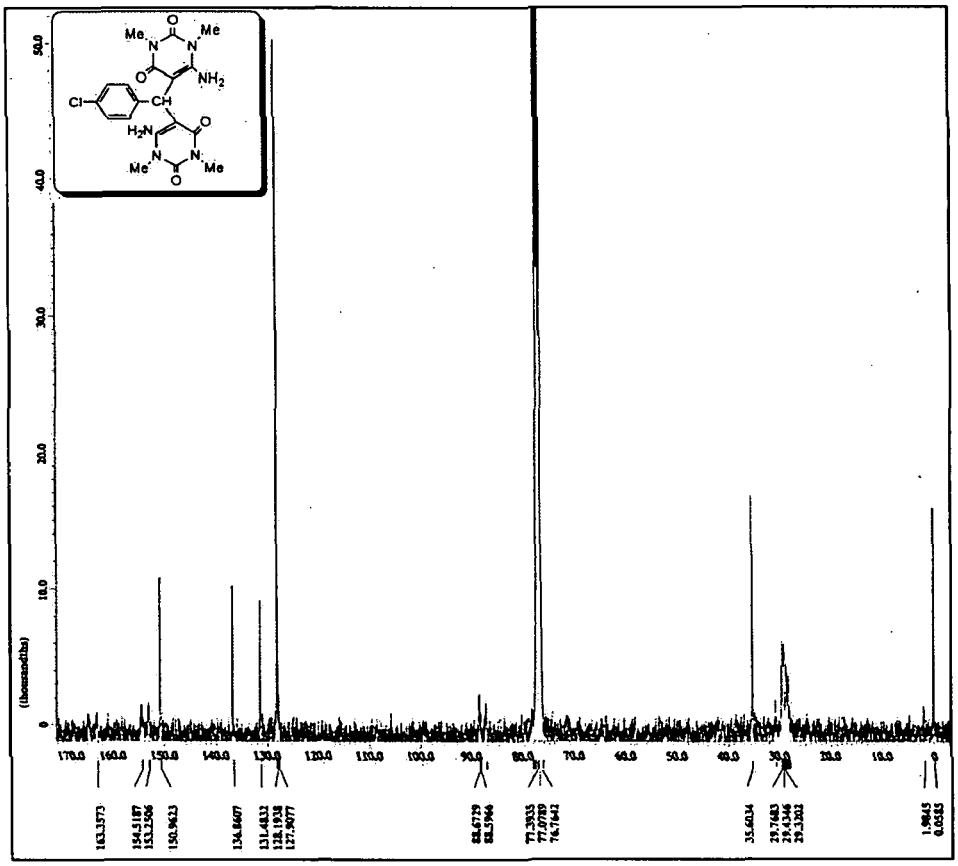
**$^{1}H$ and $^{13}C$ NMR spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(4-methoxybenzylidene)bis[pyrimidine-2,4(*1H,3H*)-dione}]**





**$^{1}H$, $^{13}C$ NMR and $D_2O$ exchanged $^{1}H$ spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(4-chlorobenzylidene)bis[pyrimidine-2,4(*1H,3H*)-dione]**

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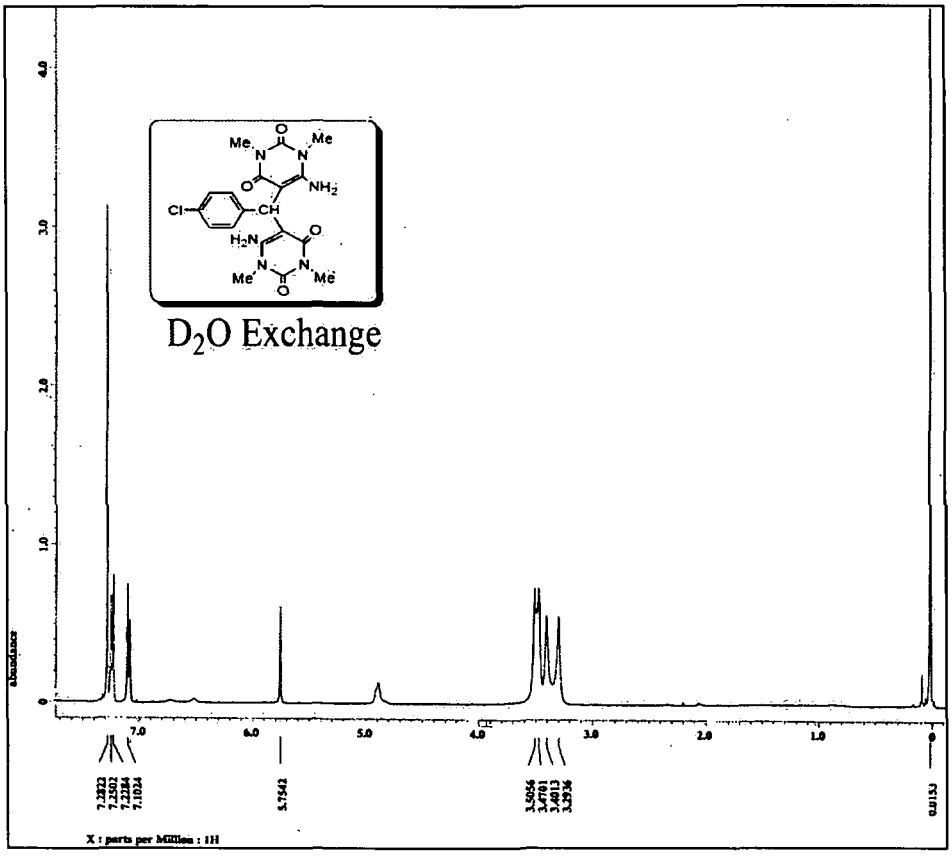
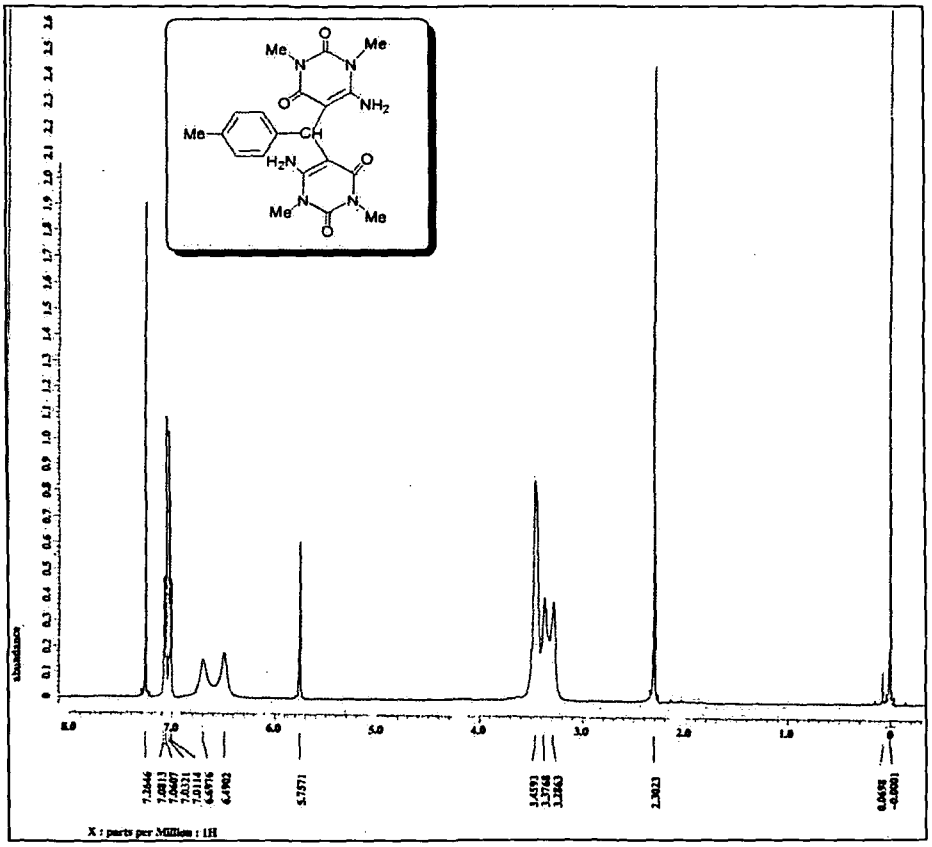
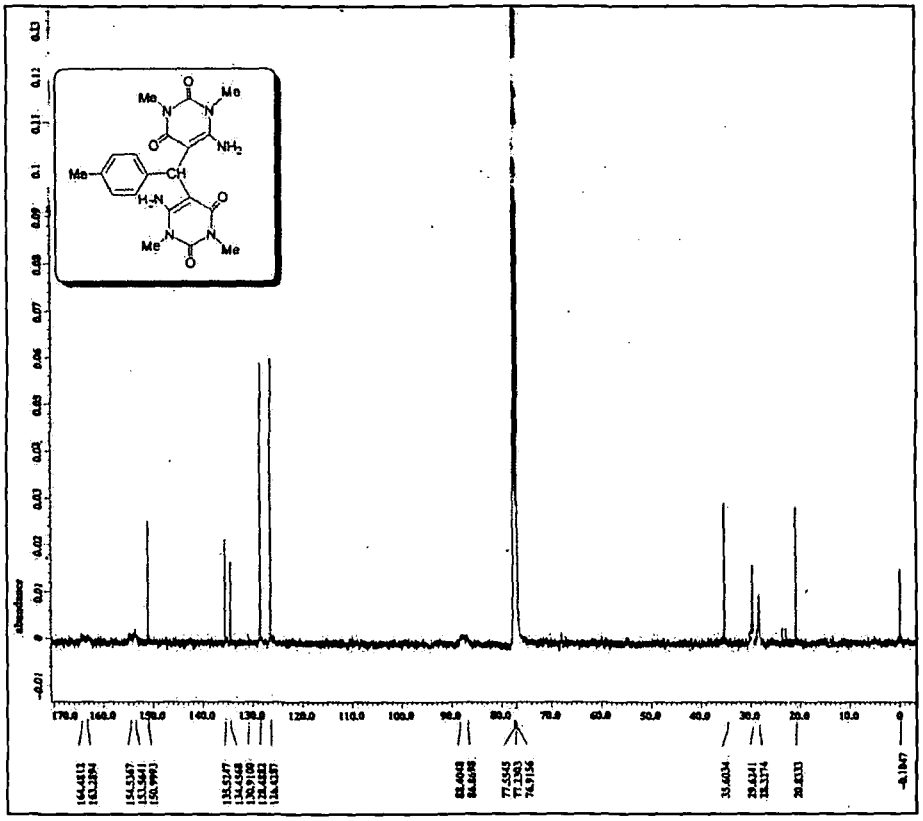
Me
N
NH2
Cl
CH
H2N
O
N
Me
O
Me

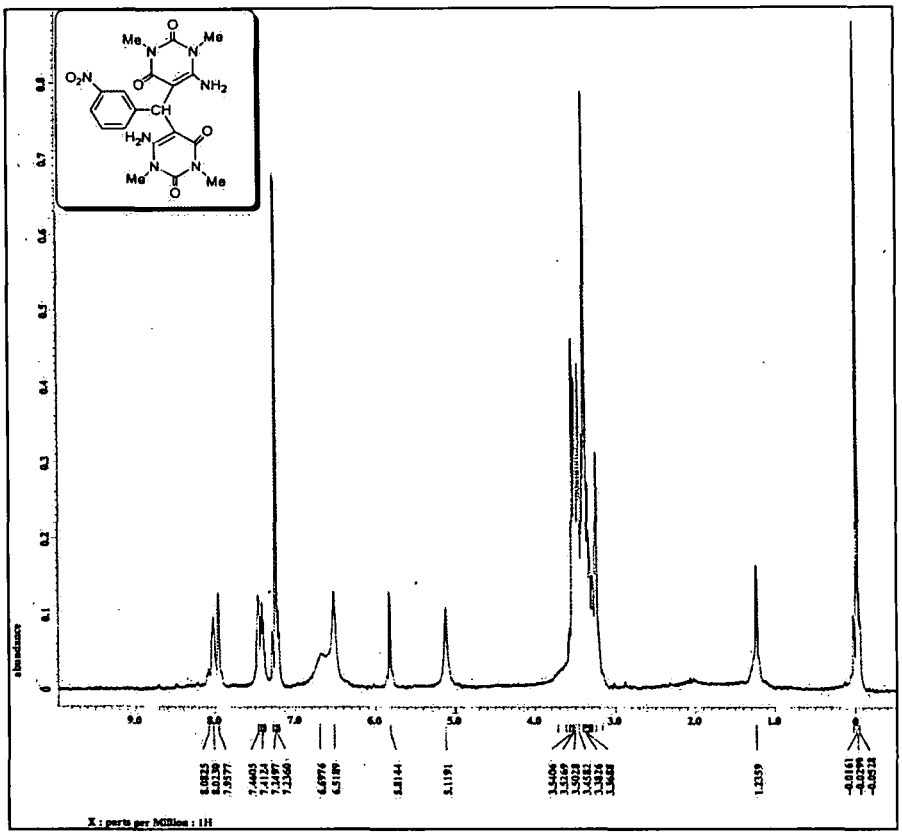

Me
N
O
Me
N
NH2
Cl
CH
H2N
O
N
Me
O
Me
D2O Exchange
X : parts per Million : 1H

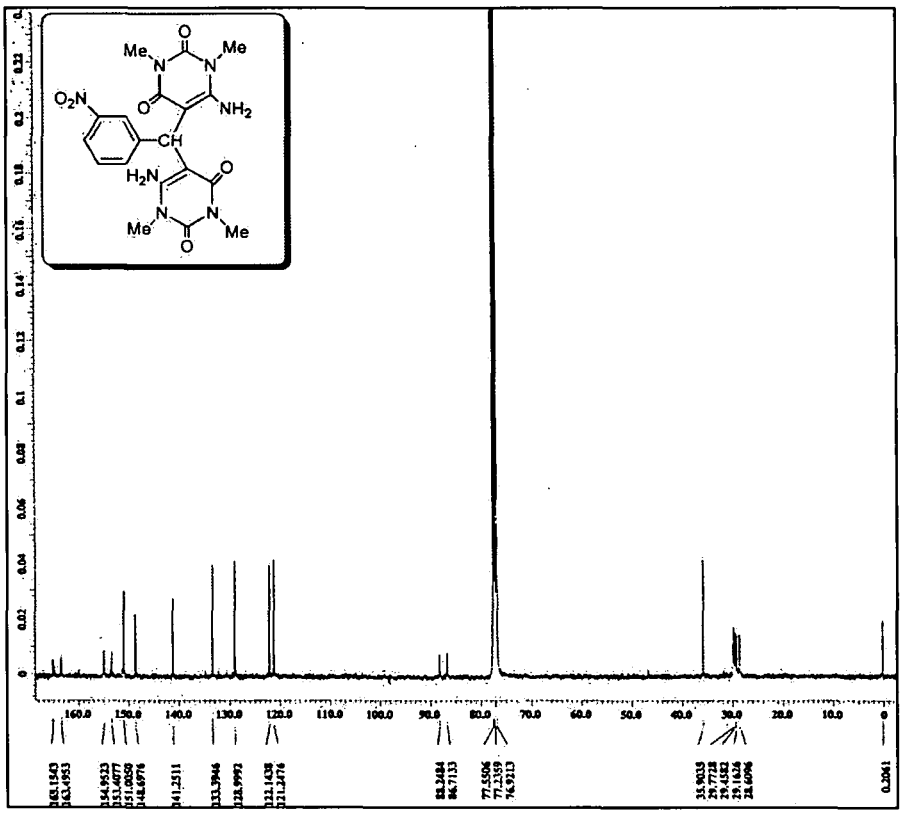
**$^{1}H$ and $^{13}C$ NMR spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(4-methylbenzylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**



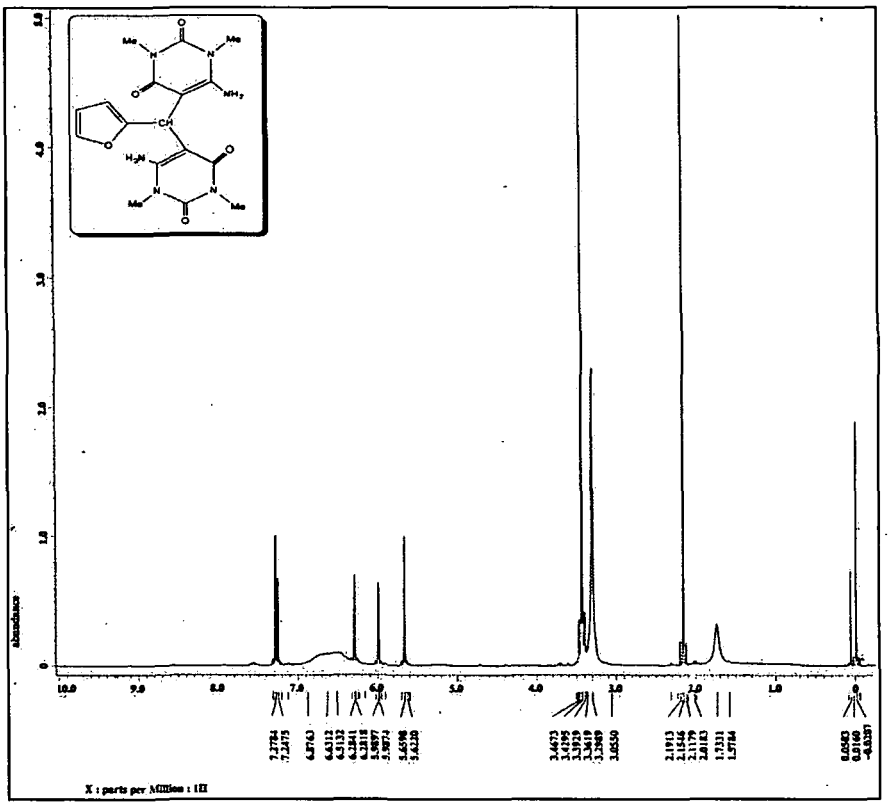



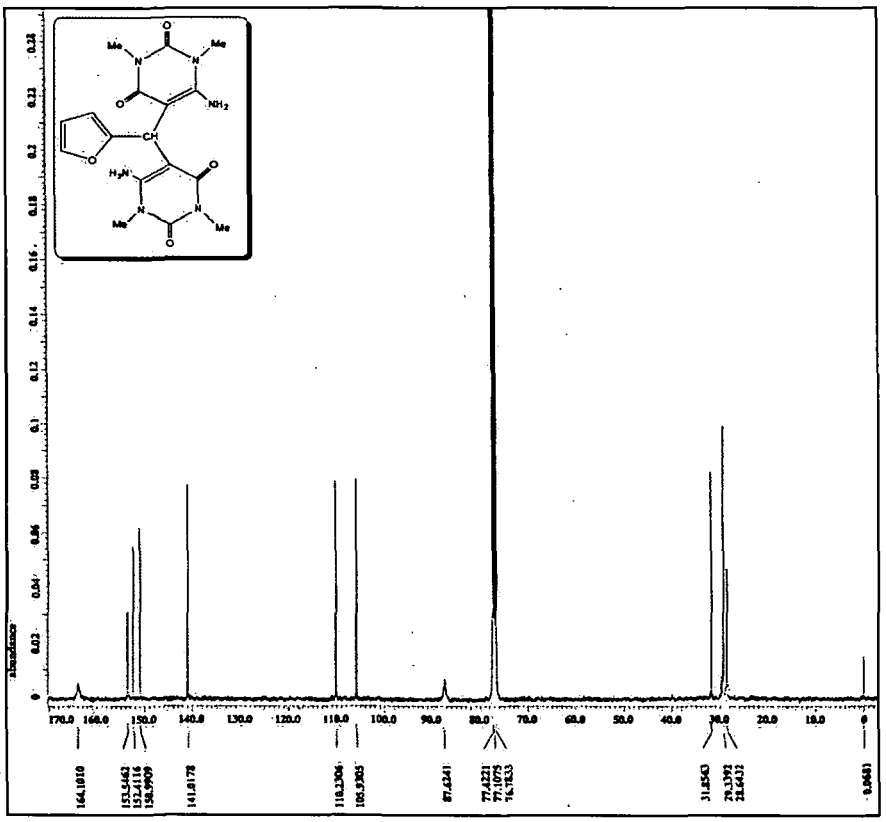
**$^{1}H$ and $^{13}C$ NMR spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(3-nitrobenzylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**

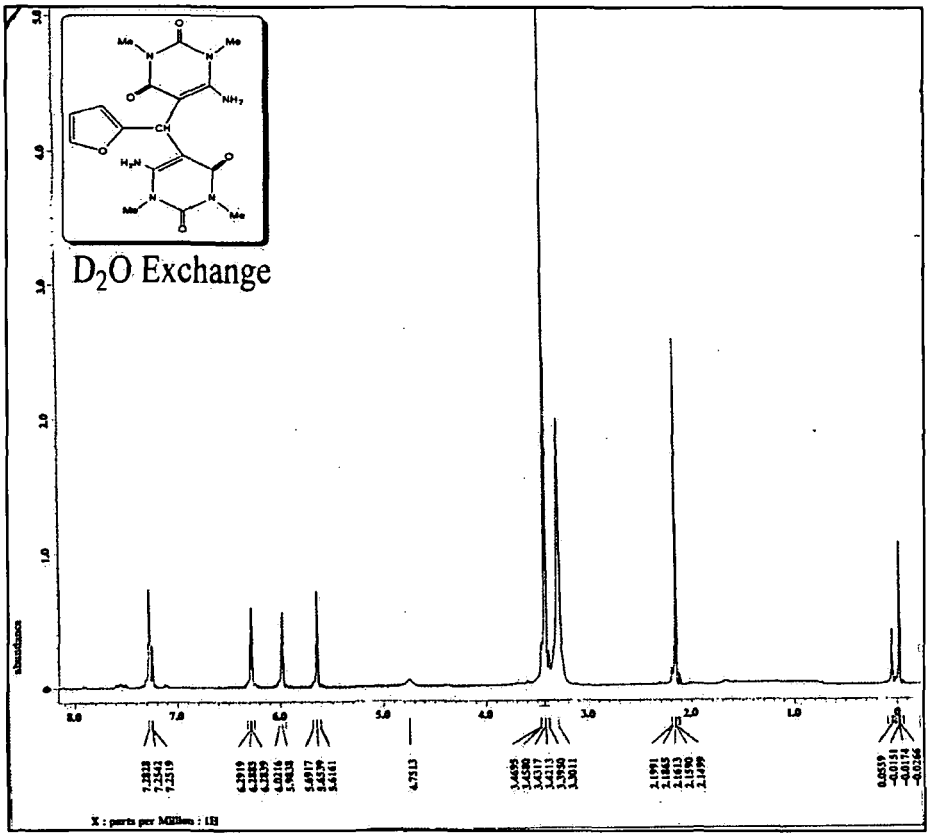




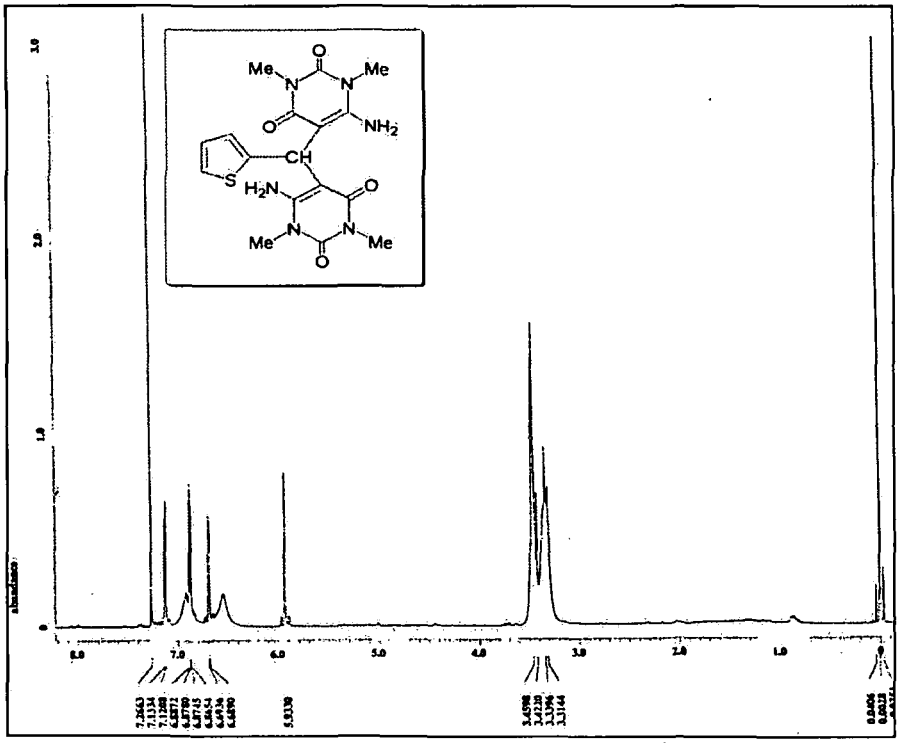
**$^{1}H$, $^{13}C$ NMR and $D_2O$ exchanged $^{1}H$ spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(furayl)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**

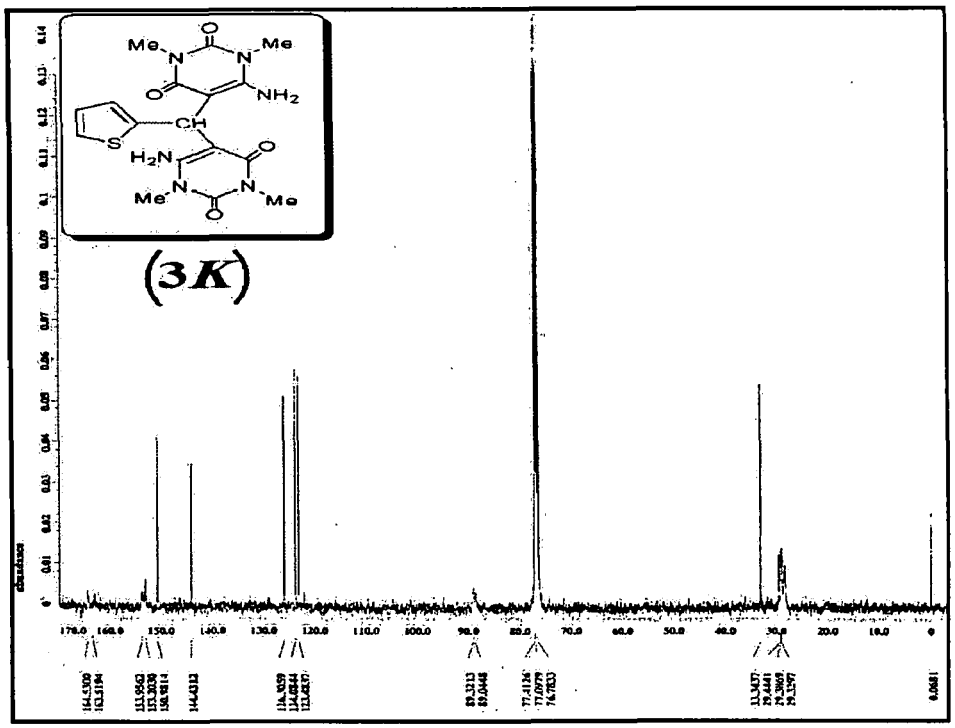




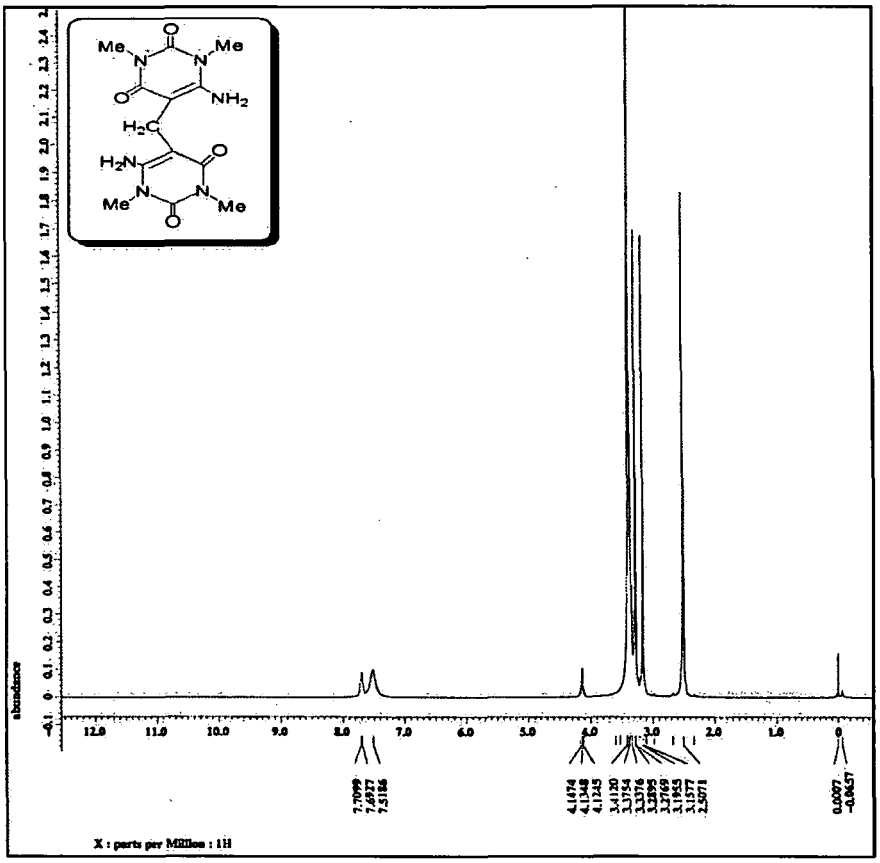


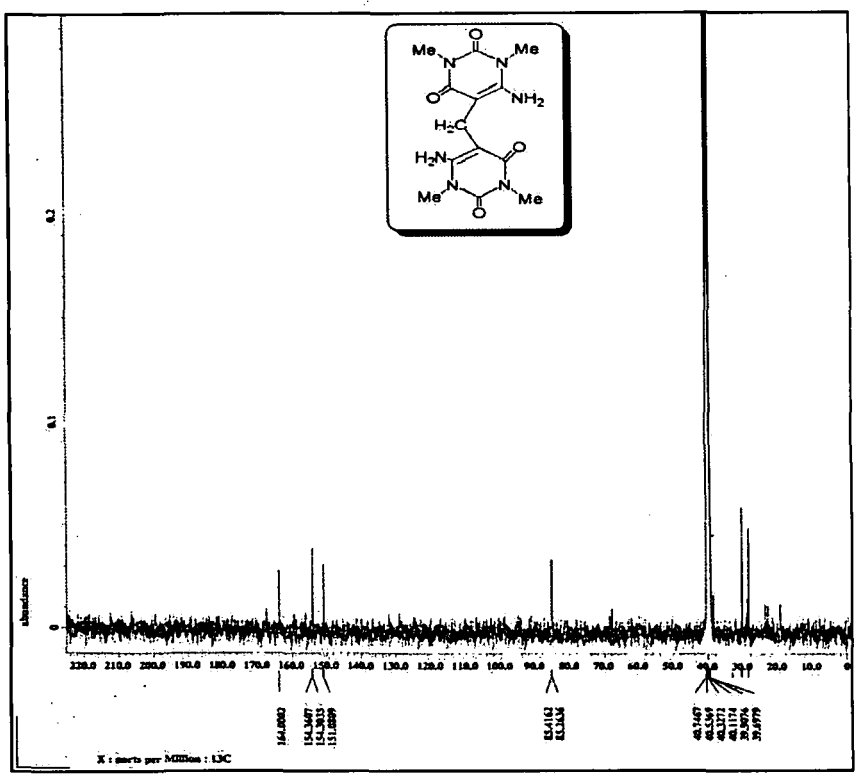

**$^{1}H$ and $^{13}C$ NMR spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(thiopheneyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**



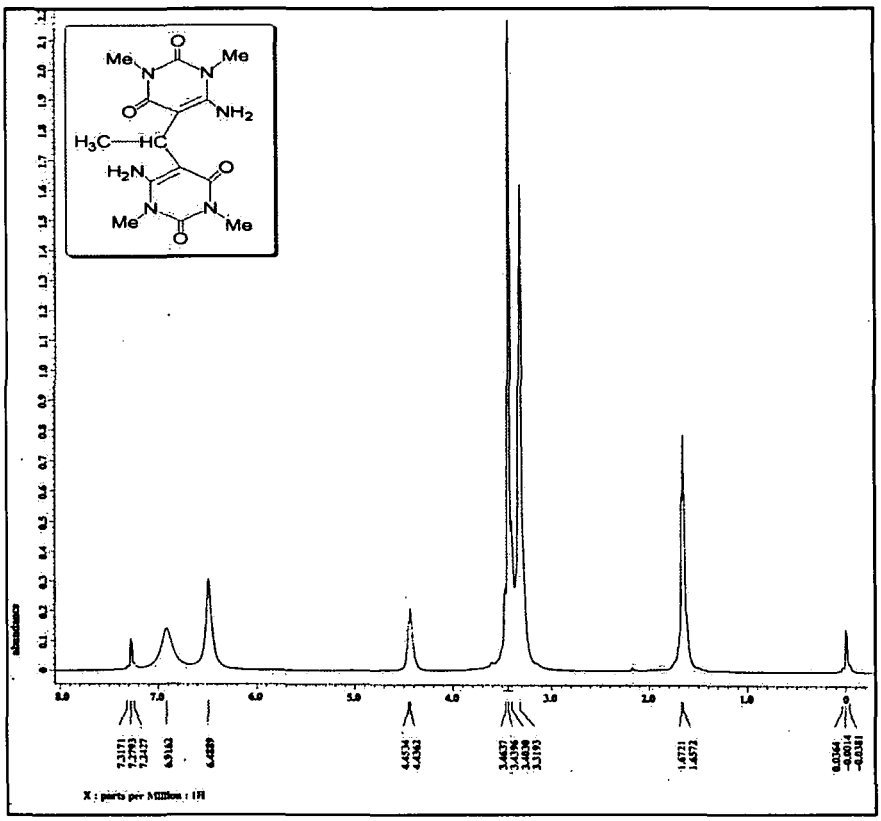


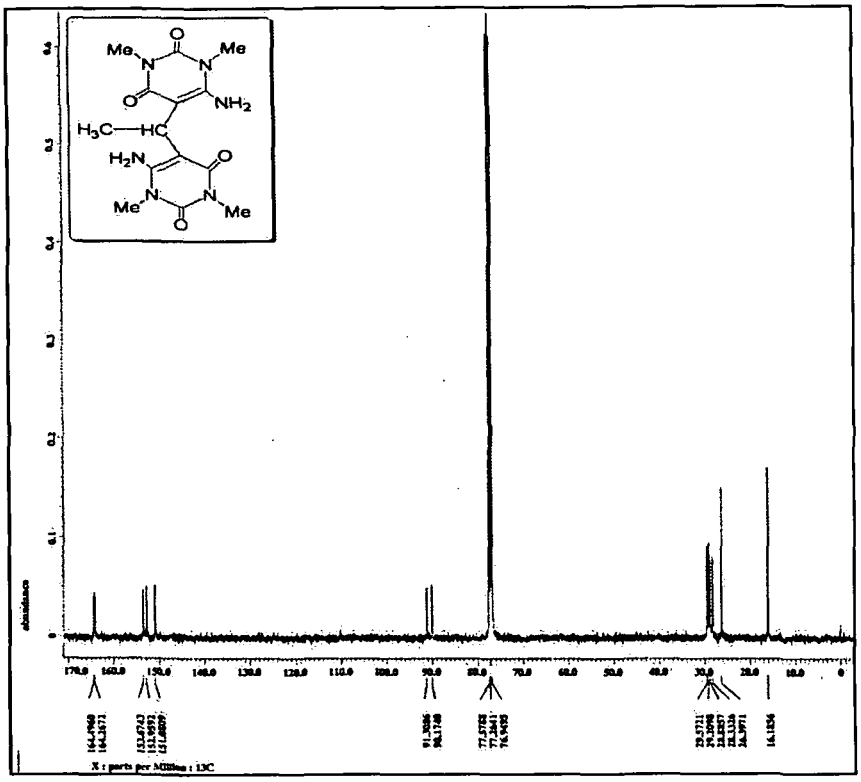

**$^{1}H$ and $^{13}C$ NMR spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(methyl)bis-[pyrimidine-2,4(1*H*,3*H*)-dione]**



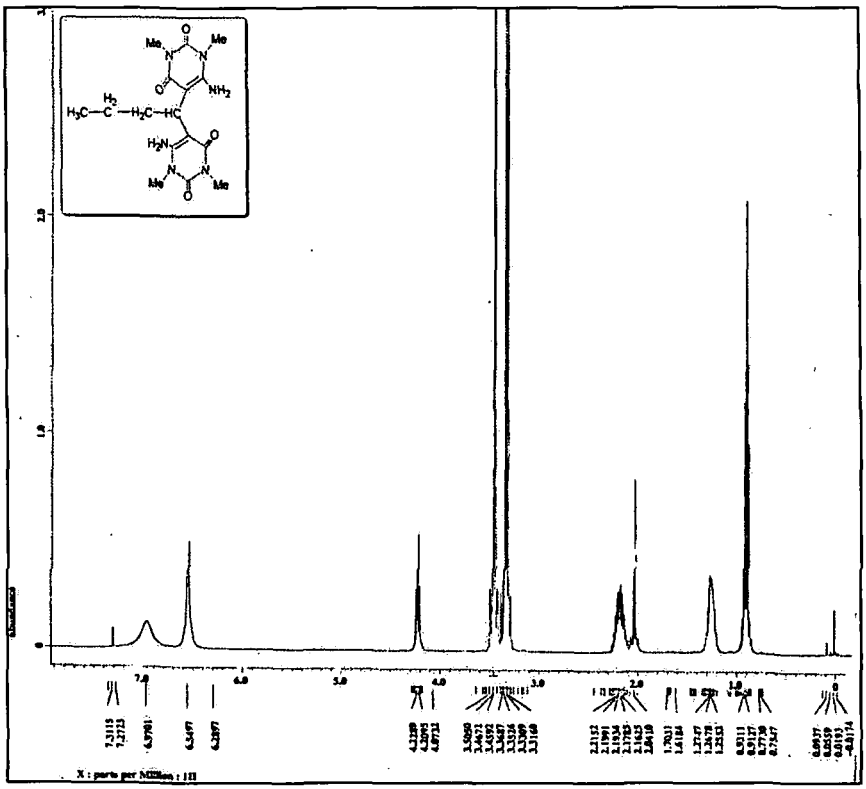


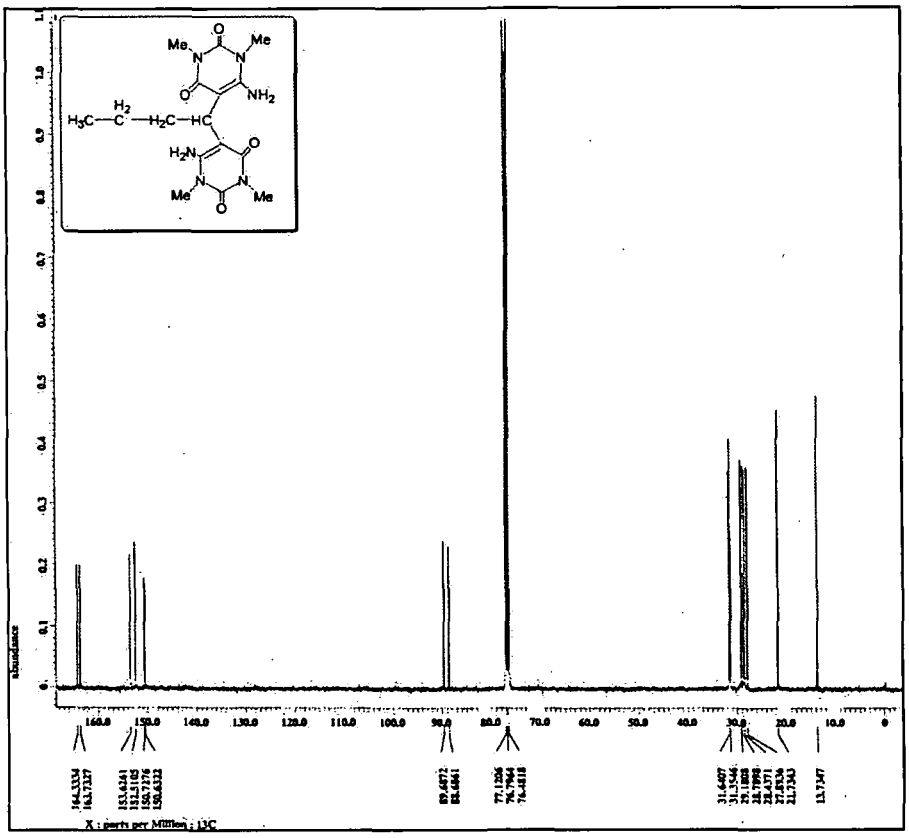
**$^{1}H$ and $^{13}C$ NMR spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(acetanyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**





**$^{1}H$, $^{13}C$ NMR and $D_2O$ exchanged $^{1}H$ spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(butanyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**

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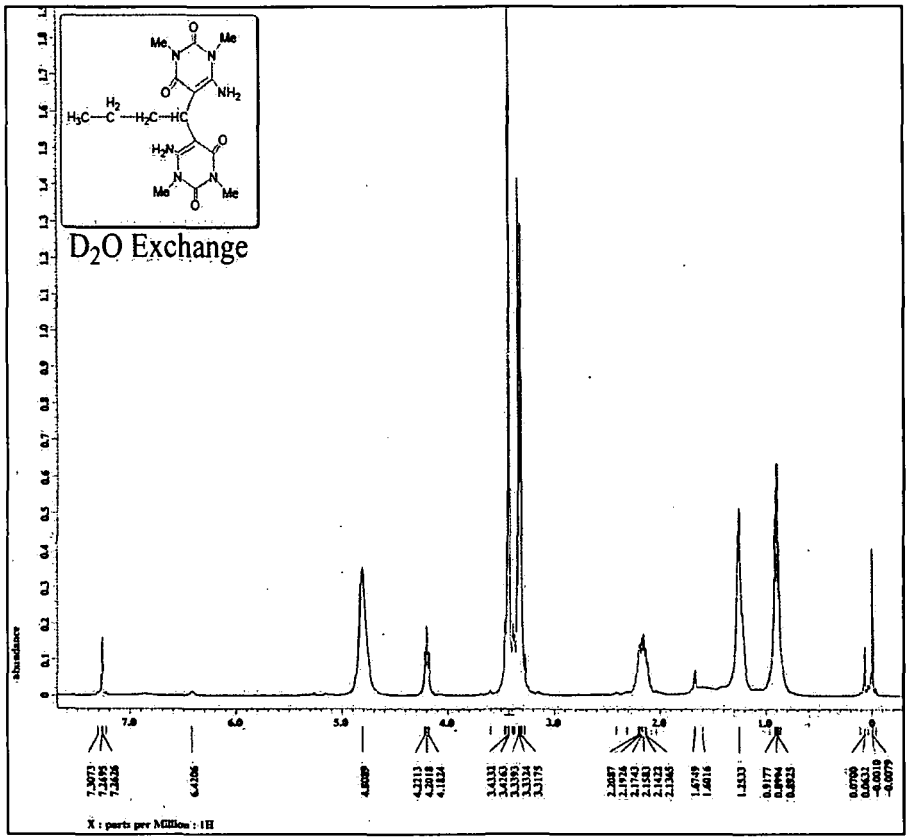
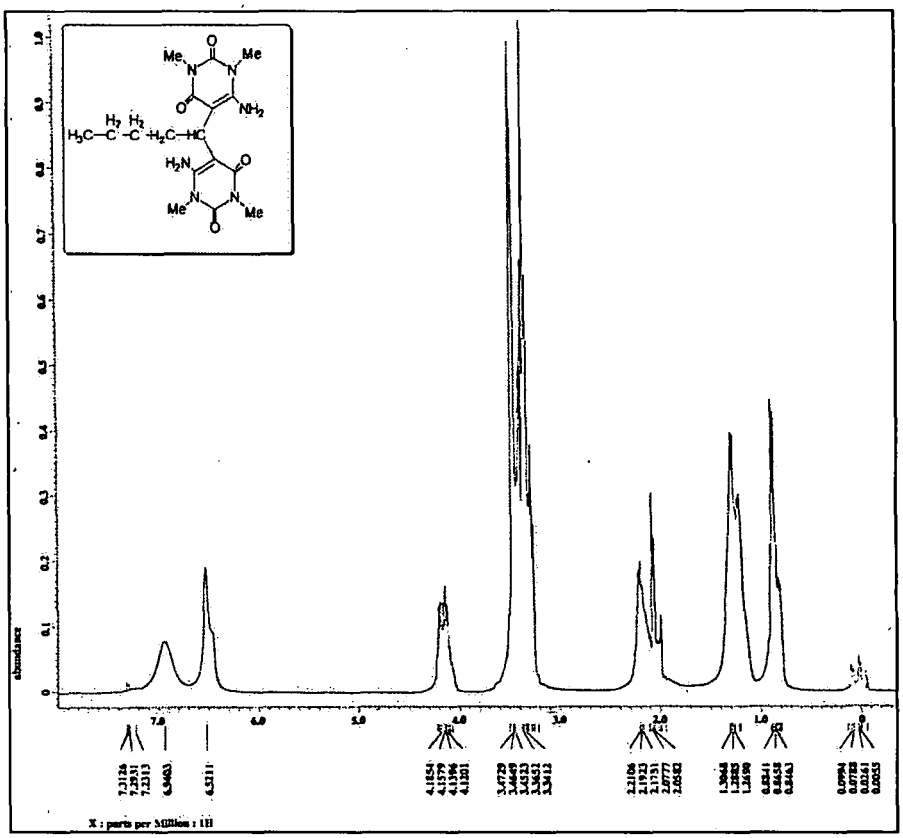
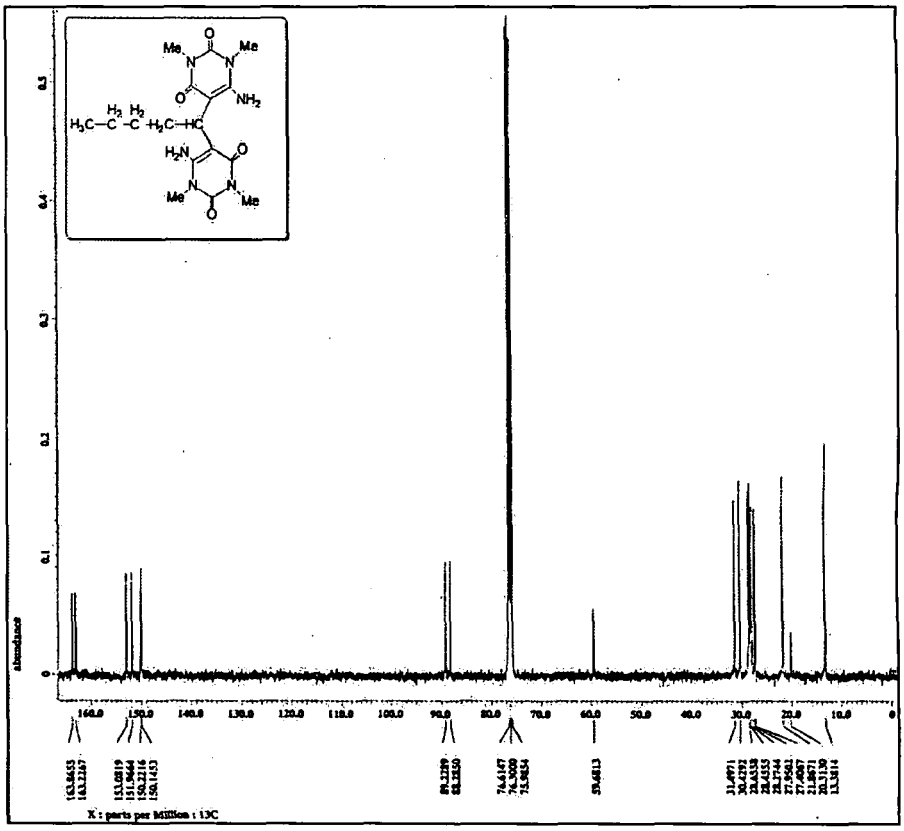
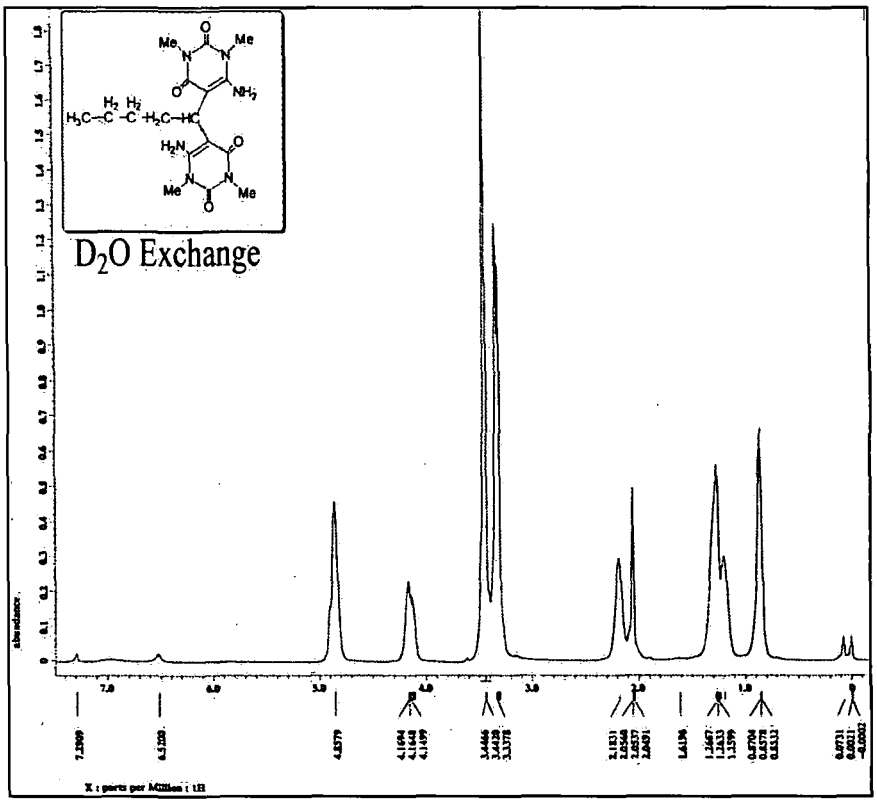
Me
NH2
H3C—C—H2C—HC
H2
H2N
Me
Me
O
X : parts per Million : 13C


Me
Me
NH2
H3C—C—H2C—HC
H2
H2N
Me
Me
O
D2O Exchange
X : parts per Million : 1H

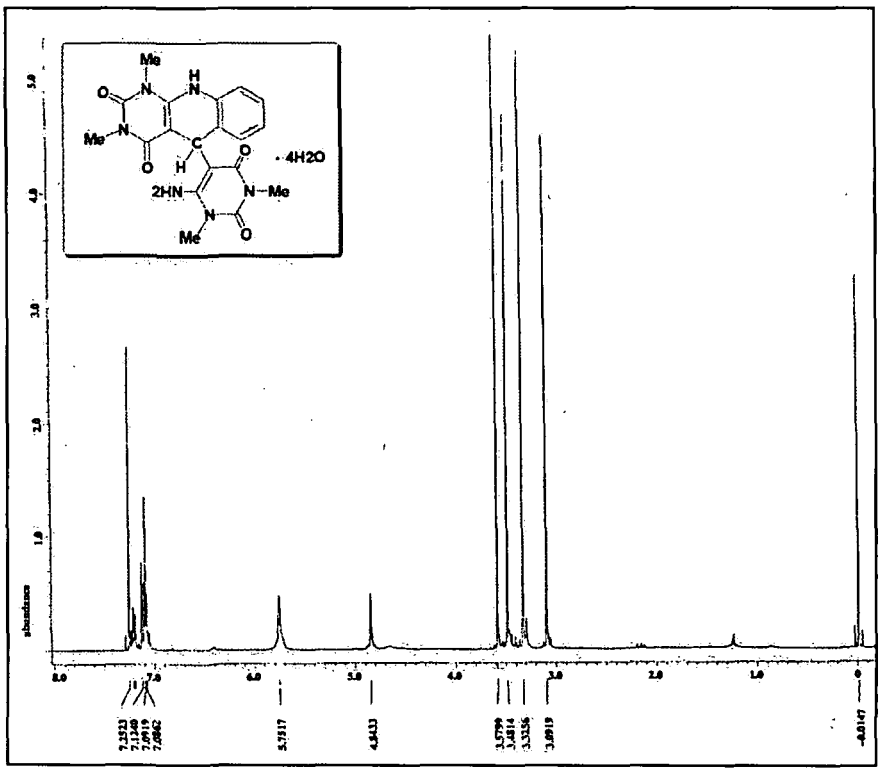
**$^{1}H$, $^{13}C$ NMR and $D_2O$ exchanged $^{1}H$ spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(pentanyl)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**

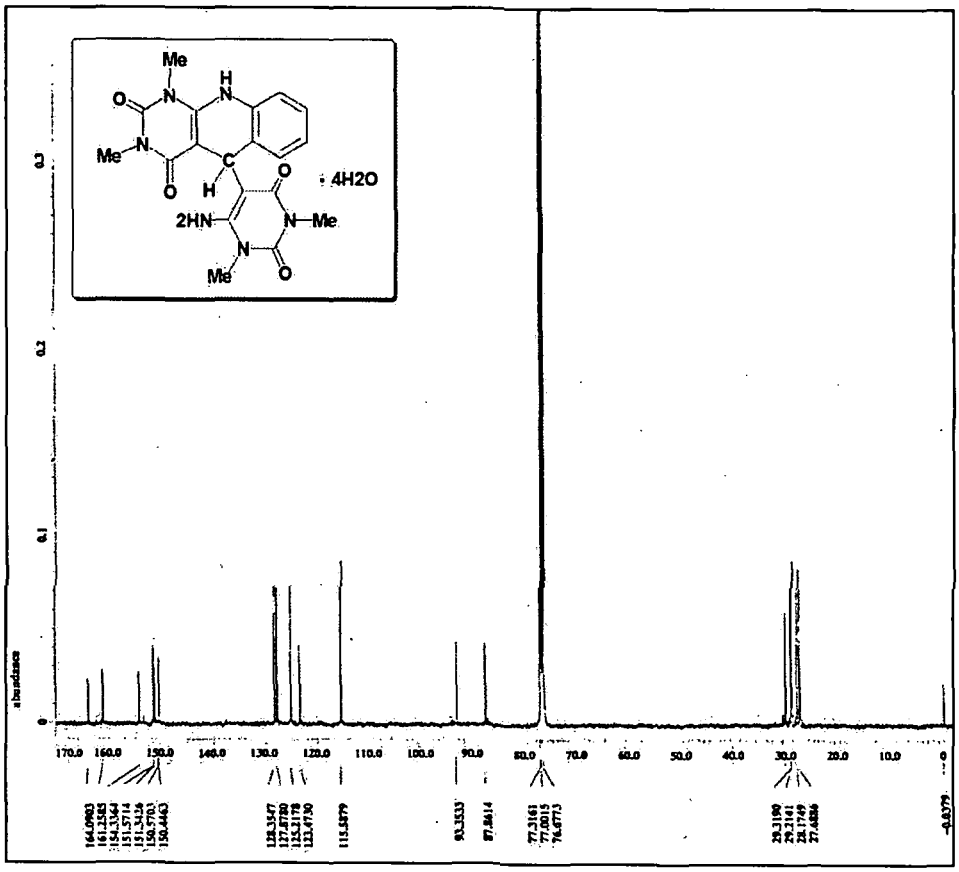







**$^{1}H$, $^{13}C$ NMR and $D_2O$ exchanged $^{1}H$ spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(2-hydroxybenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione]**




Me
N
H
O
N
Me
N
C
H
O
O
· 4H2O
2HN
N—Me
N
Me
O
abundance
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
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161.3585
154.3364
151.5774
151.1136
150.9703
150.4463
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125.2178
123.4750
115.4879
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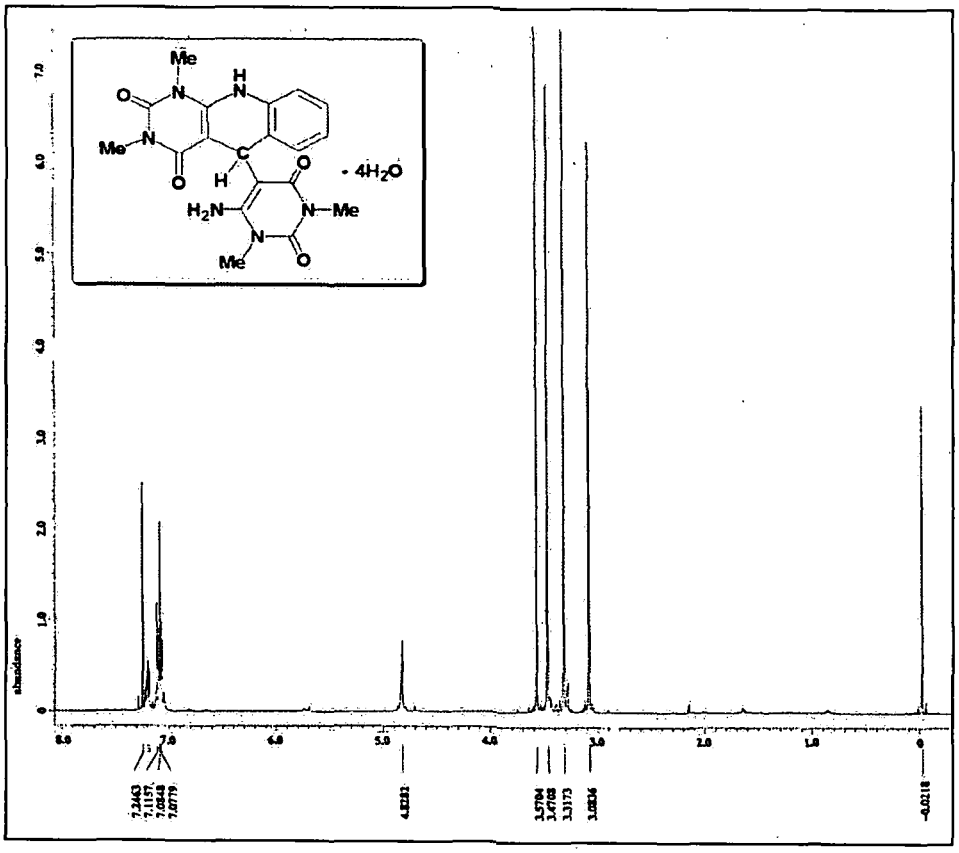
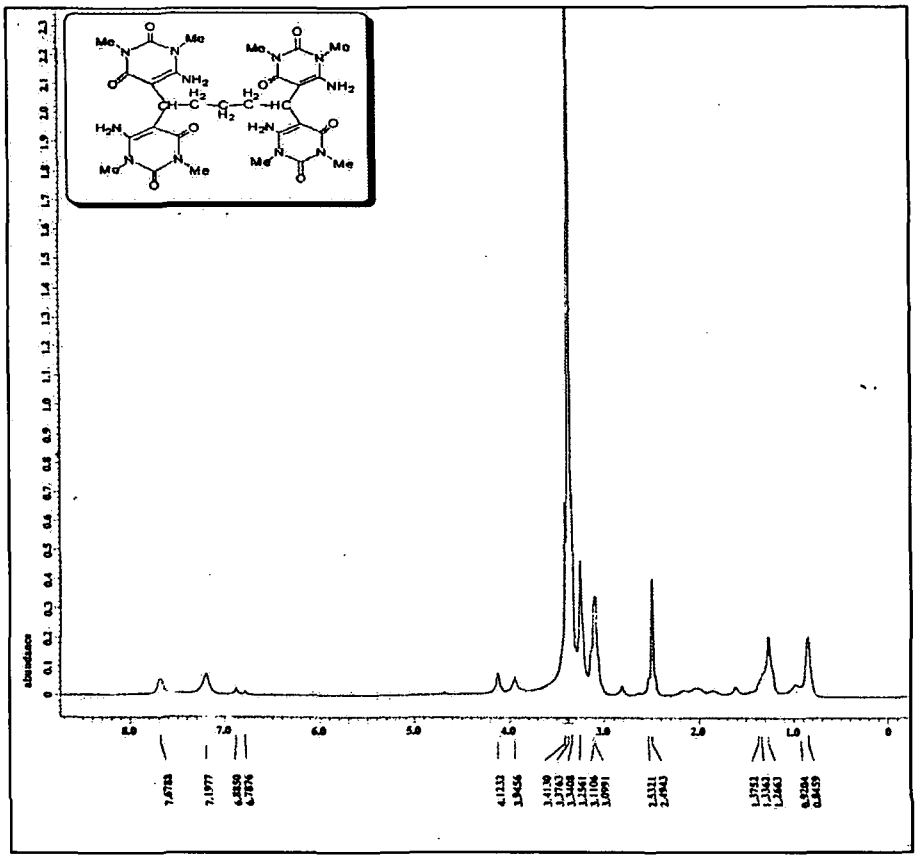
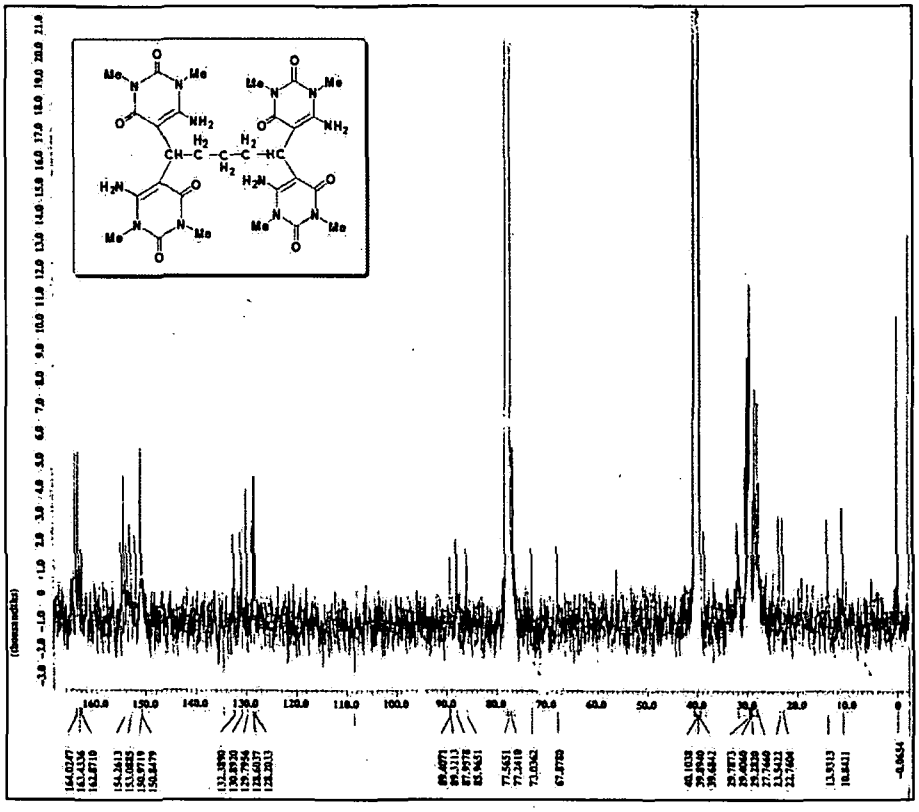
-0.0379

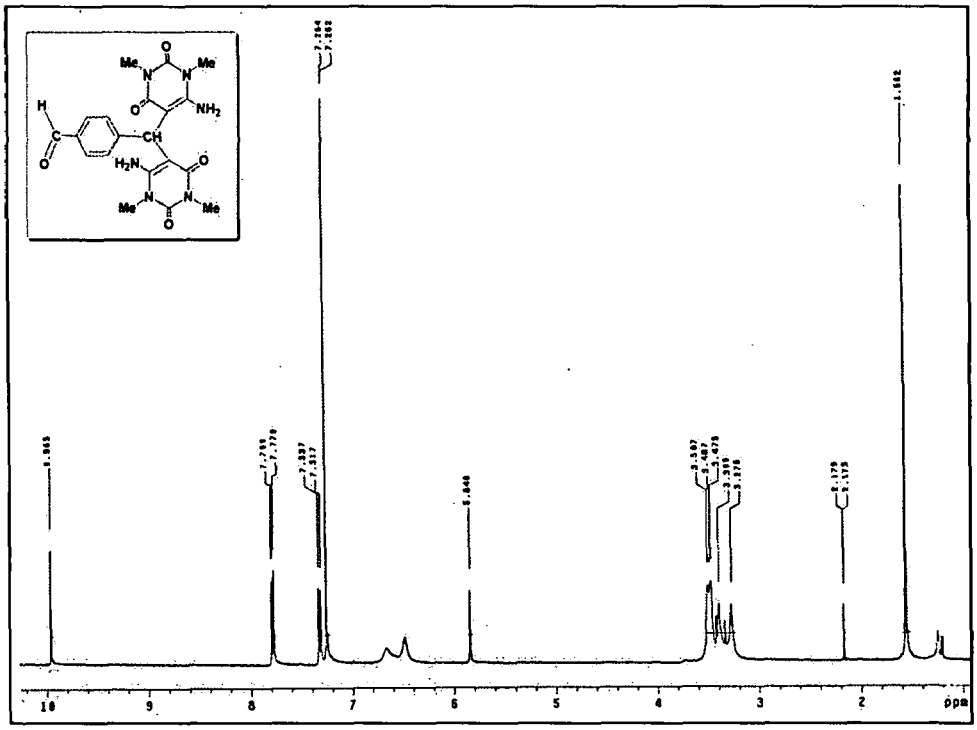

Me
N
H
O
N
Me
N
C
H
O
O
· 4H2O
H2N
N—Me
N
Me
O
abundance
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.2463
7.1157
7.0848
7.0779
4.8221
3.5704
3.4768
3.3173
3.0836
-0.0218

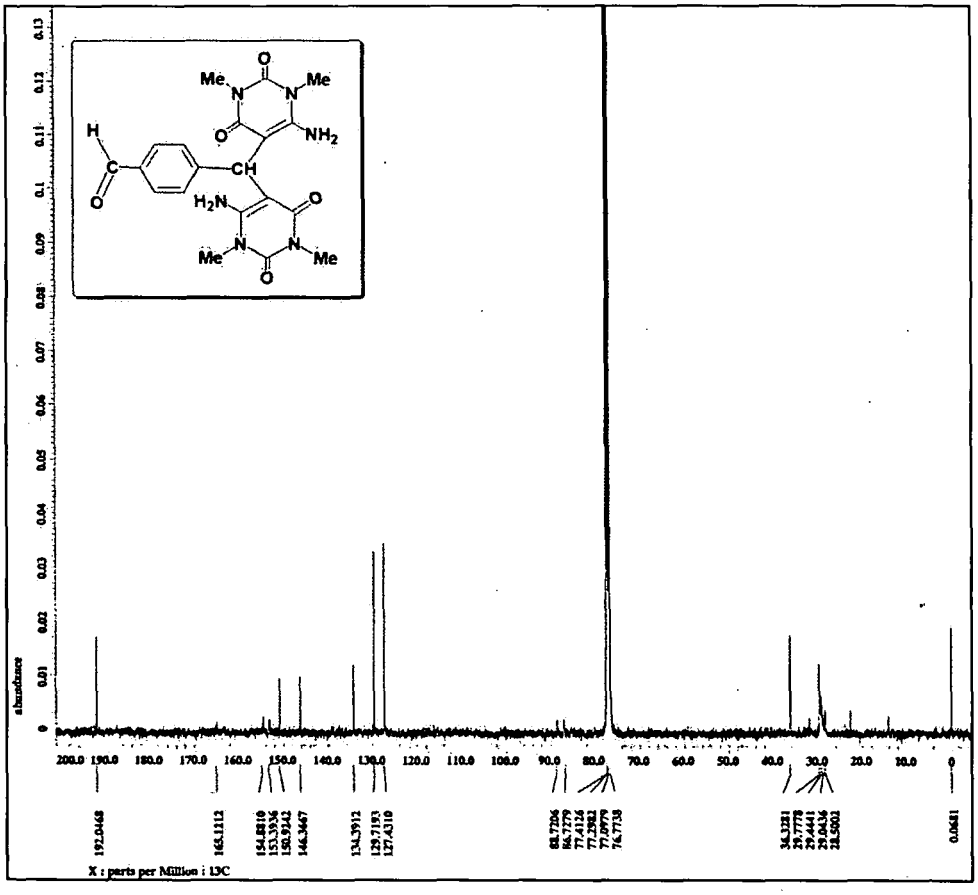
**$^{1}H$ and $^{13}C$ NMR spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(glutarylidene) -bis[pyrimidine-2,4(1*H*,3*H*)-dione]**



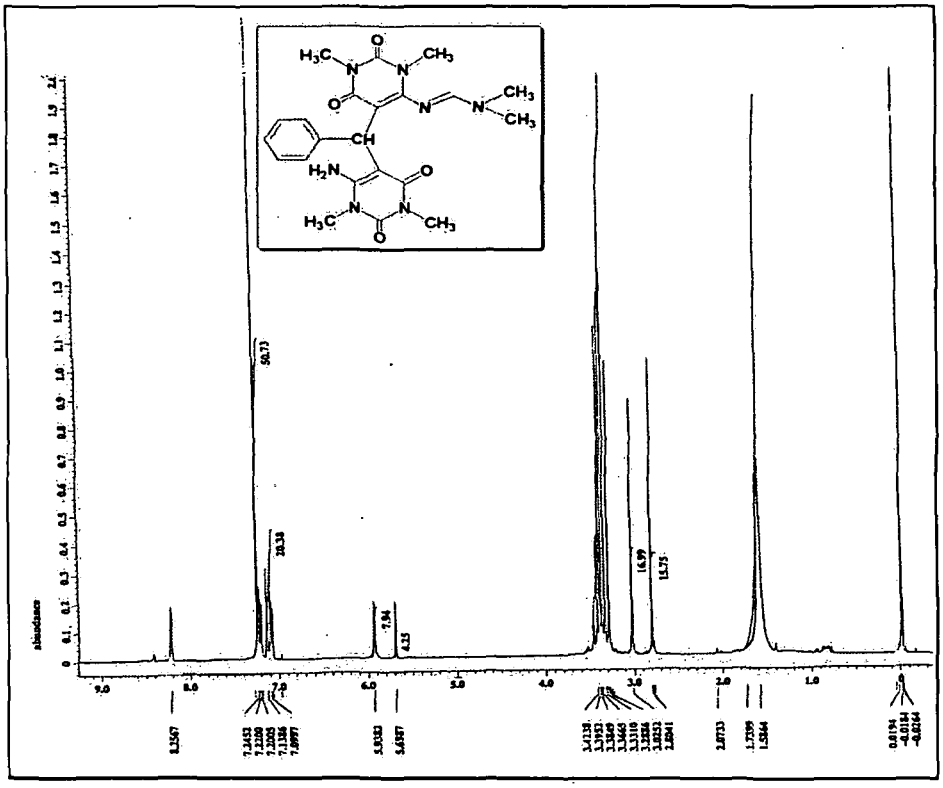


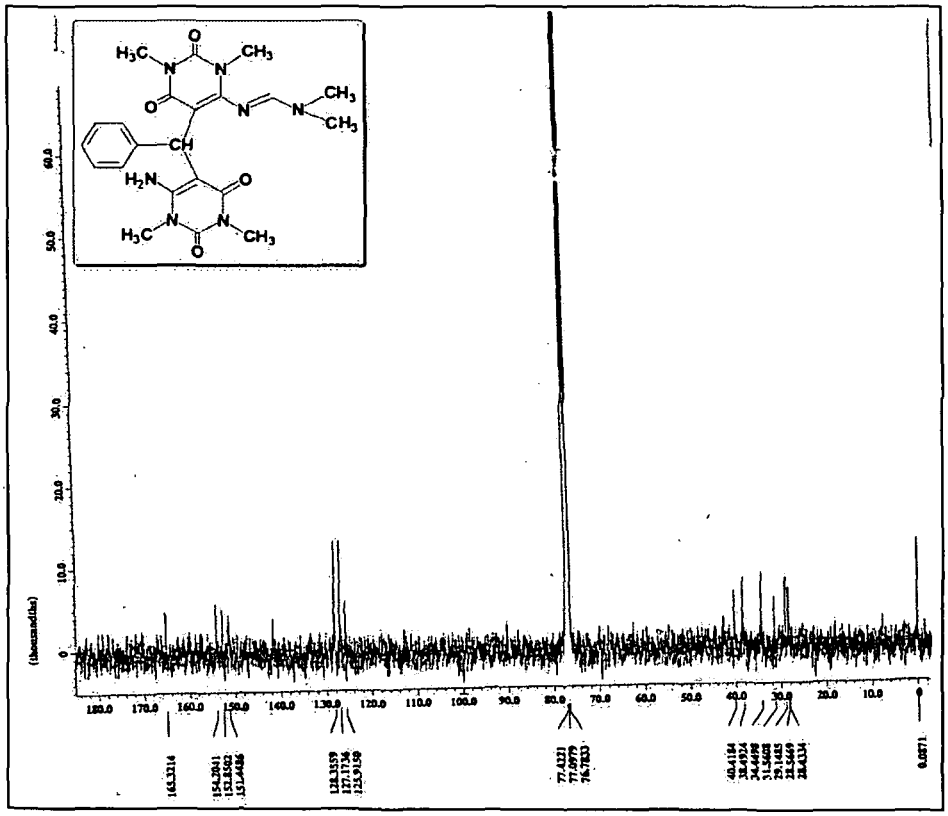
**$^{1}H$ and $^{13}C$ NMR spectra for 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(terepthaldibenzylidene)bis[pyrimidine-2,4(1*H*,3*H*)-dione]**



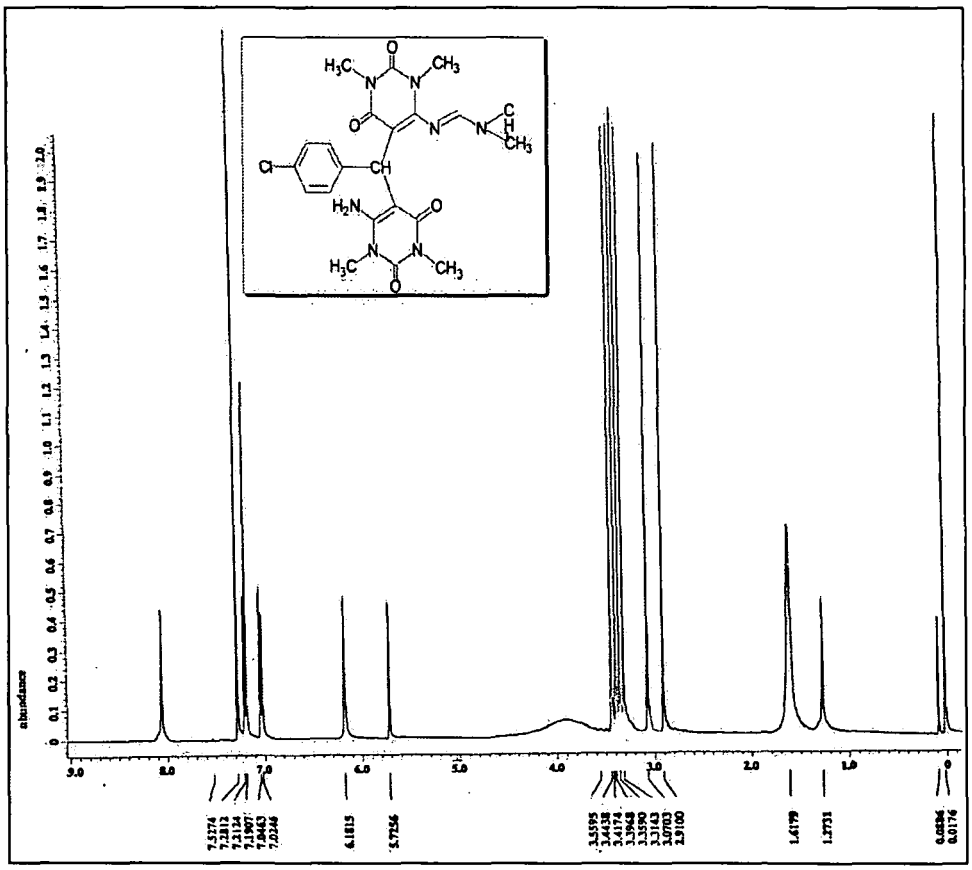



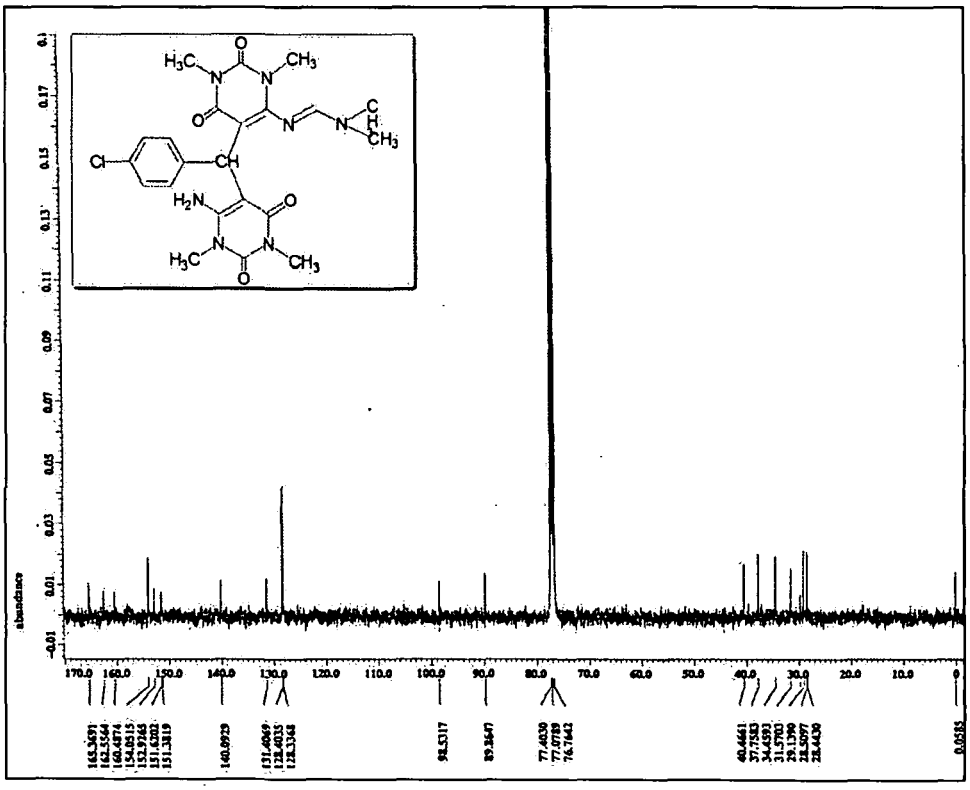
**$^{1}H$ and $^{13}C$ NMR spectra for 6-amino-6´-(dimethylamino) methyleneamino-1,1',3,3'-tetramethyl-5,5'-(benzylidene)bis[pyrimidine -2,4(1*H*,3*H*)-dione]**



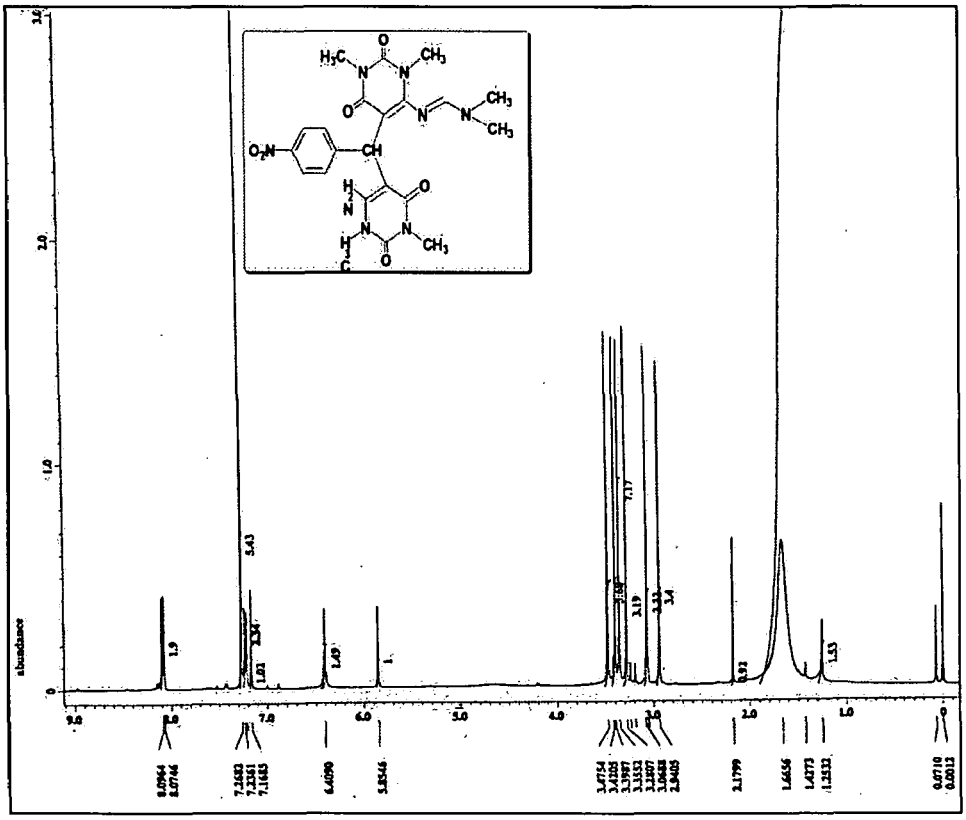


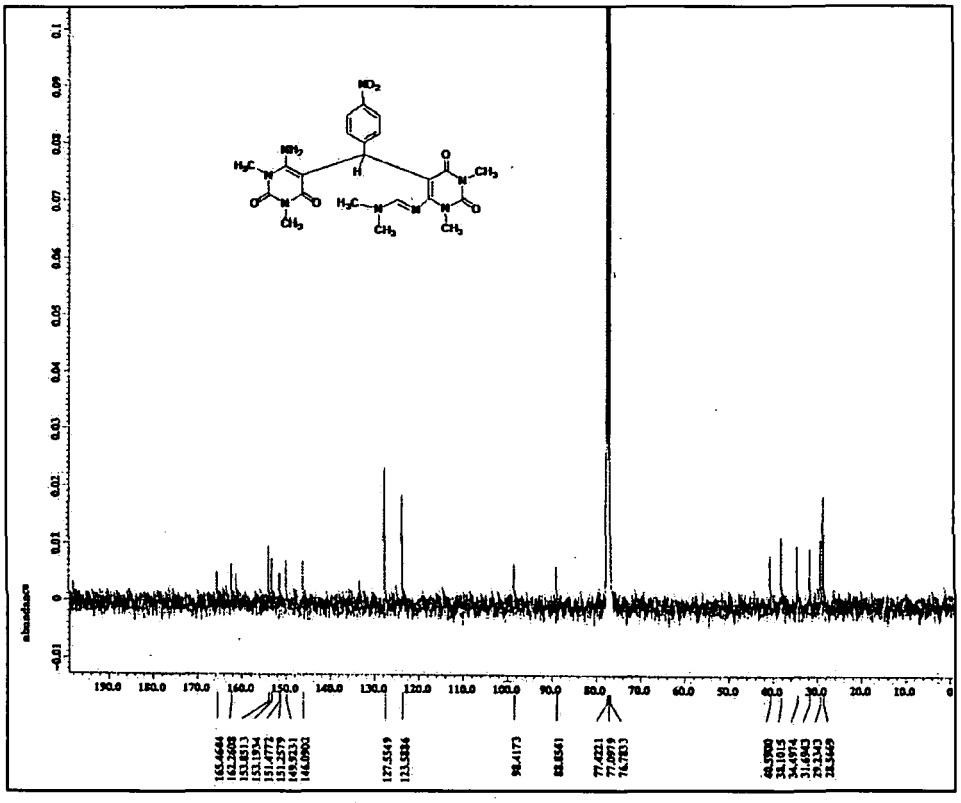
**$^{1}H$ and $^{13}C$ NMR spectra for 6-amino-6´-(dimethylamino) methyleneamino-1,1',3,3'-tetramethyl-5,5'-(4-chlorobenzylidene)-bis-[pyrimidine-2,4(1*H*,3*H*)-dione]**



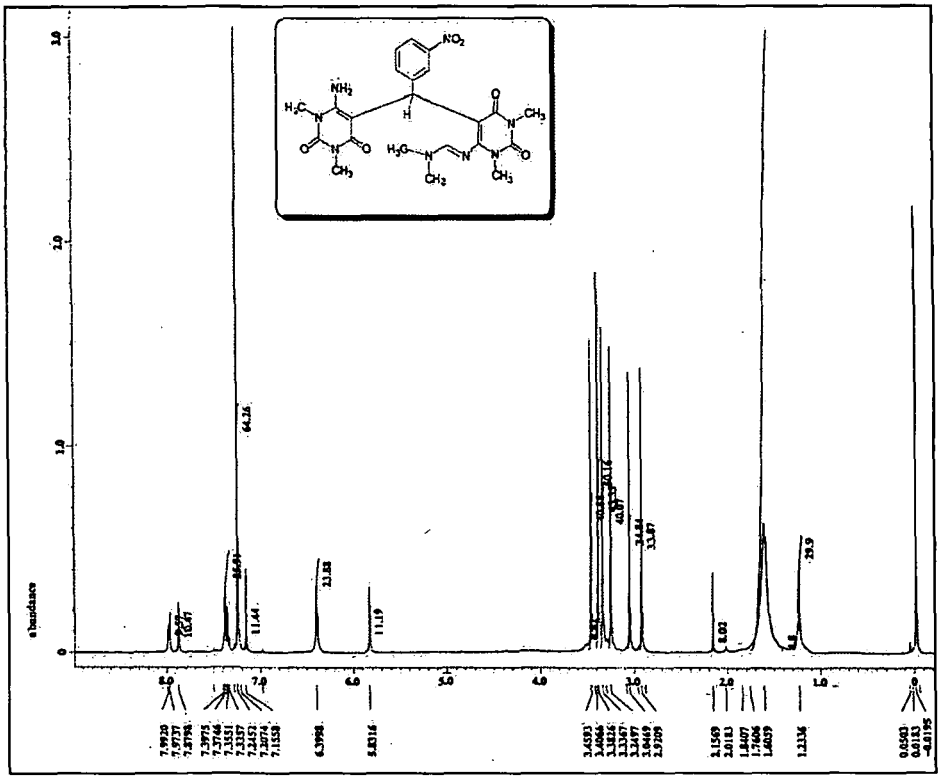


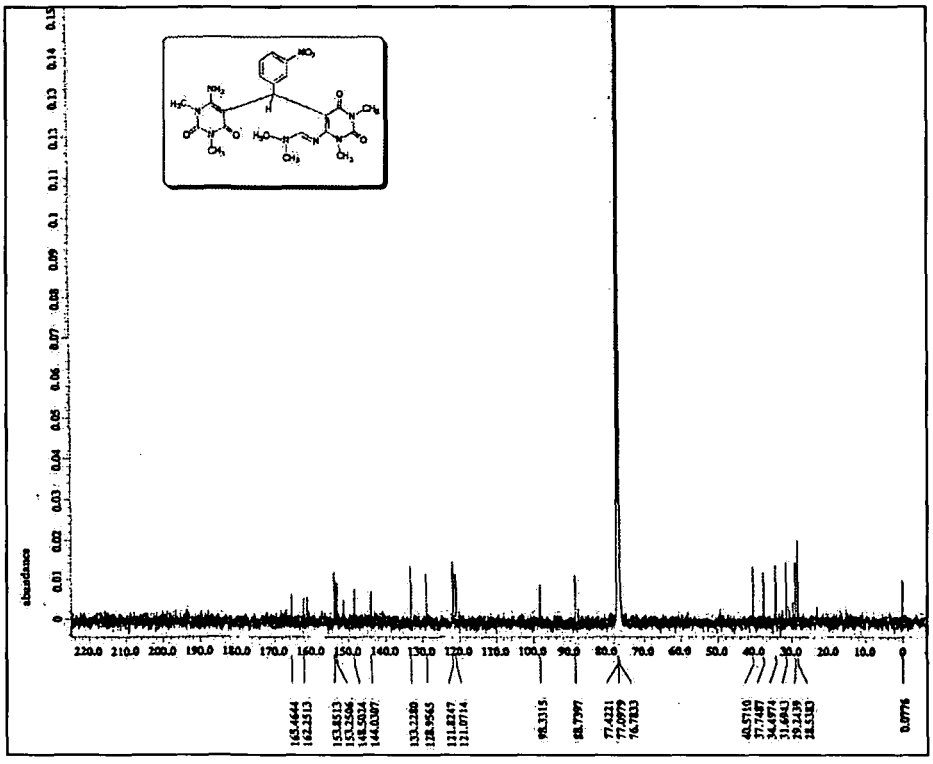
**$^{1}H$ and $^{13}C$ NMR spectra for 6-amino-6´-(dimethylamino) methyleneamino-1,1',3,3'-tetramethyl-5,5'-(4-nitrobenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione]**



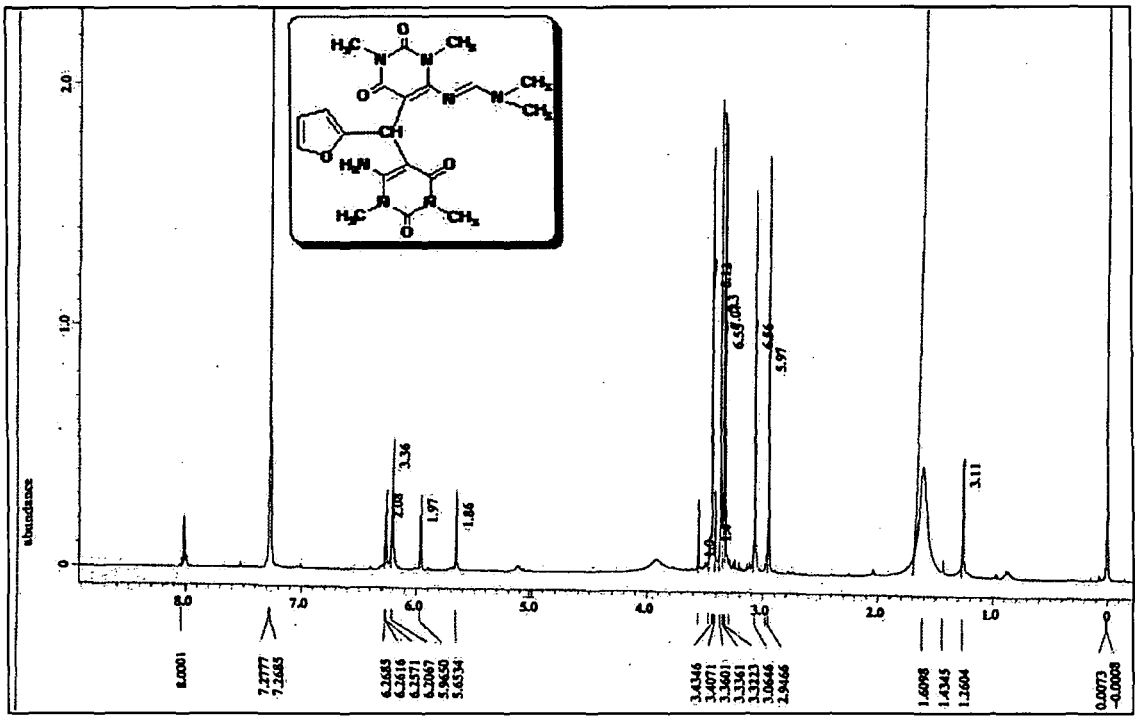


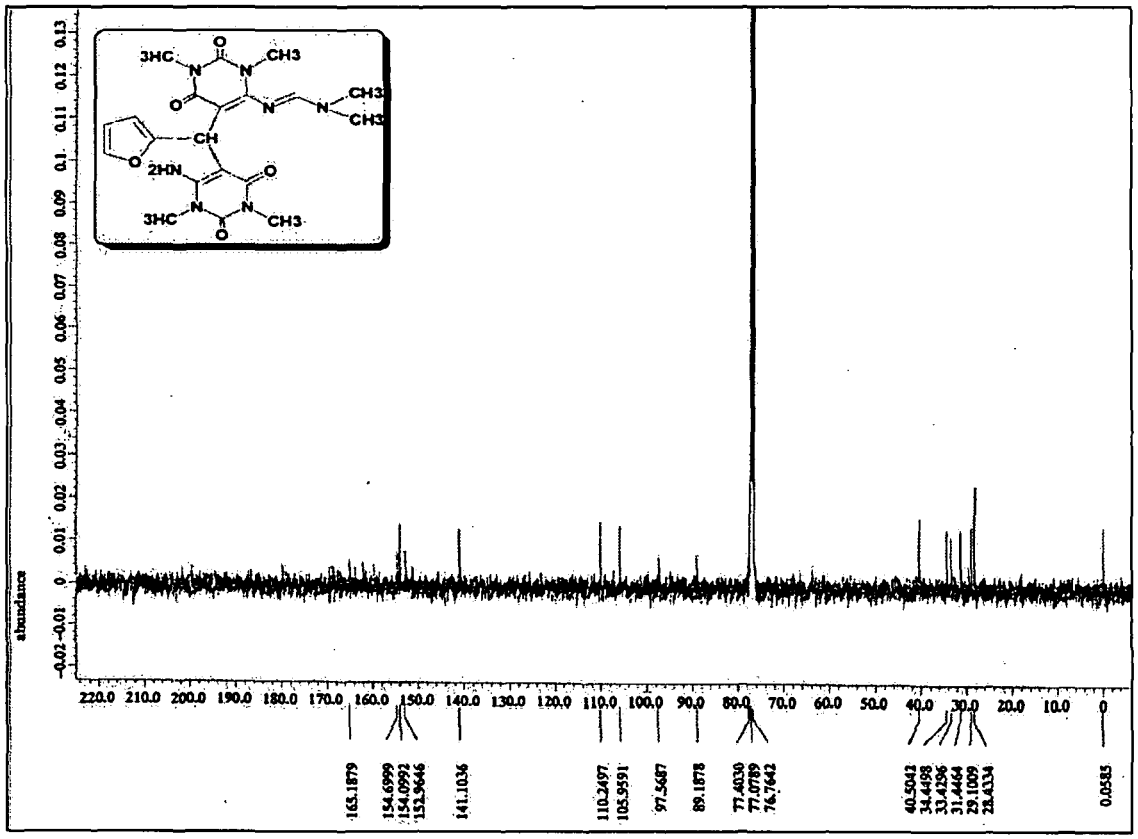
**$^{1}H$ and $^{13}C$ NMR spectra for 6-amino-6´-(dimethylamino) methyleneamino-1,1',3,3'-tetramethyl-5,5'-(3-nitrobenzylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione]**



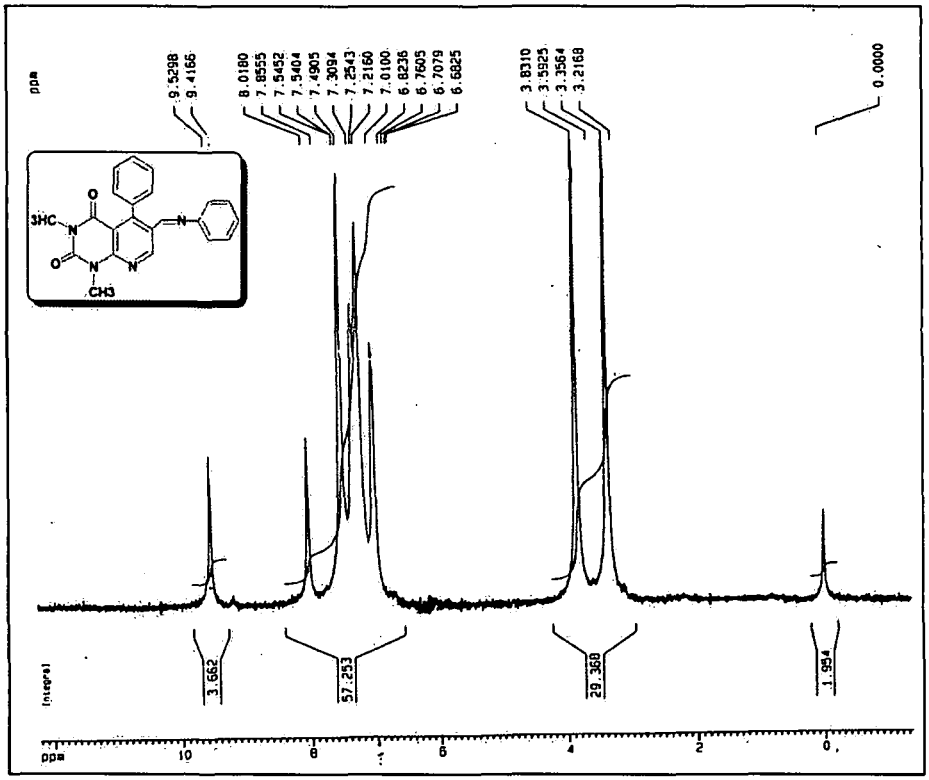


**$^{1}H$ and $^{13}C$ NMR spectra for 6-amino-6´-(dimethylamino) methyleneamino-1,1',3,3'-tetramethyl-5,5'-(2-furyl)bis-[pyrimidine-2,4(1*H*,3*H*)-dione]**

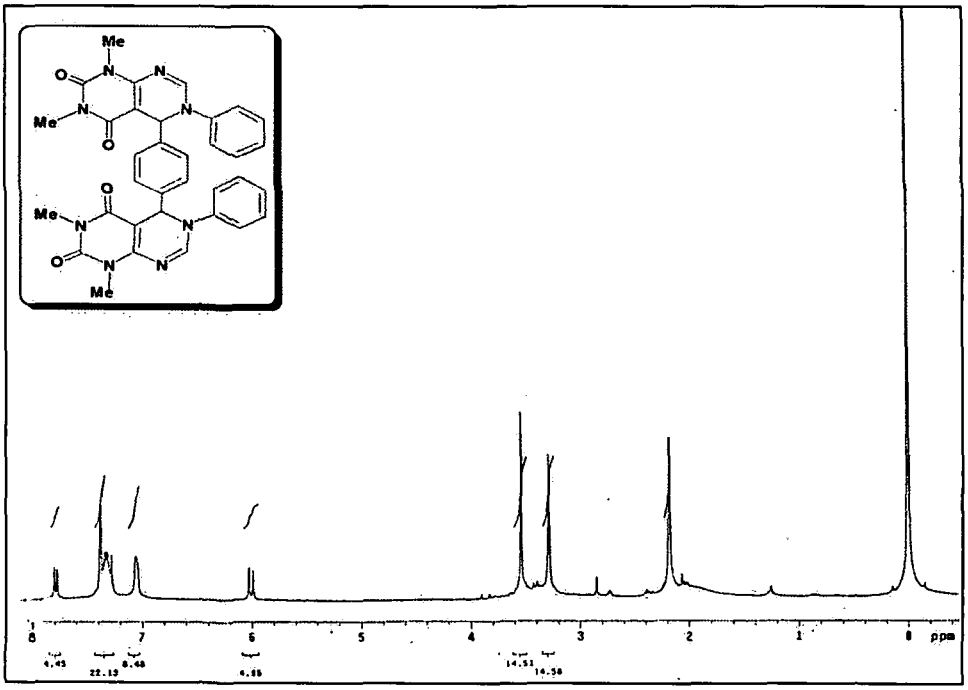




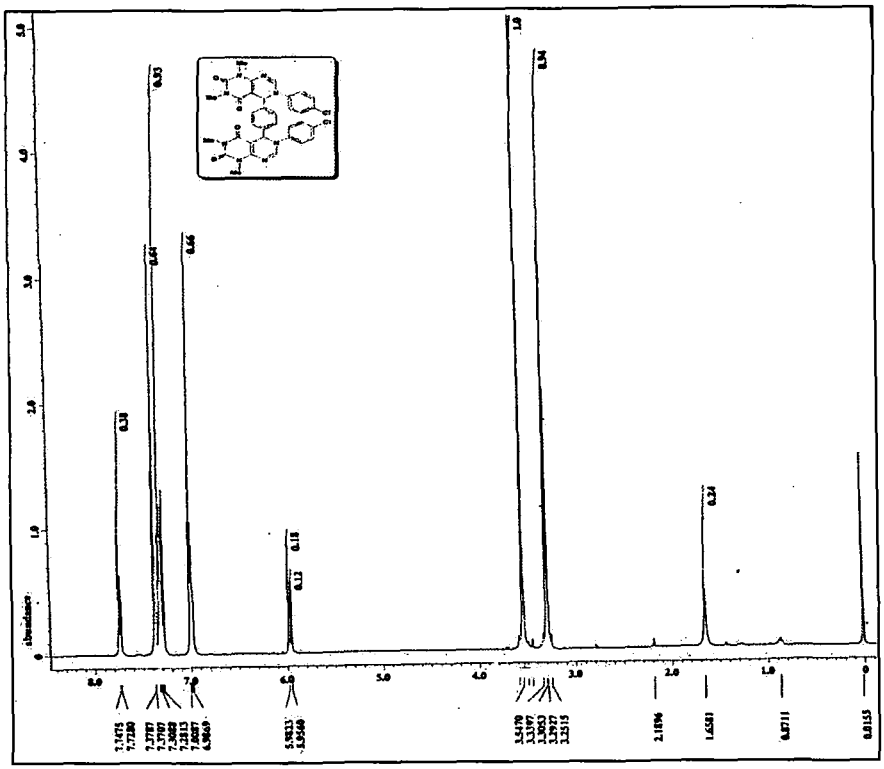
**$^1$H, spectra for 1,3-dimethyl-5-phenyl-6-((phenylimino)methyl) pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione**

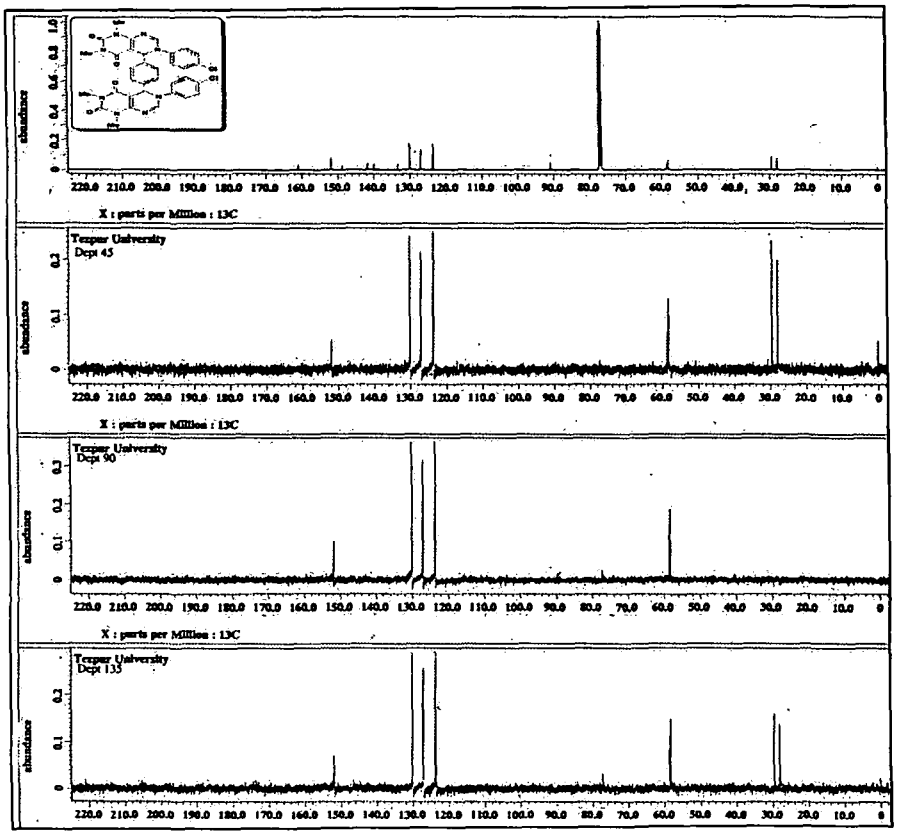


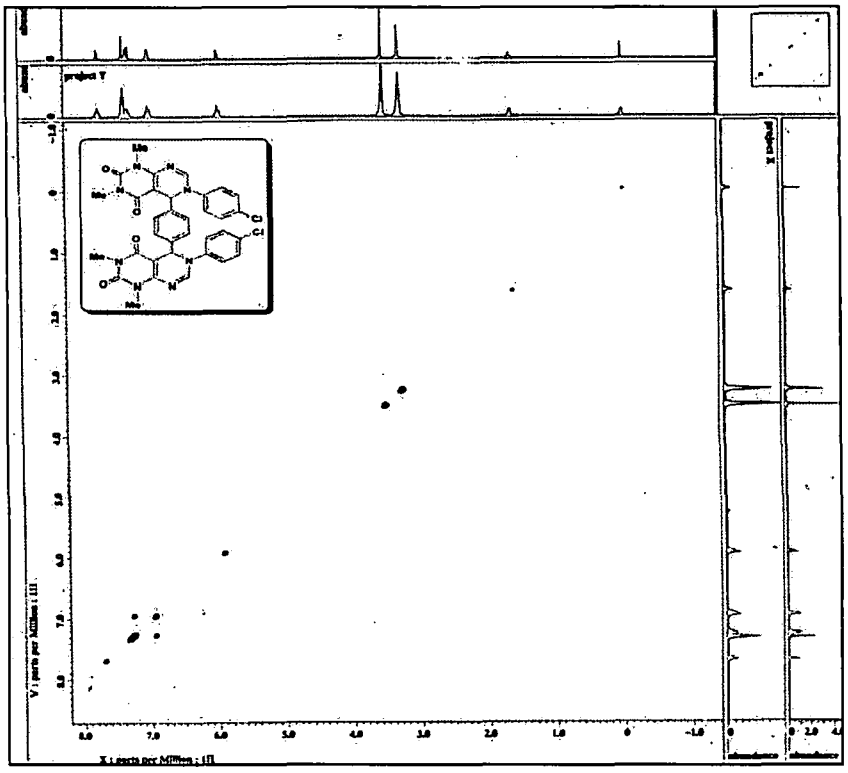

**$^1$H, spectra for 5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxo-6-phenylpyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-phenylpyrimido[4,5-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione**



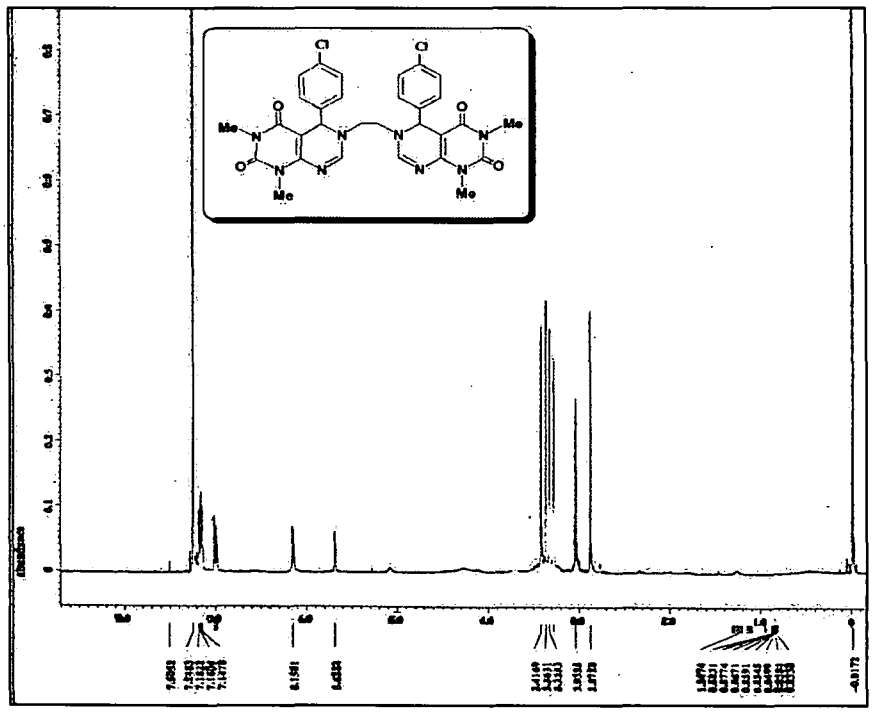
**$^{1}H$ $^{13}C$ NMR and 2D cosy spectra for 6-(4-chlorophenyl)-5-(4-(6-(4-chlorophenyl)-1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxopyrimido [4,5-*d*]pyrimidin-5-yl)phenyl)-5,6-dihydro-1,3-dimethylpyrimido[4,5-*d*] -pyrimidine-2,4(1*H*,3*H*)-dione**

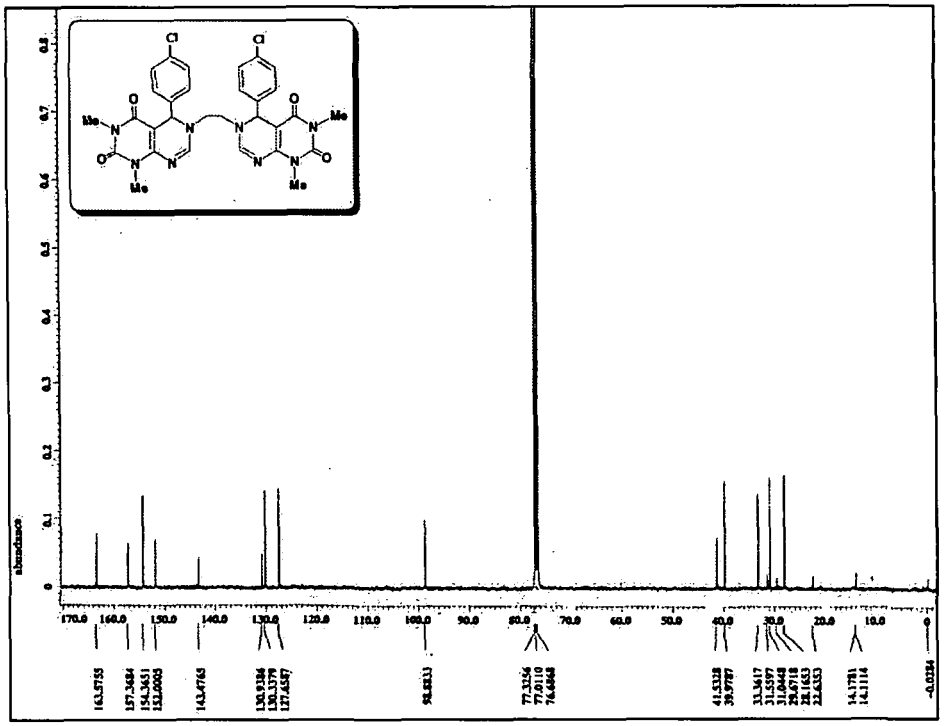




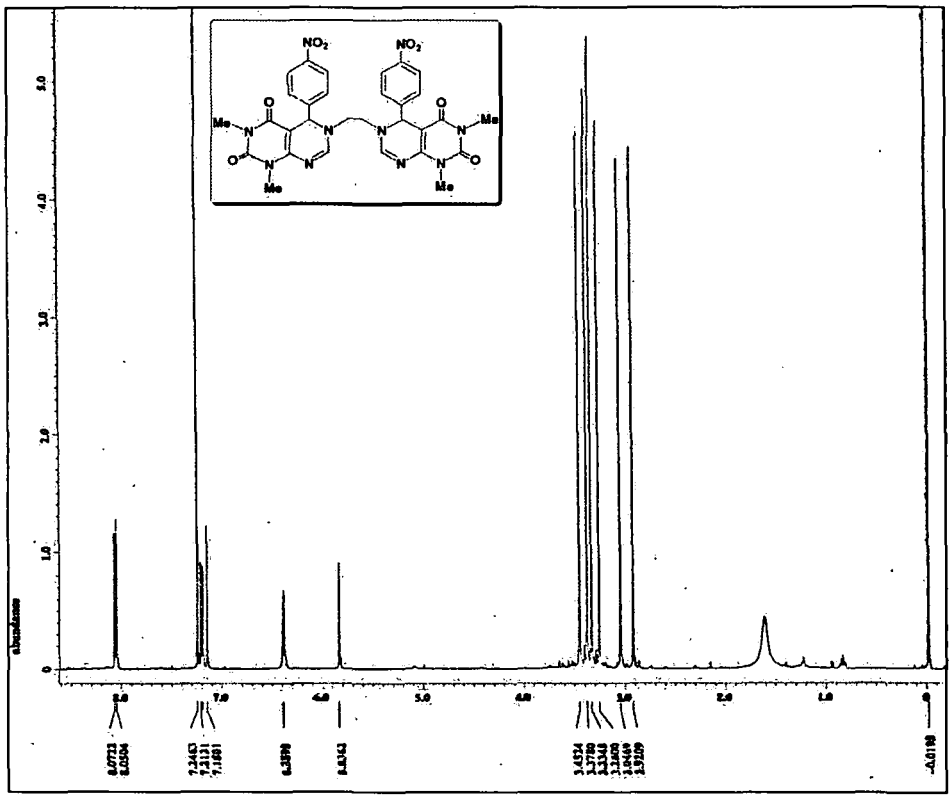


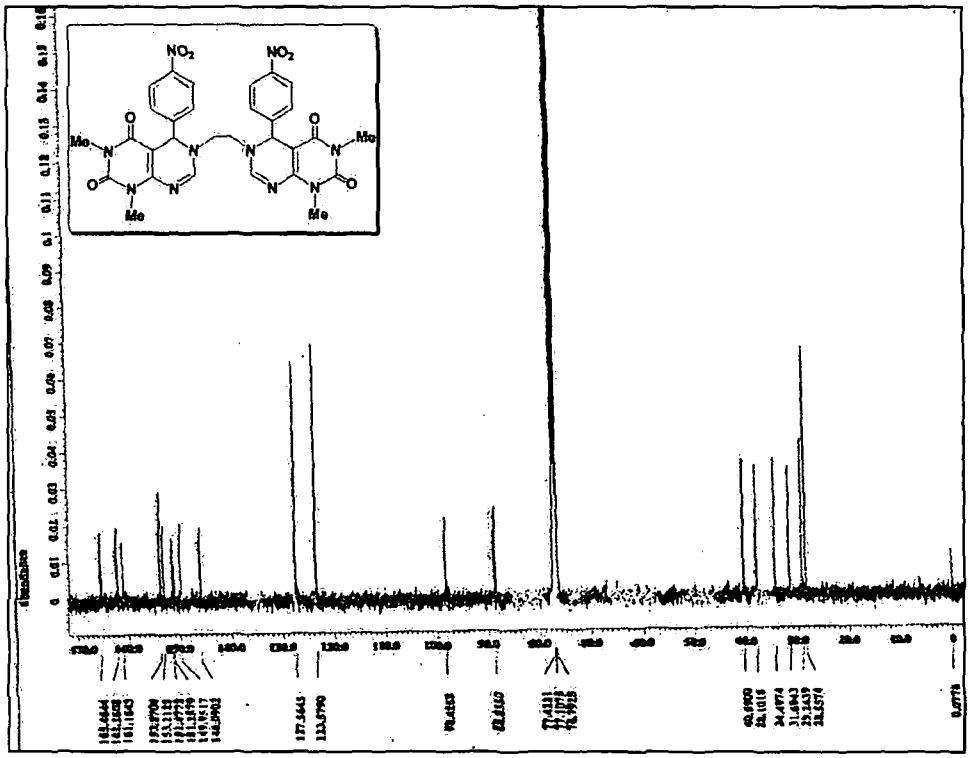
**$^{1}$H and $^{13}$C NMR spectra for 5-(4-chlorophenyl)-6-(2-(5-(4-chlorophenyl)-1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxopyrimido[4,5-*d*]pyrimidin-6(5*H*)-yl)ethyl)-5,6-dihydro-1,3-dimethylpyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione**





**$^{1}$H and $^{13}$C NMR spectra for 5,6-dihydro-6-(2-(1,2,3,4-tetrahydro-1,3-dimethyl-5-(4-nitrophenyl)-2,4-dioxopyrimido[4,5-*d*]pyrimidin-6(5*H*)-yl)ethyl)-1,3-dimethyl-5-(4-nitrophenyl)pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione**

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## List of Publications:

1. A clean, highly efficient and one-pot green synthesis of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimethyluracil-5-yl)methanes in water.
   **Das, S.** and Thakur, A.J.
   ***Eur. J. Org. Chem.*** **2011,** 2301-2308.

2. A green development of Bernthsen 9-substituted acridine synthesis in the absence of solvent catalysed by *p*-toluenesulphonic Acid (*p* -TSA).
   **Das, S.** and Thakur, A.J.
   ***Green Chem. Lett. & Rev.*** **2011**, *4*, 131-135.

3. Crude biosurfactant from thermophilic *Alcaligenes faecalis*: Feasibility in petro-spill bioremediation.
   Bharali, P.; **Das, S.**; Konwar, B.K. and Thakur, A.J.
   ***Int. Biodeter. Biodegr.*** **2011**, doi:10.1016/j.ibiod.2011.04.001 (in press)

4. Production and physico-chemical characterization of a biosurfactant produced by *Pseudomonas aeruginosa* OBP1 isolated from petroleum sludge.
   Bharali, P. and Konwar, B.K. (Acknowledge to **Das, S.**, for technical support)
   ***Appl. Biochem. Biotechnol.*** **2011**, DOI 10.1007/s12010-011-9225-z

5. Deprotection chemistry mediated by ZrOCl2.8H2O: An efficient and mild green method for the conversion of oximes to carbonyl compounds in aqueous acetone.
   Saikia, L.; **Das, S.** and Thakur, A.J.
   ***Synth. Commun.*** **2011**, *41*, 1071-1075.

6. *N, N*-Dimethyl urea,
   **Das, S.,**
   ***Synlett Spotlight*****, 2010**, *7*, 1138-1139.

7. Replay of amide resonance in 6-[(dimethylamino)methylene]1,3-dimethyl aminouracil: A dynamic NMR and density functional theory study.
   Thakur, A.J.; **Das, S.** and Phukan, A.K.
   ***J. Mol. Struc.*** **2009,** *929*, 134-140.

8. 6,6'-Diamino-1,1',3,3'-tetramethyl-5,5'-(4-chlorobenzylidene)bis[pyrimidine-2,4-(*1H,3H*)-dione]
   **Das, S.;** Saikia, B.K.; Das, B.; Saikia, L and Thakur, A.J.
   ***Acta Cryst. 65E*** **2009**, o2416-o2417.

9. Molecular iodine in protection and deprotection chemistry.
   **Das, S.**; Borah, R.; Devi, R.R. and Thakur, A.J.
   ***Synlett* 2008,** *18*, 2741-2762.

10. 6-[(Dimethylamino)methyleneamino]-1,3-dimethyl-pyrimidine-2,4(1*H*,3*H*)-dione dihydrate.
    **Das, S.**; Saikia, B.K.; Sridhar, B. and Thakur, A.J.
    ***Acta Cryst. 64E* 2008**, p01662.

11. Protection and deprotection chemistry by $ZrOCl_2.8H_2O$.
    Das, V.; **Das, S.** and Thakur, A.J. (Under minor rivision)

12. Environment-friendly and solvent free synthesis of symmetrical bis-imines under microwave irradiation.
    **Das, S.**; Das, V.; Saikia, L. and Thakur, A.J. (Communicated)

13. Feasible formation of C-5 and C-6 substituted 1, 3-dimethyl-6-amino uracil architectures.
    Saikia, B.K.; **Das, S.**; Prajapati, R.; Sridhar, B. and Thakur, A.J. (Communicated)

14. Isolation of indigenous biosurfactant producing *Pseudomonas aeruginosa* strains from the oil drilling sites of Assam.
    Bharali, P.; **Das, S.**; Konwar, B.K. and Thakur, A.J. (Communicated)

15. Nucleophilic addition of 6-aminouracil derivatives using 'green surfactant' isolated from *P. aeruginosa* OBP1 in water at room temperature
    **Das, S.**; Kalita, S.J.; Bharali, P.; Konwar, B.K.; Thakur, A.J. (Communicated)

16. Biological studies of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimthyluracil-5-yl)methanes as potential antioxidant, antibacterial and antifungal agents and studies their QSAR.
    **Das, S.**; Roy, A.; Bharali, P.; Thakur, A.J.; Deka, R.C. and Konwar, B.K. (To be communicated)

17. 6,6'-diamino-1,1',3,3'-tetramethyl-5,5'-(cinnamylidene)bis-[pyrimidine-2,4(1*H*,3*H*)-dione; Trans isomer is stable one.
    **Das, S.** and Thakur, A.J. (To be communicated)

18. One-pot, multi-component synthesis and biological evaluation of 1,3-dimethyl-5-phenylpyrido[2,3-*d*]pyrimidine-2,4-dione derivatives.
    **Das, S.**, Thakur, A.J. and Medhi, T. (To be communicated)

19. Hetero-Diels-Alder methodology for the synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives.
**Das, S.** and Thakur, A.J. (To be communicated)

## Seminar / Workshop / Symposium / Summer School Etc. Attended / Predented

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1. Poster in **3rd RSC (Royal Society of Chemistry) -11th National Symposium in Chemistry (NSC-11)** held in National Chemical Laboratory, Pune, India, under the auspices of **Chemical Research Society of India (CRSI)**, Bangalore, 5 – 8th Feb 2009.
   Title: A benign approach for the synthesis of Bis-imines
   **Subrata Das** and Ashim J. Thakur

2. Poster in **2nd RSC-10th National Symposium in Chemistry (NSC-10)** held in Indian Institute of Science (IISc), Bangalore, India, under the auspices of **CRSI**, Bangalore, 31st Jan – 3rd Feb 2008.
   Title: Synthesis of 9- substituted acridines under microwave irradiation
   **Subrata Das** and Ashim J. Thakur

3. Poster in **9th National Symposium in Chemistry (NSC-9)** held in Delhi University, Delhi, India, under the auspices of **CRSI**, Bangalore, 2nd – 4th Feb 2007.
   Title: Experimental Determination of rotational barrier in 6-[(Dimethylamino) -methylene]-1, 3-dimethylaminouracil by dynamic NMR spectroscopy
   **Subrata Das** and Ashim J. Thakur

4. Winter School on "**Crystallography**" held at the School of Chemistry, University of Hyderabad, Hyderabad, under the auspices of **UGC Networking Centre (UGC)**, India, 22nd Nov – 4th Dec 2010.

5. DST (Department of Science and Technology), Govt. of India and Council for the Lindau Nobel Laureate Meetings, Govt. of Germany award to attend the **59th meeting of Nobel Laureates and students in Chemistry in Lindau, Germany** during 29 – 3rd July and to visit the premier institutions/ universities in Germany during 4 – 11th July, 2009.

6. Participation in the **International Seminar On Frontiers in Polymer Science and Technology (Poly-2007)** at Guwahati organised jointly by Prof. Sukumar Maiti Polymer Award Foundation, Kolkata, India and Tezpur University, Napaam, Assam, India. Jadavpur University, Kolkata, India, November-2007.

7. Summer School on "**Frontiers in Polymer Chemistry**" held at the Dept. of Inorganic and Physical chemistry, Indian Institute of Science (IISc), Bangalore, Karnataka, India, June 2006 – July 2006

8. **Frontier Lecture Series** at Tezpur University organized by Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore in collaboration with the Department of Chemical Sciences, Tezpur University, India. (20 - 22 Nov-2009)

9. National workshop in **Nuclear and Atomic Techniques Based Pure and Applied Sciences,** Tezpur University, jointly organized by Tezpur University and UGC-DAE Consortium for Scientific Research, Kolkata, India. (1 - 3 Feb-2011)

10. One day workshop on **Intellectual Property Rights Sensitization: IPRSW-2010** organized by Tezpur University, India. (23 Dec-2010)

11. **14th National Workshop on Catalysis** at Tezpur University organized by Department of Chemical Sciences, Tezpur University, India. (21 - 23 Dec-2009)

## Patent

- Biological activities of aryl/alkyl/heteroaryl bis(6-amino-1,3-dimthyluracil-5-yl)methanes.
  **Das, S.**; Bharali, P.; Roy, A.; Konwar, B. K.; Thakur, A. J.
  (Under Preparation)