CHAPTER 3

A one pot, two-step synthesis of 5-arylpyrrolo[2,3-*d*]pyrimidines and their preliminary bioassay studies

3.1 Introduction

Among various fused heterocycles, pyrrolo[2,3-*d*]pyrimidine has been considered as one of the most important classes of organic compounds. Various compounds of this family have been screened and established to possess diverse biological activities like antifolate [1], antitumour [2], antibacterial [3], antiviral [4], antimicrobial [5], anti HCV [6], receptor tyrosine kinase (RTK) inhibitor [7], firefly luciferase inhibitor [8], microtubule inhibitor[9], affinity against α_1-adrenosine receptor (α_1-AR) [10] etc. Alimta (**1**, **Fig. 1**), a 5-substituted pyrrolo[2,3-*d*]pyrimidine has been established as a unique antifolate with remarkable activity against a broad spectrum of solid tumors [11]. The scaffold doesn't attract the attention of synthetic and medicinal chemists only, but becomes a topic of interest for material chemist also, due to its redox ability [12] and fluorescent property [13].

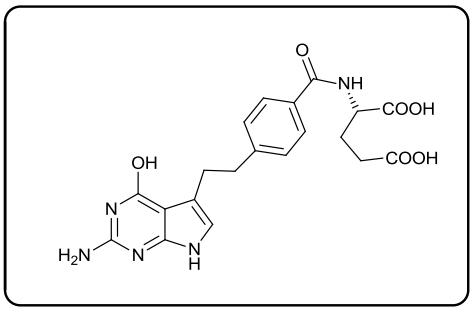
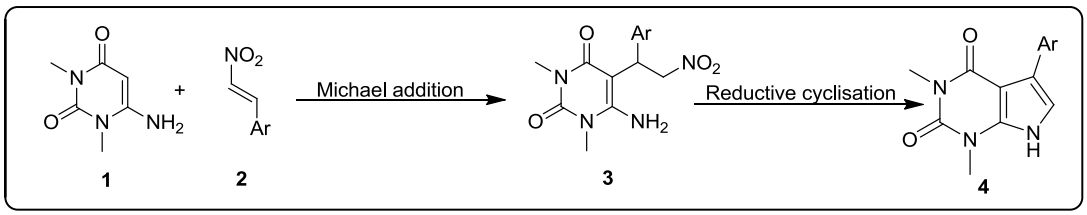


Fig. 1: Structure of Alimta, an antifolate

Although, the classical routes for synthesizing the unit were Knorr [14] and Paal-Knorr [15] synthesis, later on numerous other methods with different strategies have been developed and reported subsequently for the synthesis of pyrrolo[2,3-*d*]pyrimidines [16]. A few of these methods use aminopyrimidine and nitrostyrene as substrates to carry out the synthesis either in a single or in a multistep process. Very recently, a three component route has been reported to synthesize the same starting from aminopyrimidine, nitromethane and aldehydes [17]. In spite of having a plethora of synthetic methods for 5-substituted-pyrrolo[2,3-*d*]pyrimidines, chemists are still in search of efficient newer methodologies, as the

reported methods possess various drawbacks like, involving multiple steps, requiring longer reaction time, associated lower yield etc.

In view of the extreme medicinal as well as material importance of pyrrolo[2,3-*d*]pyrimidines and the need to develop newer synthetic methodology for the same, we sought to design an efficient, one pot synthetic methodology for 5-arylpyrrolo[2,3-*d*]pyrimidines from aminopyrimidine and nitrostyrenes. A keen literature survey resulted only two reports [16d, 17] of this type, both of which mainly emphasis on the catalytic properties of two acid catalysts with either Lewis or Brønsted acid site to carry out the conversions. We targeted to achieve the same conversion in a one pot two-step process by exploiting: (i) the favorability of Michael addition reaction in alkaline medium and (ii) the strong reducing capability of alkaline $Na_2S_2O_4$ solution. These two facts led us to the idea that there could be a useful synthetic route for pyrrolo[2,3-*d*]pyrimidine starting from aminopyrimidine and nitrostyrene by carrying out the Michael type addition (first step) in alkaline medium followed by reductive cyclisation of the Michael adduct (second step) using alkaline $Na_2S_2O_4$ solution (**Scheme 1**). The complete process was expected to be carried out in a single pot experiment without isolating the intermediate. Strategically, if both the steps would become successful in a common solvent that would be an added advantage.

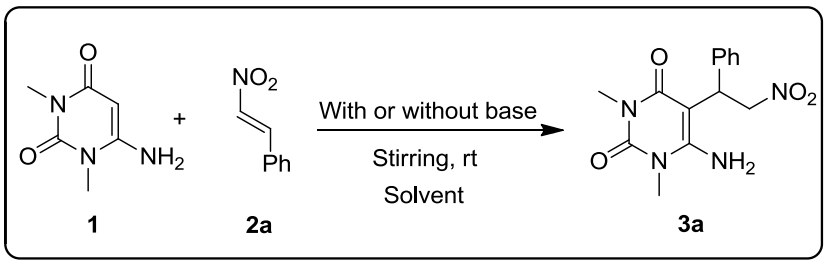


Scheme 1: Proposed route for pyrrolo[2,3-*d*]pyrimidine

3.2 Results and discussion

To start with, 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**1**) was treated with (*E*)-(2-nitrovinyl)benzene (**2a**) in equimolar ratio in water at room temperature (**Scheme 2**). To our delight, the starting materials were observed to be consumed completely within 6 hours resulting a single product which was later

on confirmed as the Michael adduct (**3a**) by single crystal X-ray analysis (**Fig. 2**). The same reaction was then tested in presence of 1 equivalent of aqueous NaOH solution and to our delight, it worked sufficiently well to reduce the time required for complete consumption of the substrates from 6 hrs to 2 hrs. Other solvent-base combinations were also examined under identical conditions and the results are summarized in a bar diagram (**Fig. 3**). **Fig. 3** clearly signifies the suitability of polar protic solvents over polar aprotic as well as non-polar solvents and EtOH-NaOH is established as the best solvent-base combination for the purpose. EtOH is found to



Scheme 2: Michael addition of **1** & **2a**

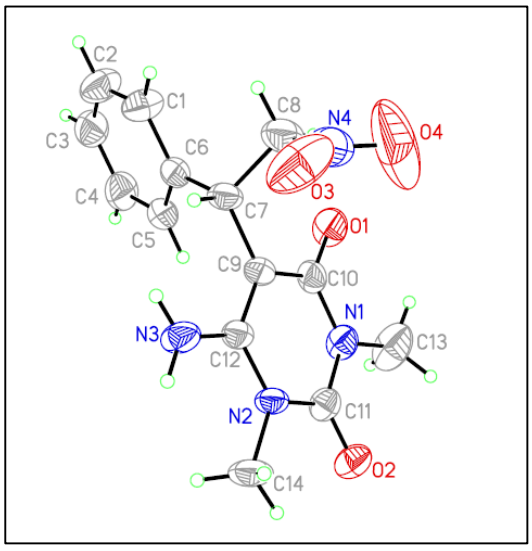


Fig. 2: ORTEP diagram of **3a**

be more effective than H_2O (A-Q and B-Q in **Fig. 3**), which may be attributed to the greater solubility of starting materials in EtOH than in H_2O. Optimization of NaOH added was carried out and an amount of 1.5 equivalent was established as the most suitable as it reduced the time requirement for complete consumption of **1** to

1 hour only. Work up was carried out to isolate the crude **3a**, which was then subjected to reductive cyclisation using alkaline $Na_2S_2O_4$solution (**Scheme 3**). Effect of solvent, amount of NaOH as base and temperature on the yield of product (**4a**) were also studied and the results are summarized in **Table 1**. Solvents like

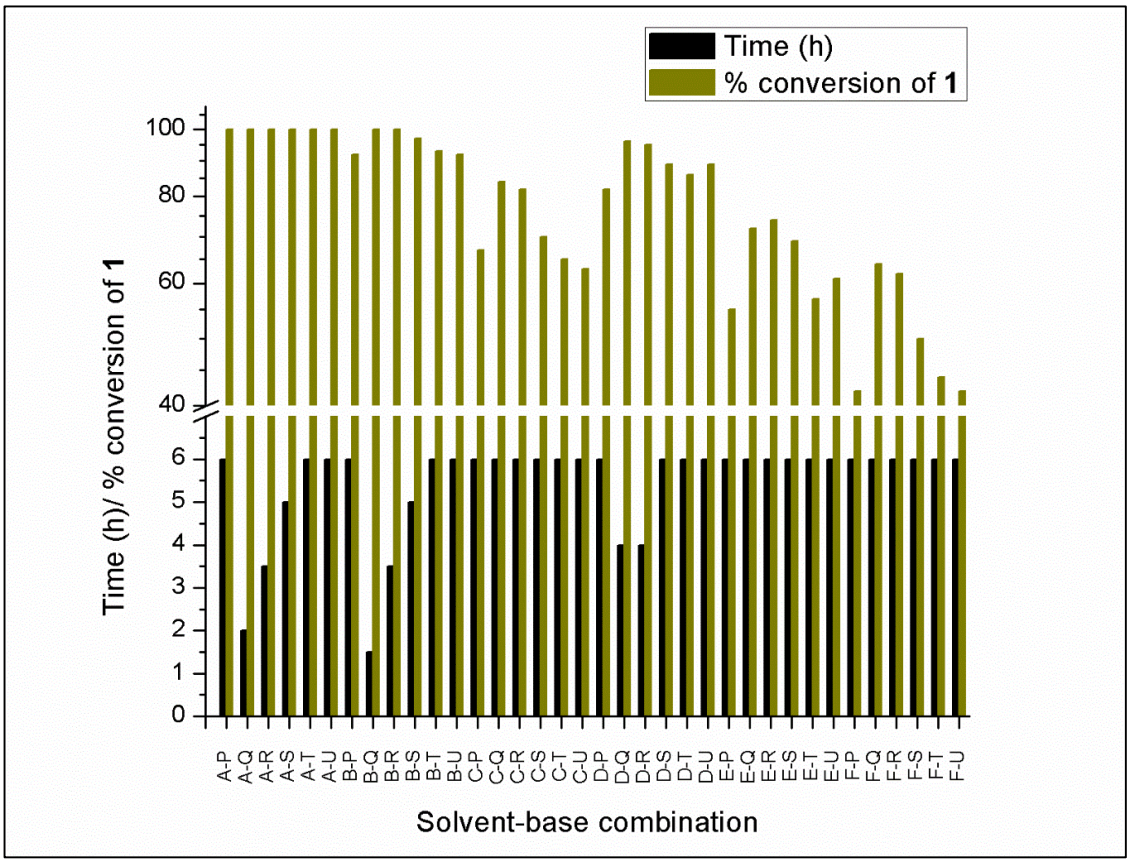
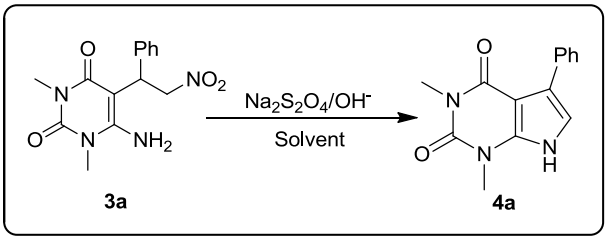


Fig. 3: Bar diagram showing the effect of solvent-base combination on **Scheme 2**. Here, A=H_2O; B=EtOH; C=CH_3CN; D=DMF; E=CH_3COCH_3; F=$CHCl_3$; P=No base; Q=NaOH; R=Na_2CO_3; S=CH_3COONa; T=Et_3N; U=Ph_3P (% conversions were calculated from NMR spectra)



Scheme 3: Reductive cyclisation of Michael adduct (**3a**)

Table 1: Effect of reaction condition on **Scheme 3**

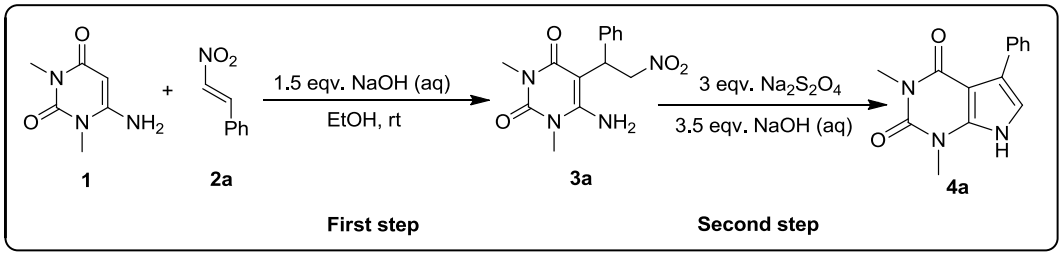
Entry	Solvent	**3a**:$Na_2S_2O_4$:NaOH	Temperature [^oC]	Time [h]	% conversion of **3a** [%][a]	Yield of **4a** [%][b]
1	H_2O	1:1:1	r.t.	6	32	32
			60	6	42	40
			Reflux	6	48	45
		1:2:3	r.t.	6	45	43
			60	6	60	60
			Reflux	6	64	57
		1:3:4	r.t.	6	46	45
			60	6	72	68
			reflux	6	80	72
		1:3:5	r.t.	6	48	46
			60	6	70	69
			reflux	6	82	67
2	EtOH	1:1:1	r.t.	6	30	30
			60	6	45	43
			reflux	6	58	52
		1:2:3	r.t.	6	43	40
			60	4	70	68
			reflux	4	75	70
		1:3:4	r.t.	6	60	58
			60	1	100	92
			reflux	1	100	84
		1:3:5	r.t.	6	62	56
			60	1	100	92
			reflux	1	100	86
3	CH_3CN	1:1:1	r.t.	6	25	25
			60	6	45	50
			reflux	6	55	53
		1:2:3	r.t.	6	50	48
			60	6	62	60
			reflux	6	63	62
		1:3:4	r.t.	6	52	50

			60	6	88	85
			reflux	6	88	82
		1:3:5	r.t.	6	54	52
			60	6	86	84
			reflux	6	90	85
4	$(CH_3)_2CO$	1:1:1	r.t.	6	35	32
			60	6	45	44
			reflux	6	55	53
		1:2:3	r.t.	6	40	36
			60	4	70	68
			reflux	4	75	70
		1:3:4	r.t.	6	65	63
			60	1	100	90
			reflux	0.5	100	86
		1:3:5	r.t.	6	65	63
			60	1	100	91
			reflux	0.5	100	79

[a] Calculated from NMR spectra; [b] isolated yield

DMF, DMSO were not considered due to their high boiling point. **Table 1** clearly indicates the suitability of EtOH and $(CH_3)_2CO$ for the said purpose. However, EtOH becomes the first choice considering the fact that it was the most suitable solvent for the **Scheme 2** also which would offer the opportunity to carry out both **Scheme 2** and **Scheme 3** in a common solvent. The **Table 1** also establishes 1:3:4 as the best stoichiometry for **3a**, $Na_2S_2O_4$ and NaOH as well as 60 ^{0}C as the optimum temperature for the reaction. After optimizing the reaction conditions for both **Scheme 2** and **Scheme 3**, an attempt was made to carry out the synthesis of pyrrolo[2,3-*d*]pyrimidine via a single pot two steps route without isolating the intermediate Michael type product. Optimization for this overall route was also carried out which concluded with a reduction of the amount of NaOH added in the second step from 4 equivalent to 3.5 equivalent The overall process has been expressed as **Scheme 4**.

The final product (**4a**) was confirmed as 1,3-dimethyl-5-phenyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione by single crystal X-ray analysis (**Fig. 3**). The



Scheme 4: One pot two- step synthesis of **4a**

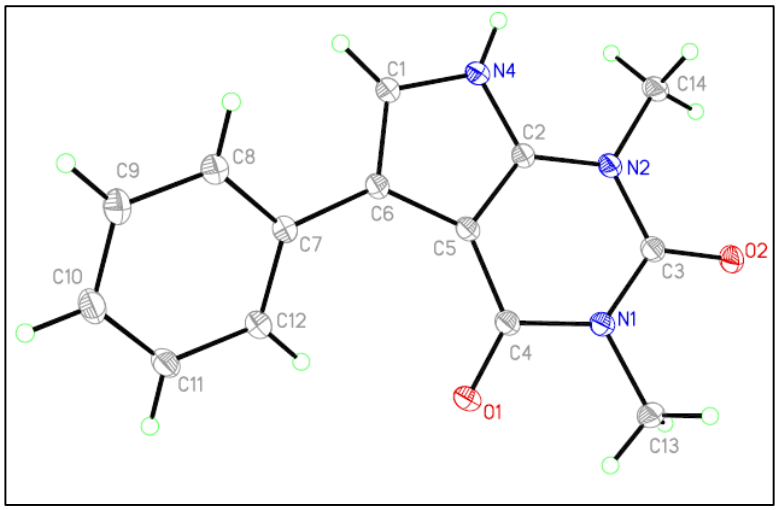
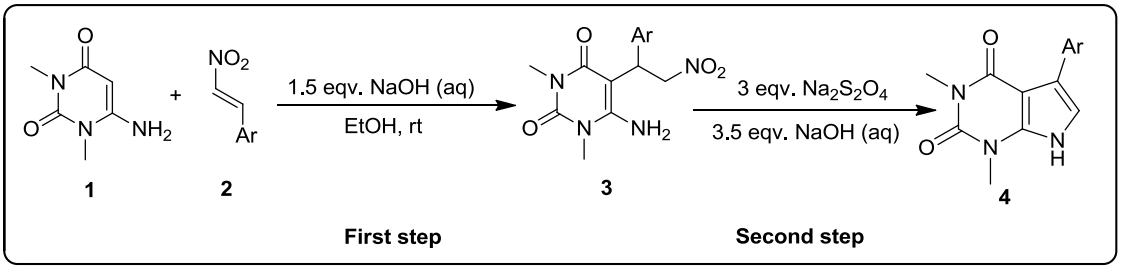


Fig. 4: ORTEP diagram of **4a**

complete transformation can be studied by ^{1}H NMR spectra as shown in **Fig. 5**. To generalize the methodology, it was applied to a series of nitrostyrene derivatives (**Scheme 5**) and the results were summarized in **Table 2**. **Table 2** clearly reflects that the methodology is applicable to a wide variety of nitrostyrene derivatives resulting in excellent to moderate yield. Presence of weakly electron withdrawing & electron donating groups (Entries b, c, d & h, **Table 2**) in the aromatic ring of the



Scheme 5: General reaction scheme for synthesis of **4**

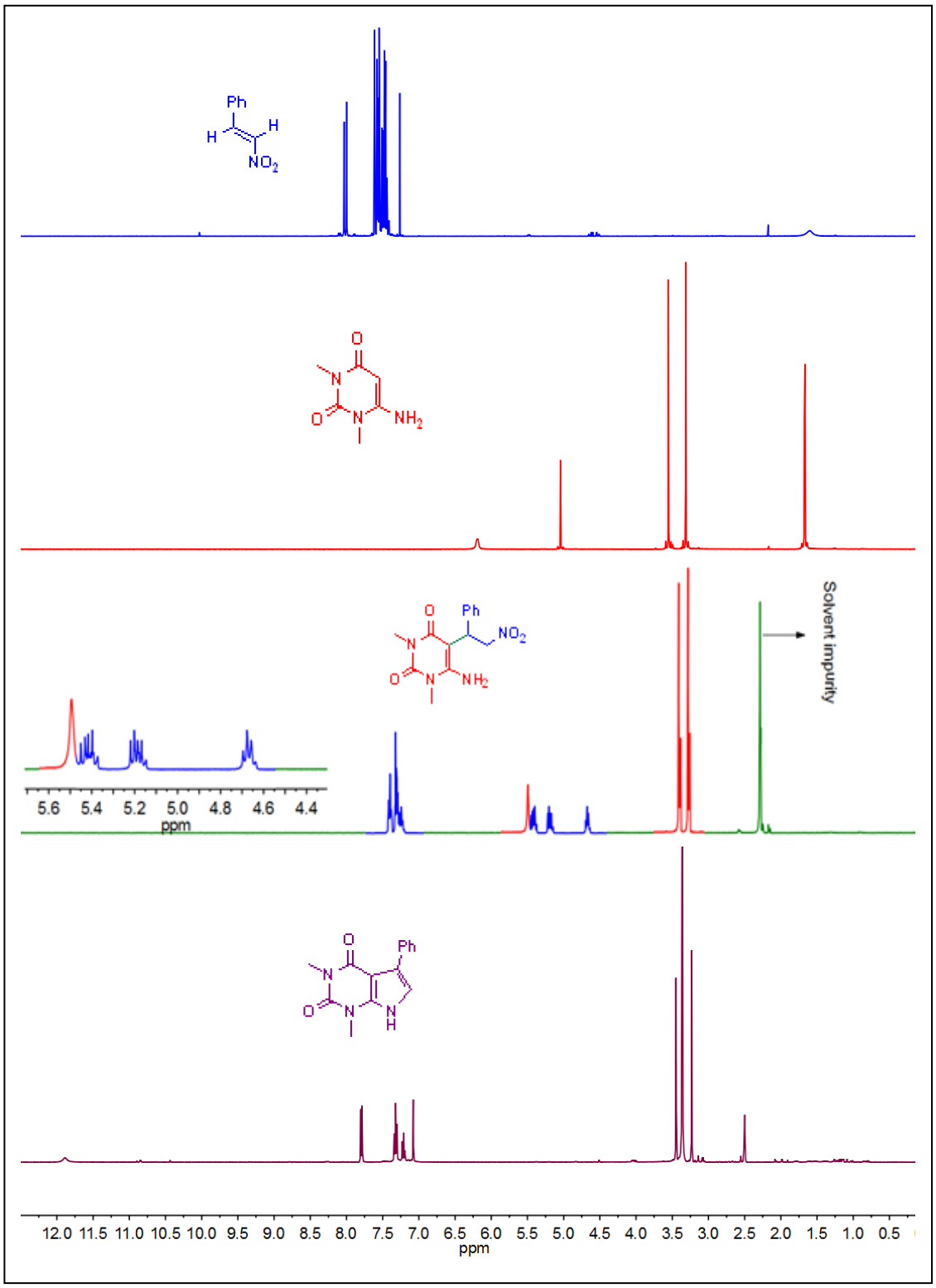
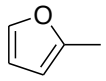
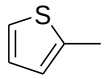
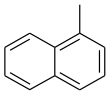
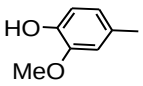
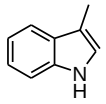
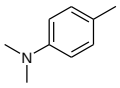


Fig. 5: Comparison of ^{1}H NMR spectra of substrates, intermediate and product

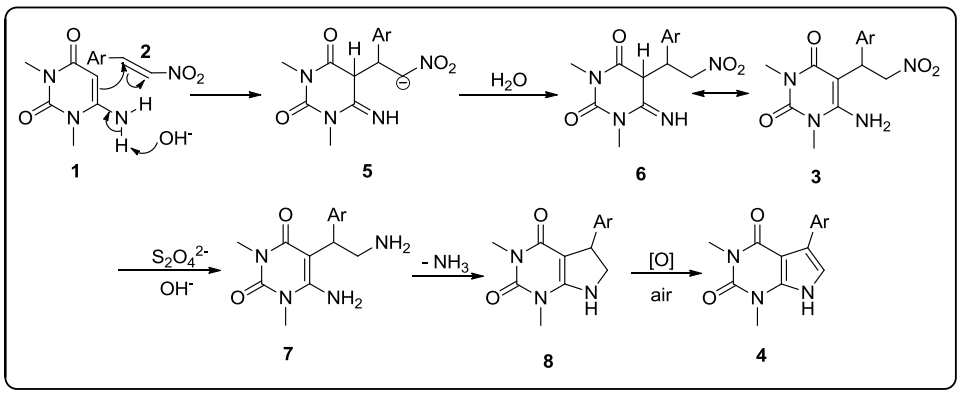
nitrostyrene derivatives don't alter the yield of **4** to a great extent. However, the consumption of **1** as well as the yield of **4** is observed to be comparatively low in

presence of strongly electron donating groups (Entries i & k, **Table 2**). Presence of heteroaryl units in the nitrostyrene derivatives also seemed to lower the yield of

Table 2: Synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives via **Scheme 5**

Entry	Ar-in **2**&**4**	Time required for 1st step [h]	Conversion of **1** [%][a]	Time required for 2nd step [h]	Yield of **4** [%][b]	mp of **4** [°C]
a	C_6H_5-	1	100	1	92	191
b	*p*-ClC_6H_4-	1.5	100	1.2	87	227
c	*o*-HOC_6H_4-	1.5	100	2	82	278
d	*p*-$MeOC_6H_4$-	2	100	1.5	72	246
e		1.5	100	1.5	48	262
f		1.5	100	1.5	53	251
g		1.5	100	1	83	276
h	*p*-MeC_6H_4-	1.5	100	1	82	237
i		4	55	3	--	--
j		4	64	2	42	230
k		4	62	2	46	218

[a] Calculated from NMR spectra; [b]isolated yield

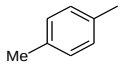
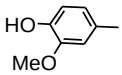
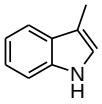
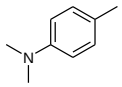


Scheme 6: Plausible mechanism for the formation of **4**

the final product **4** (Entries e, f & j, **Table 2**).

Although detailed mechanistic studies were not performed, a plausible mechanism has been suggested based on our knowledge towards Michael type addition reaction as well as literature reports available for reduction using alkaline $Na_2S_2O_4$ (**Scheme 6**). Once **7** is formed, it eliminates one molecule of NH_3 to generate **8**, which undergoes aerial oxidation to result its aromatic counterpart **4**.

Table 3: Michael type addition of **1** with electron deficient olefins via 1st step of **Scheme 5**

Entry	Ar-in **2** & **3**	Time [h]	Conversion of **1** [%][a]	Yield of **3**[%][a]	mp of **3**
a	C_6H_5-	1	+100	95	202
b	*p*-ClC_6H_4-	1.5	100	92	154
c	*o*-HOC_6H_4-	1.5	100	92	126
d	*p*-$MeOC_6H_4$-	2	100	88	242
e		1.5	100	64	195
f		1.5	100	62	191
g		1.5	100	88	137
h		1.5	100	86	202
i		4	55	38	216
j		4	64	54	160
k		4	62	54	195
l	Acrylic acid	6	--	--	--
m	Acryl amide	6	--	--	--
n	PhCH=CHCOOH	6	--	--	--
o	PhCH=CHCOPh	6	--	--	--

[a] Calculated from NMR spectra; [b] isolated yield

Being benefitted by the fastness of the methodology, we synthesized, isolated and characterized the Michael products (**3**) by stopping the reaction at the first step in a separate set of reactions. An attempt was made to extend the methodology for electron deficient olefins other than nitrostyrenes by taking acrylamide, acrylic acid, cinnamic acid and chalcone as electron deficient system, but went in vain. The results are summarized in **Table 3**.

The synthesized pyrrolo[2,3-*d*]pyrimidine (**4a-h, 4j** & **4k**) were screened for very preliminary antimicrobial activity against six bacteria namely *E. coli*. (*Ec*), *Bacilus subtilis* (*Bs*), *Proteus mirabilis* (*Pm*), *Pseudomonous aeruginosa* (*Pa*), *Yersinia enterocolitica* (*Ye*), and *Bacillus mycoides* (*Bm*) at a concentration of 5mg/ml by taking streptomycin sulfate (1 mg/mL) as reference compound. The bacterial zones of inhibition (mm) values were evaluated using the well diffusion method and the results are summarized in **Table 4**. It is observed from **Table 4** that some of the synthesized compounds (**4e-4h, 4j**&**4k**) possess good antibacterial activity against *P. aeruginosa* and *B. subtilis*. While compounds **4a-d** are found to be totally inactive to all of the microbes tested, not a single compound is found to bear any

Table 4. Bacterial zones of inhibition (in mm) by synthesized pyrrolo[2,3-*d*]pyrimidines

Entry	Compounds	Microorganisms					
		Ec	*Bm*	*Pa*	*Bs*	*Pm*	*Ye*
1	4a	--[a]	--	--	--	--	--
2	4b	--	--	--	--	--	--
3	4c	--	--	--	--	--	--
3	4d	--	--	--	--	--	--
5	4e	12	--	18	20	--	--
6	4f	13	13	20	20	--	--
7	4g	14	17	20	18	--	--
8	4h	15	12	20	20	--	--
9	4j	12	--	20	20	--	--
10	4k	11	12	20	15	--	--

[a] No activity

activity against *P. mirabilis* and *Y. enterocolitica*.

3.3 Conclusion

In this chapter, we have described a one pot two-step methodology for the synthesis of 5-arylpyrrolo[2,3-*d*]pyrimidines which involves Michael type addition followed by reductive cyclisation of the adduct. The methodology is non-catalytic, however still attractive as it is quite general and covers a wide spectrum of nitrostyrenes. The methodology enjoys additional advantage of utilizing cheaper and easily available reagent system in comparison to methods reported earlier [17]. To the best of our knowledge, the described methodology is the first to carry out reductive cyclisation of a molecule possessing an aliphatic nitro group, although numerous reports are available for similar conversions in molecules having aromatic nitro group. From biological assay studies, it is found that six of the synthesized compounds show satisfactory activity against *P. aeruginosa* and *B. subtilis*.

3.4 Experimental section

3.4.1 General information

All the starting materials (**1** & **2**) were prepared by using already avilable standard procedures [18]. All other reagents and chemicals were of reagent grade (AR grade) and were used as purchased without further purification. Melting points were determined with a Büchi 504 apparatus and are uncorrected. IR spectra were recorded as KBr pallets with a Nicolet (Impact 410) FT-IR spectrophotometer with frequencies expressed in wave numbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded in a JNM ECS 400 MHz NMR spectrophotometer (JEOL) using tetramethylsilane (TMS) as the internal standard. Chemical shift values are expressed in ppm. Coupling constants are expressed in hertz. X-ray intensity data were collected with a Bruker SMART APEX CCD area-detector diffractometer with Mo-*K*α radiation (λ = 0.71073 Å). The structures were solved by SHELX97 and refined by full-matrix least-squares on F^2(SHELX97) (Sheldrick, G. M. *Acta Crystallogr., Sect. A* 2008, *64*, 112-122). Reactions were monitored by thin-layer

chromatography (TLC) using aluminium sheets with silica gel $60F_{254}$ (Merck). UV light and Iodine vapour were used as visualizer for TLC. Elemental analyses were carried out with a Perkin-Elmer CHN analyzer (2400 series II). Mass spectrometric analysis were performed using a Waters Q-TOF Premier & Aquity UPLC spectrometer.

3.4.2 Screening of 5-arylpyrrolo[2,3-*d*]pyrimidines towards preliminary antibacterial studies

The well diffusion method was used to calculate the bacterial zones of inhibition (mm) values. The microbes were cultured in nutrient broth for 24 h. This bacterial culture was used as an inoculum for the antimicrobial assay. The pour plating was carried out by transferring bacterial suspension (10^6 CFU/ ml) to sterile petri plate and mixed with molten nutrient agar medium and allowed to solidify. About 50 µL of each of the compounds was placed in the wells and plates were incubated at 37 °C for 24 h and the activity was determined by measuring the diameter of inhibition zones.

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

A mixture of 1,3-dimethylurea (8.81 g, 0.1 mol), cyanoacetic acid (8.5 g, 0.1 mol) and acetic anhydride (12.5 ml) were heated with exclusion of moisture under stirring at 60°C for 3 h. The excess of acetic anhydride and acetic acid formed during the reaction were removed under reduced pressure. A 5% cold sodium hydroxide solution (50 ml) was slowly added to the cooled residue with stirring whereby 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**1**) precipitated out. The precipitate was recrystallized from water to give a pure compound (Yield: 85%, mp 290-292 ^{0}C).

3.4.4 General procedure for the synthesis of 4a-k via Scheme 5

To a stirred mixture of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**1**; 1 mmol) and nitrostyrene (**2**; 1 mmol) in 10 mL of EtOH, 1.5 mmol of NaOH was

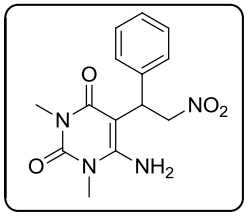
added as 1M NaOH solution (aq.). The reaction was monitored using TLC. After complete consumption of **1** (or after the time period, mentioned in **Table 2**), an alkaline solution of $Na_2S_2O_4$ (3 mmol $Na_2S_2O_4$ and 3.5 mmol NaOH in 5 mL H_2O) was added to the reaction vessel and then the content was allowed to stir at 60 ^{0}C. The reaction was again monitored by TLC. After the completion of the reaction, EtOH was removed under vacuum and the residue was neutralized using 2N HCl. Extraction was done using EtOAc (3×20 mL), the organic part was washed with brine and dried over anhydrous Na_2SO_4. Ethyl acetate was evaporated under vacuum and the residue was purified by column chromatography using silica gel (100-200 mesh) as adsorbent and EtOAc-Hexane as eluent.

3.4.5 General procedure for the synthesis of 3a-o via Scheme 5 (First step)

To a stirred mixture of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**1**; 1 mmol) and nitrostyrene (**2**; 1 mmol) in 10 mL of EtOH, 1.5 mmol of NaOH was added as 1M NaOH solution (aq.). The reaction was monitored using TLC. After complete consumption of **1** (or after the time period, mentioned in **Table 2**), EtOH was removed under vacuum and the residue was neutralized using 2N HCl. Extraction was done using EtOAc (3×20 mL), the organic part was washed with brine and dried over anhydrous Na_2SO_4. Ethyl acetate was evaporated under vacuum and the residue was purified by column chromatography using silica gel (100-200 mesh) as adsorbent and EtOAc-Hexane as eluent.

3.4.6 Spectral data

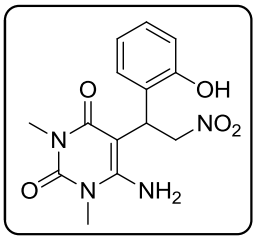
6-amino-1,3-dimethyl-5-(2-nitro-1-phenylethyl)pyrimidine-2,4(1*H*,3*H*)-dione (3a)



FT-IR (KBr): ν_{max}= 3411, 3260, 2365, 1963, 1617, 1483, 1424, 1374cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.39 (t, *J*= 8 Hz, 2H), 7.30 (m, 3H), 5.49 (bs, 2H), 5.41 (m, 1H), 5.18 (m, 1H), 4.6 (m, 1H), 3.41 (s, 3H), 3.28 (s, 3H) ppm;^{13}C NMR (100 MHz, $CDCl_3$):

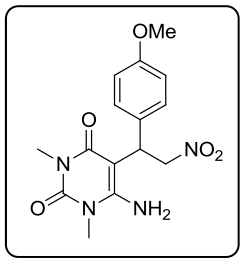
δ= 164.1, 155.3, 153.2, 141.3, 129.8, 129.0, 128.3, 87.1, 78.4, 41.3, 31.2, 28.6 ppm; MS(ESI): m/z= 304 $[M]^+$; Anal. Calcd (%) for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.28; H, 5.33; N, 18.46.

6-amino-5-(1-(2-hydroxyphenyl)-2-nitroethyl)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (3c)



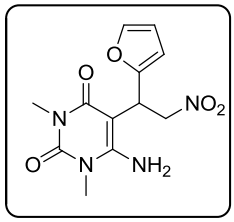
FT-IR (KBr): ν_{max}= 3389, 3238, 2956, 2378, 2276, 1599, 1497, 1451, 1373cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.51(d, *J*= 8 Hz, 1H), 7.06 (t, *J*= 8 Hz, 1H), 6.79 (m, 2H), 5.4 (m, 1H), 5.23 (m, 1H), 4.99 (m, 1H), 4.64 (bs, 1H), 3.39 (s, 3H), 3.23 (s, 3H) ppm;^{13}C NMR (100 MHz, DMSO-d_6): δ= 155.4, 130.4, 129.6, 121.7, 116.5, 101.7, 35.2, 30.8, 28.7 ppm; MS(ESI): m/z= 320 $[M]^+$; Anal. Calcd for $C_{14}H_{16}N_4O_5$: C, 52.50; H, 5.03; N, 17.49. Found: C, 52.54; H, 5.05; N, 17.53.

6-amino-5-(1-(4-methoxyphenyl)-2-nitroethyl)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (3d)



FT-IR (KBr): ν_{max}= 3257, 3223, 2959 2371, 2271, 1605, 1499, 1457, 1377cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.36 (d, *J*= 8 Hz, 2H), 6.94 (bs, 2H), 6.81 (d, *J*= 8 Hz, 2H), 5.39 (m, 1H), 5.27(m, 1H), 4.67 (m, 1H), 3.69 (s, 3H), 3.29 (s, 3H), 3.07 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ=167.0, 161.0, 158.0, 152.4, 150.7, 132.2, 128.8, 113.5, 84.5, 77.7, 55.0, 39.5, 30.1 ,27.4, 22.4 ppm; MS(ESI): m/z= 334 $[M]^+$; Anal. Calcd (%) for $C_{15}H_{18}N_4O_5$: C, 53.89; H, 5.43; N, 16.76. Found: C, 53.86; H, 5.47; N, 16.81.

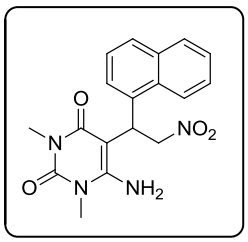
6-amino-5-(1-(furan-2-yl)-2-nitroethyl)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (3e)



FT-IR (KBr): ν_{max}= 3412, 3237, 2952, 1616, 1495, 1384cm^{-1}; ^{1}H NMR (400 MHz, Methanol-d_4): δ= 7.38 (s,1H), 6.32-6.30(m,1H), 6.16-6.15 (m, 1H), 5.23 (m, 2H), 5.02(m, 1H), 3.42 (s, 3H), 3.22 (s, 3H) ppm; ^{13}C NMR (100 MHz, Methanol-d_4): δ= 163.7, 155.2,

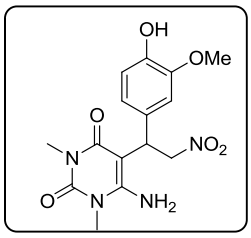
152.8, 142.9, 111.5, 107.3, 84.3, 76.2, 35.8, 30.6, 28.3 ppm; MS(ESI): m/z= 294 $[M]^+$; Anal. Calcd (%) for $C_{12}H_{14}N_4O_5$: C, 48.98; H, 4.80; N, 19.04. Found: C, 49.02; H, 4.85; N, 19.08.

6-amino-1,3-dimethyl-5-(1-(naphthalen-1-yl)-2-nitroethyl)pyrimidine-2,4(1*H*,3*H*)-dione (3g)



FT-IR (KBr): ν_{max}= 3332, 3214, 2926, 1632, 1485cm^{-1}; ^{1}H NMR (400 MHz,Methanol-*d*$_4$): δ=7.99 (d, *J*= 9.2 Hz, 1H), 7.75 (d, *J*= 9.6 Hz, 1H), 7.67(d, *J*= 8 Hz, 1H), 7.47 (d, *J*= 8 Hz, 1H) 7.37(m, 3H), 5.59 (m, 1H), 5.21 (m, 2H), 3.19 (s, 3H), 3.13 (s, 3H) ppm; ^{13}C NMR (100 MHz, Methanol-*d*$_4$): δ= 162.5, 153.4, 151.3, 134.3, 131.5, 128.7, 127.9, 126.1, 125.6, 124.7, 124.1, 123.1, 85.0, 75.0, 36.3, 28.9, 27.1 ppm; MS(ESI): m/z= 376 $[M]^+$; Anal. Calcd (%) for $C_{20}H_{16}N_4O_4$: C, 63.82; H, 4.28; N, 14.89. Found: C, 63.81; H, 4.31; N, 14.93.

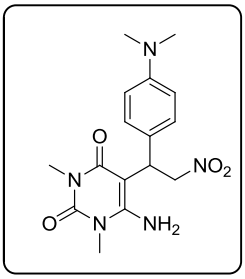
6-amino-5-(1-(4-hydroxy-3-methoxyphenyl)-2-nitroethyl)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (3i)



FT-IR (KBr): ν_{max}= 3462, 3359, 3266, 2935, 1707, 1592, 1489, 1353 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ= 9.3 (bs, 1H), 7.06 (s, 1H), 6.84 (d, *J*= 1.6 Hz, 1H), 6.82 (d, *J*= 2 Hz, 1H), 6.64 (s, 1H), 6.62 (s, 1H), 5.49 (bs, 2H) 5.42 (m, 1H), 5.18 (m, 1H), 4.58 (m, 1H), 3.69 (s, 3H), 3.28 (s, 3H), 3.08 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ= 161.3, 152.5, 150.8, 147.2, 145.5, 131.2, 120.3, 115.2, 112.6, 84.7, 78.0, 60.0, 55.8, 30.3, 27.6, 21.0 ppm; MS (ESI): m/z= 350 $[M^+]$; Anal. Calcd (%) for $C_{15}H_{18}N_4O_6$: C, 51.43; H, 5.18; N, 15.99. Found: C, 51.47; H, 5.27, N, 15.15.

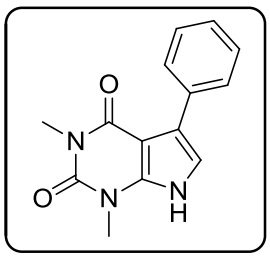
6-amino-5-(1-(4-(dimethylamino)phenyl)-2-nitroethyl)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (3k)

FT-IR (KBr): ν_{max}= 3435, 3346, 3219, 2796, 1695, 1599, 1494, 1346 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ= 7.26 (d, *J*= 8 Hz, 2H), 6.86 (bs, 2H), 6.61 (d, *J*= 8 Hz, 2H), 5.47 (m, 1H), 5.24 (m, 1H), 4.68 (m, 1H), 3.29 (s, 3H), 3.0(s, 3H), 2.82 (s, 6H) ppm;



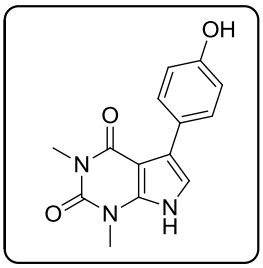
^{13}C NMR (100 MHz, DMSO-d_6): δ= 164.1, 155.3, 155.2, 141.3, 129.9, 129.0, 128.0, 87.1, 78.4, 40.9, 35.0, 30.1 ppm; MS (ESI): m/z= 347 [M$^+$]; Anal. Calcd (%) for $C_{16}H_{21}N_5O_4$: C, 55.32; H, 6.09; N, 20.16. Found: C, 55.37; H, 6.04; N, 20.17.

1,3-dimethyl-5-phenyl-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (4a)



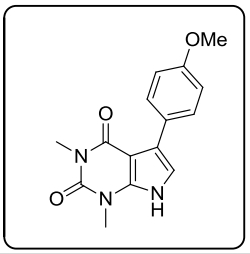
FT-IR (KBr): ν_{max}= 3423, 3178, 1703, 1659, 1355 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 11.87 (bs, 1H), 7.79 (d, *J*= 8 Hz, 2H), 7.32 (t, *J*= 8 Hz, 2H), 7.21 (t, *J*= 8 Hz, 1H), 7.07 (s, 1H), 3.44 (s, 3H), 3.23 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ= 158.9, 151.0, 140.7, 134.2, 128.3, 126.5, 122.2, 115.7, 96.1, 30.8, 28.3 ppm; MS (ESI): m/z= 255 [M$^+$]; Anal. Calcd (%) for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.84; H, 5.14; N, 16.47.

5-(4-hydroxyphenyl)-1,3-dimethyl-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (4c)



FT-IR (KBr): ν_{max}= 3478, 3253, 1704, 1678, 1344 cm^{-1}; ^{1}H NMR(400 MHz, DMSO-d_6): δ= 11.87 (bs, 1H), 9.32 (s, 1H), 7.49 (s, 1H), 7.07 (t, *J*= 8 Hz, 1H), 6.86 (d, *J*= 8 Hz, 1H), 6.78 (t, *J*= 8 Hz, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ= 159.2, 154.7, 150.6, 139.6, 131.8, 127.5, 121.5, 121.5, 118.9, 116.8, 116.3, 96.9, 30.5, 27.9 ppm; MS (ESI): m/z= 271 [M$^+$]; Anal. Calcd (%) for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.49. Found: C, 61.94; H, 4.84; N, 15.47.

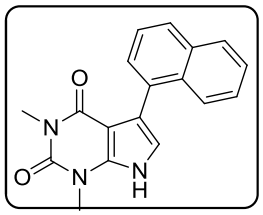
5-(4-methoxyphenyl)-1,3-dimethyl-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (4d)



FT-IR (KBr): ν_{max}= 3487, 3150, 1694, 1610, 1544, 1441 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 11.77 (bs, 1H), 7.72 (d, *J*= 8 Hz, 2H), 6.96 (s, 1H), 6.88 (d, *J*= 8 Hz, 2H), 3.75 (s, 3H), 3.41 (s, 3H), 3.22 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ= 158.7,

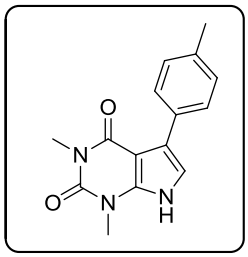
158.0, 150.6, 140.2, 129.4, 126.4, 121.6, 114.3, 113.4, 95.8, 55.2, 30.4, 27.9 ppm; MS (ESI): m/z= 285 [M⁺]; Anal. Calcd (%) for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.10; H, 5.33; N, 14.76.

1,3-dimethyl-5-(naphthalen-1-yl)-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (4g)



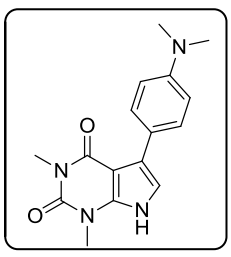
FT-IR (KBr): ν_{max}= 3443, 3160, 3041, 1695, 1624, 1534 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6):δ= 12.01 (bs, 1H), 7.90 (m, 3H), 7.46 (m, 4H), 6.9 (s, 1H), 3.51 (s, 3H), 3.13 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ=158.0, 150.7, 139.4, 133.1, 132.3, 132.1, 127.8, 127.1, 126.3, 125.7 ppm; MS (ESI): m/z= 305 [M⁺]; Anal. Calcd (%) for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76. Found: C, 70.80; H, 4.90; N, 13.77.

1,3-dimethyl-5-(p-tolyl)-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (4h)



FT-IR (KBr): ν_{max}= 3417, 3234, 3190, 1688, 1624, 1531 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ= 11.86 (bs, 1H), 7.12 (d, *J*= 8 Hz, 2H), 7.04 (s, 1H), 7.68 (d, *J*= 8 Hz, 2H), 3.43 (s, 3H), 3.22 (s, 3H), 2.29 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ= 158.5, 150.5, 140.1, 135.1, 130.9, 128.4, 127.9, 121.6, 114.8, 95.6, 30.3, 27.8, 20.8; MS (ESI): m/z= 269 [M⁺]; Anal. Calcd (%) for $C_{18}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60. Found: C, 66.92; H, 5.60; N, 5.68.

5-(4-(dimethylamino)phenyl)-1,3-dimethyl-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (4k)



FT-IR (KBr): ν_{max}= 3420, 3167, 2922, 2379, 1695, 1636, 1539 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ= 11.99 (bs, 1H); 7.81 (d, *J*=8, 2H), 7.24 (d, *J*= 8, 2H), 7.16 (s, 2H), 3.56 (s, 3H), 3.34 (s, 3H), 3.17 (s, 6H), 2.42 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ= 158.7, 150.7, 140.3, 135.3, 131.1, 128.6, 128.1, 121.8, 115.0, 95.8, 35.0, 30.5, 28.0, 21.0 ppm; MS (ESI): m/z= 298 [M⁺]; Anal. Calcd (%) for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.46; H, 6.10; N, 18.74.

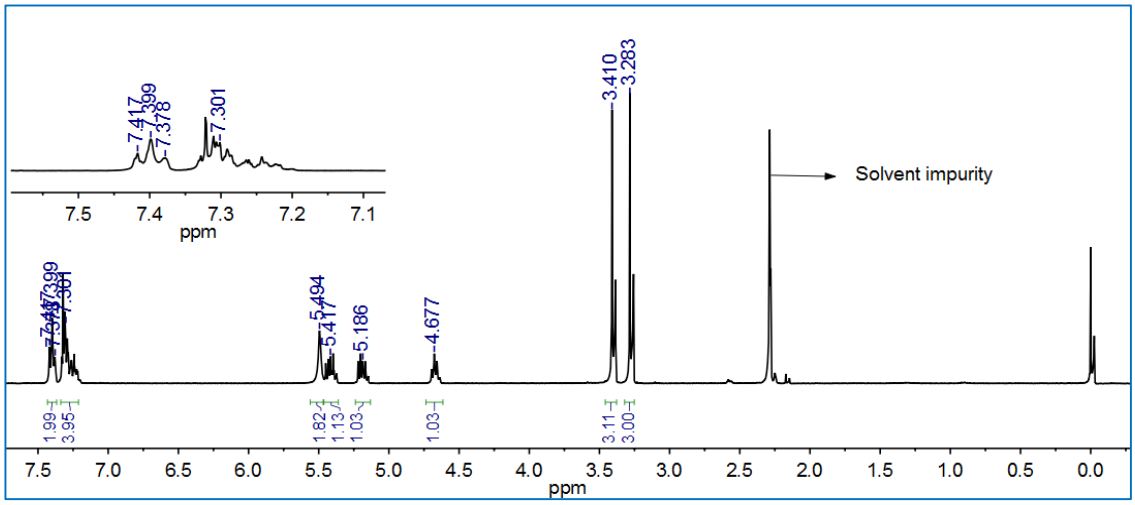


Fig. 6: ^{1}H NMR spectrum of **3a**

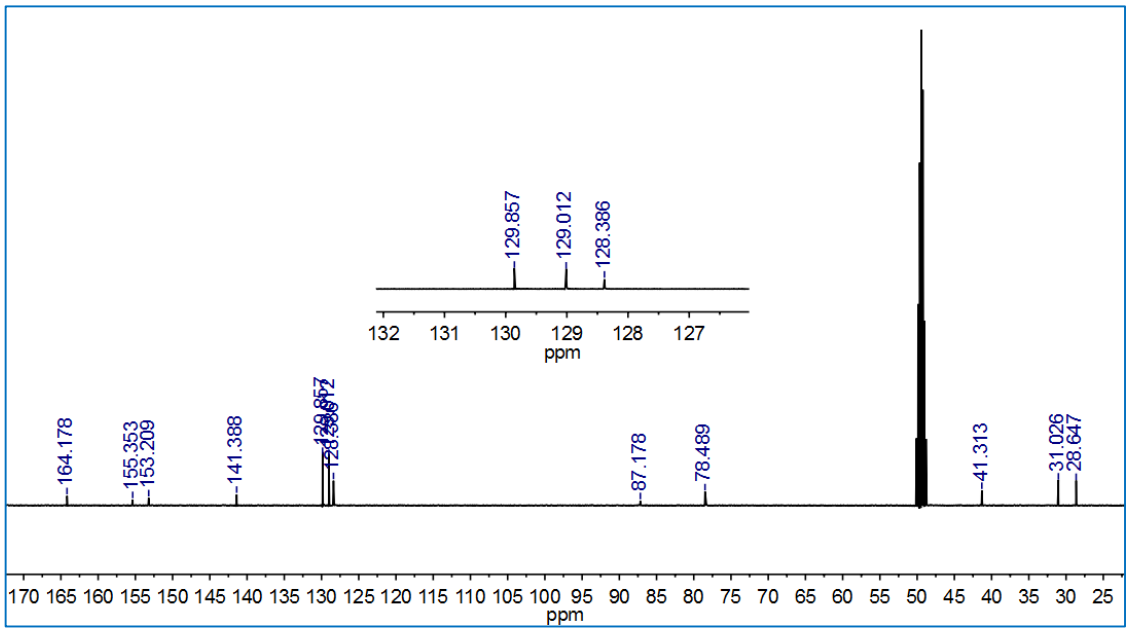


Fig. 7: ^{13}C NMR spectrum of **3a**

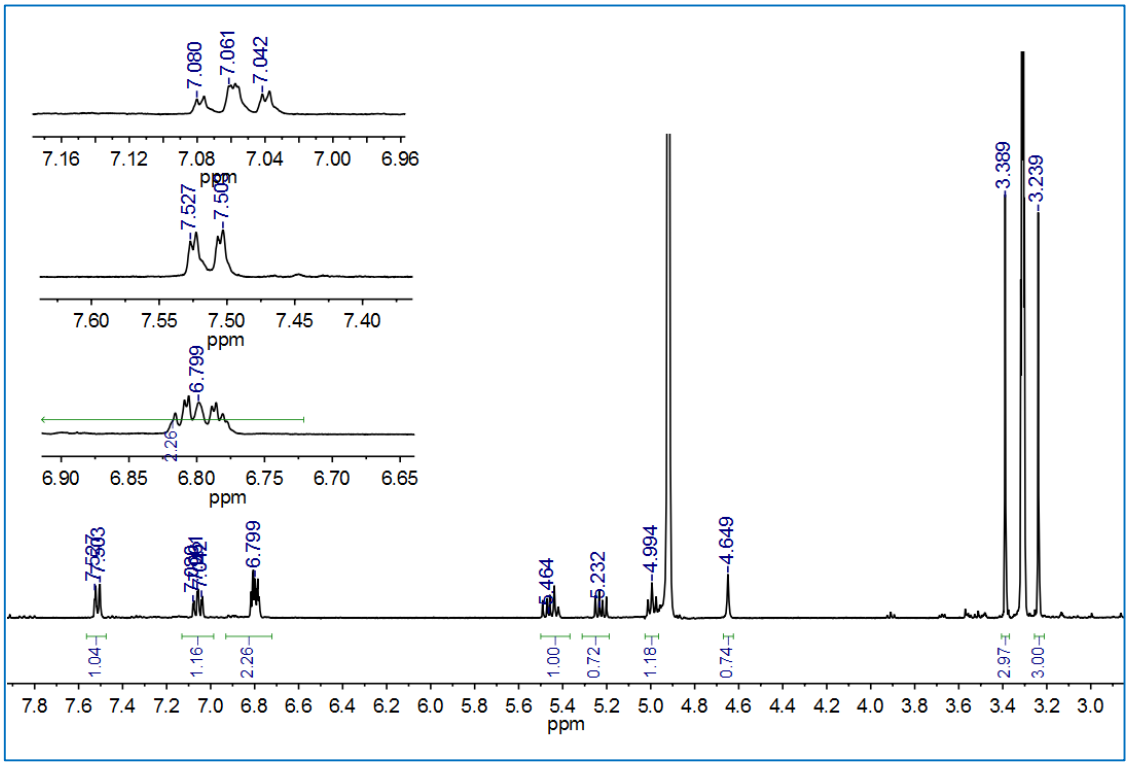


Fig. 8: ^{1}H NMR spectrum of **3c**

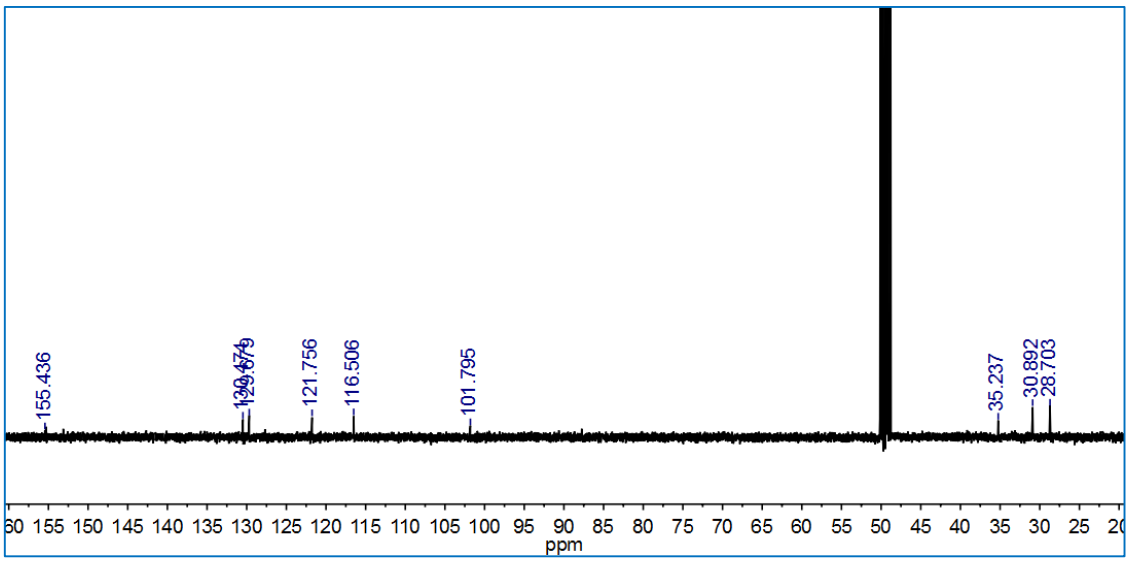


Fig. 9: ^{13}C NMR spectrum of **3c**

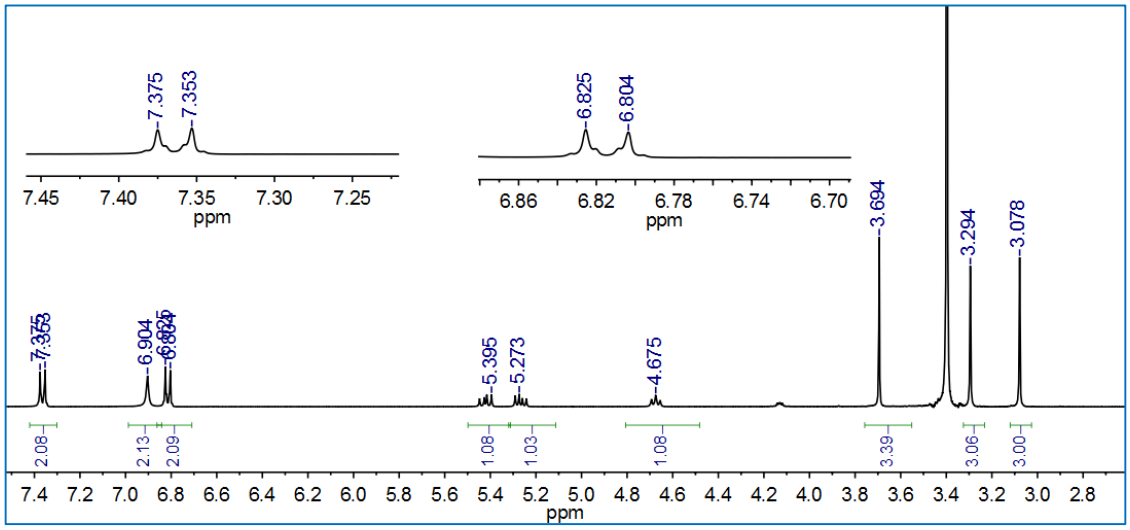


Fig. 10: ^{1}H NMR spectrum of **3d**

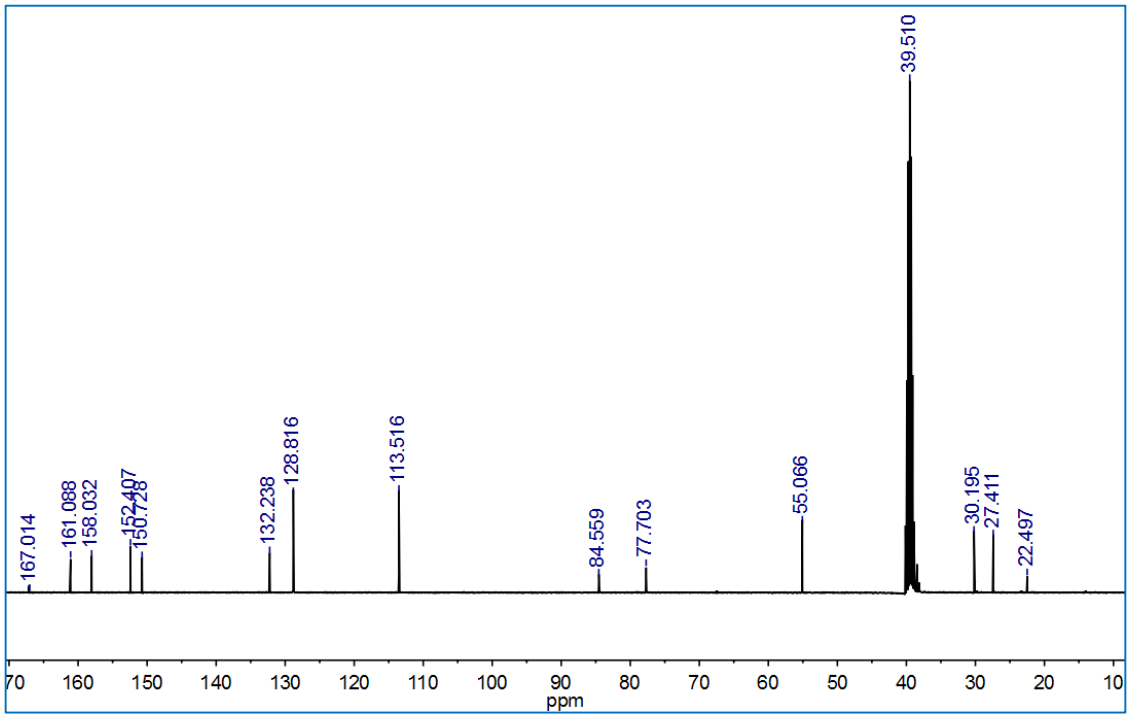


Fig. 11: ^{13}C NMR spectrum of **3d**

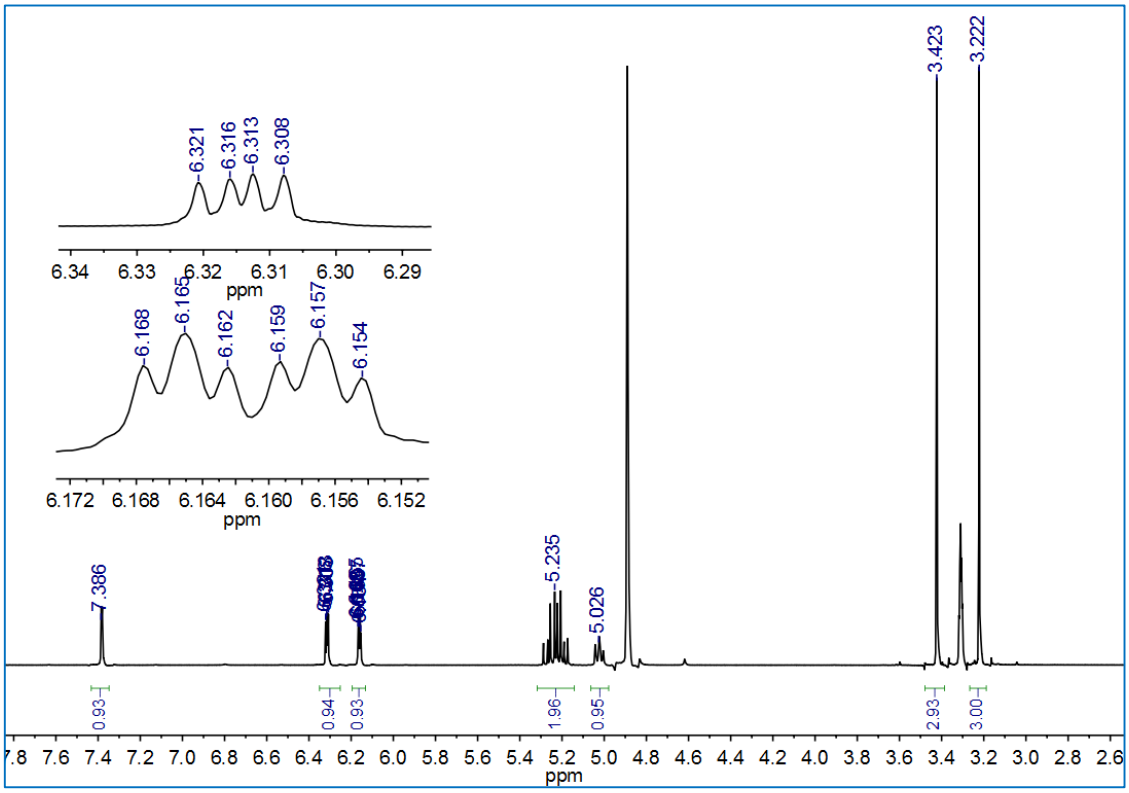


Fig. 12: ^{1}H NMR spectrum of **3e**

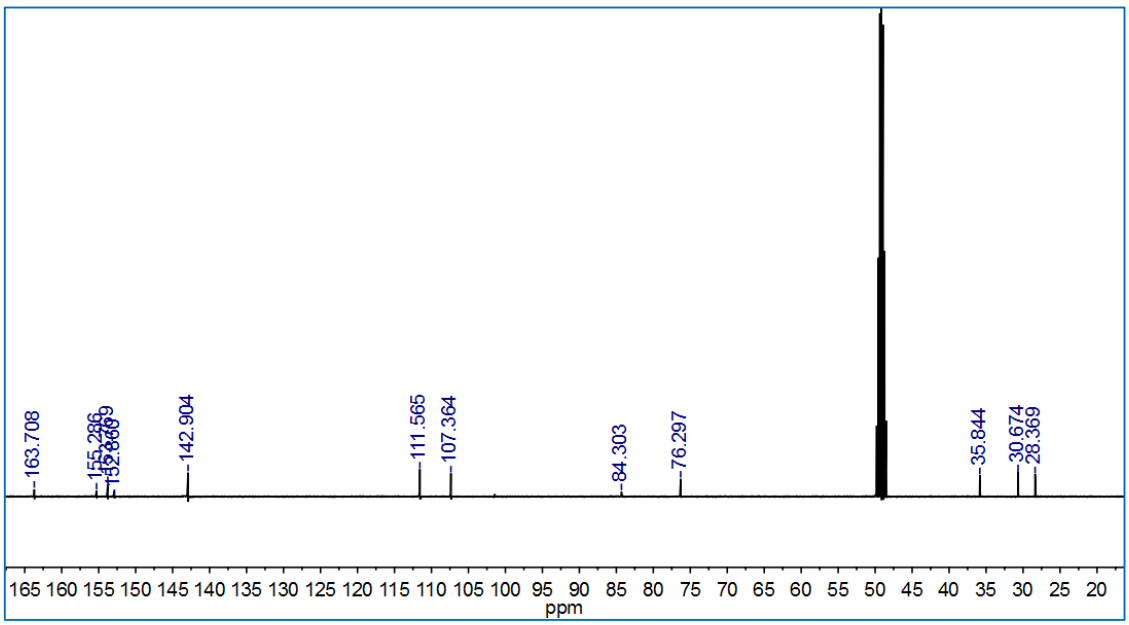


Fig. 13: ^{13}C NMR spectrum of **3e**

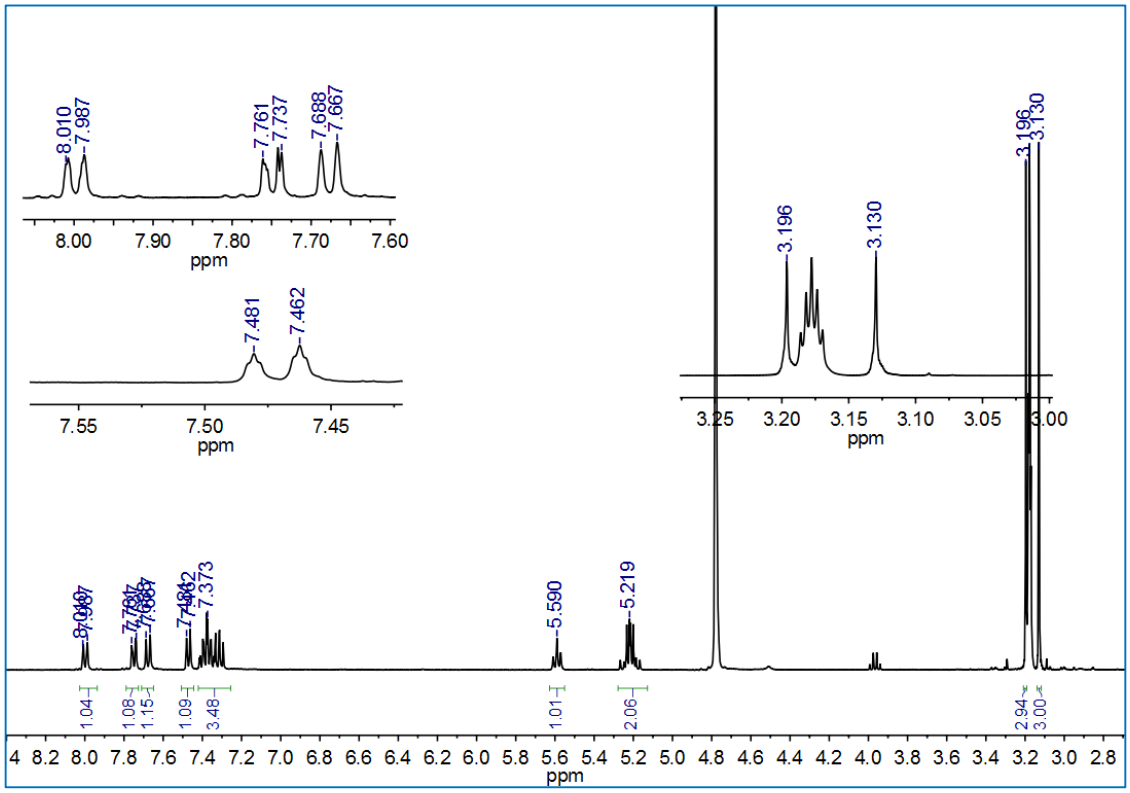


Fig. 14: ^{1}H NMR spectrum of **3g**

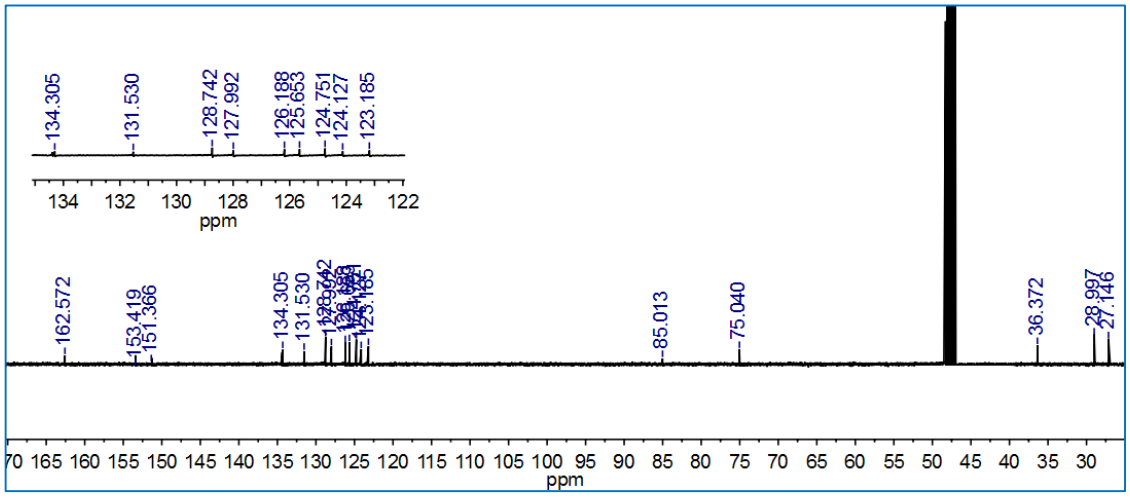


Fig. 15: ^{13}C NMR spectrum of **3g**

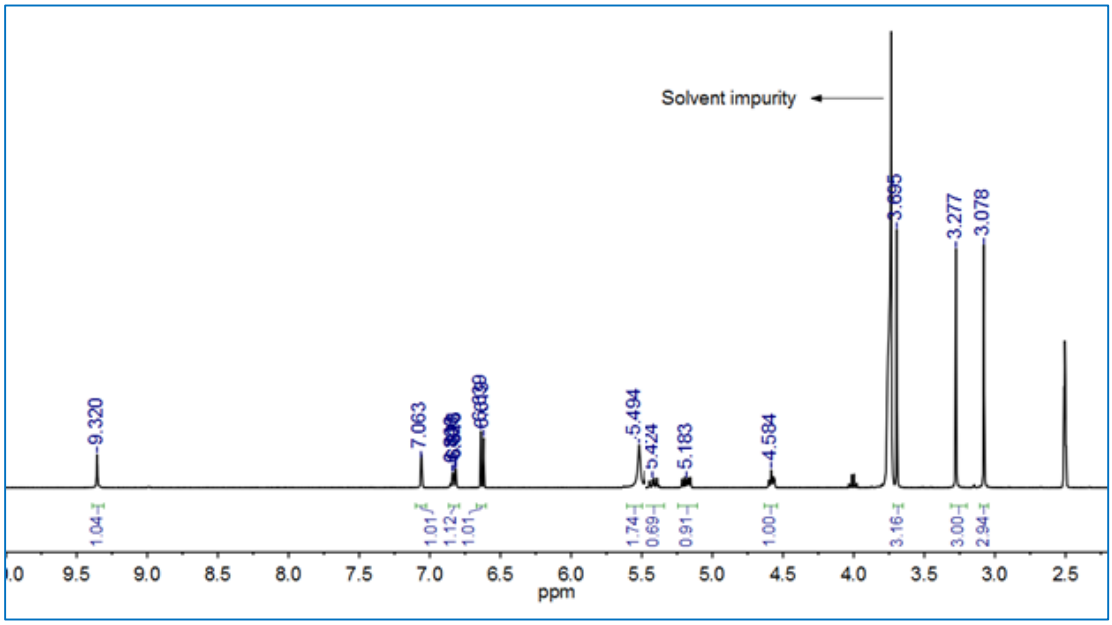


Fig. 16: ^{1}H NMR spectrum of **3i**

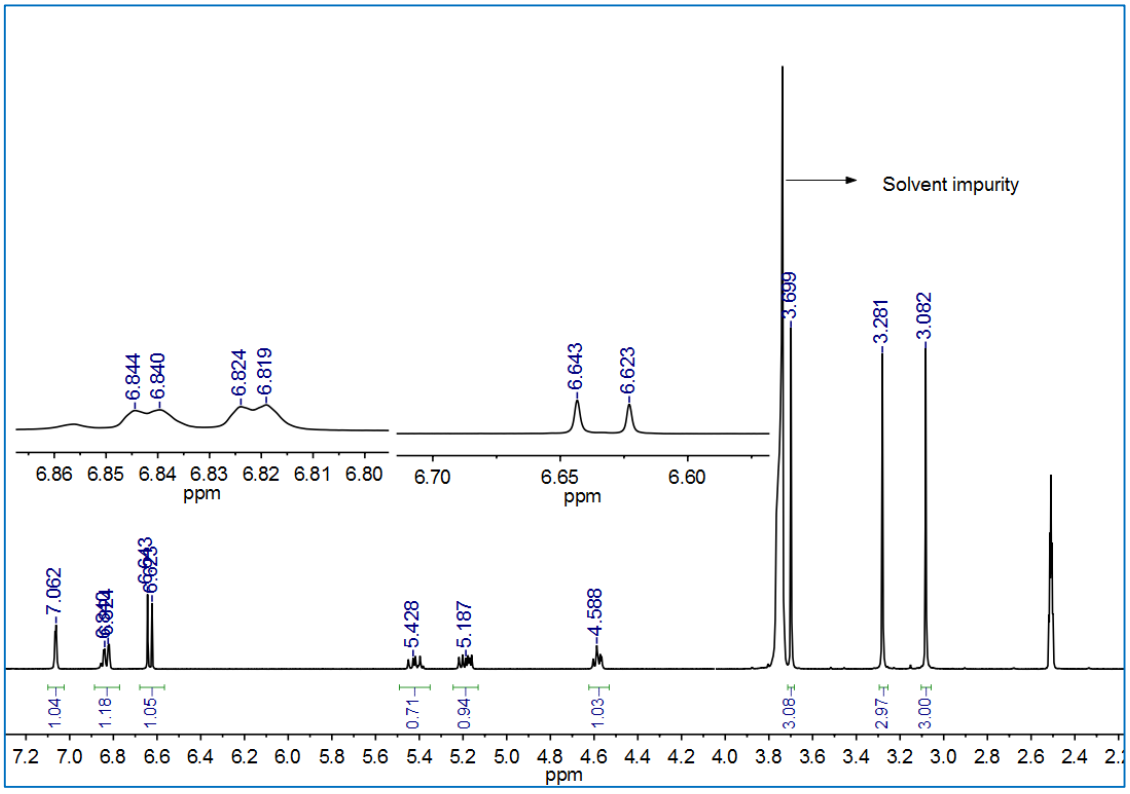


Fig. 17: D_2O exchange of ^{1}H NMR spectrum of **3i**

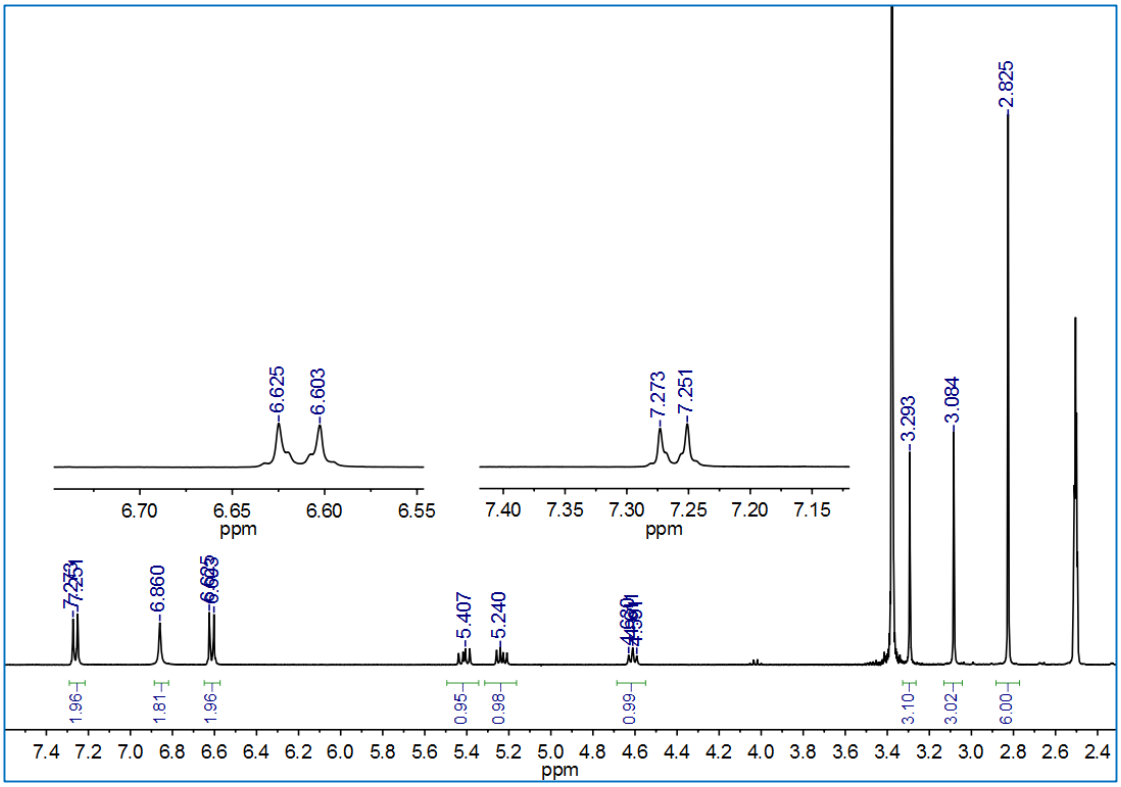


Fig. 18: ^{1}H NMR spectrum of **3k**

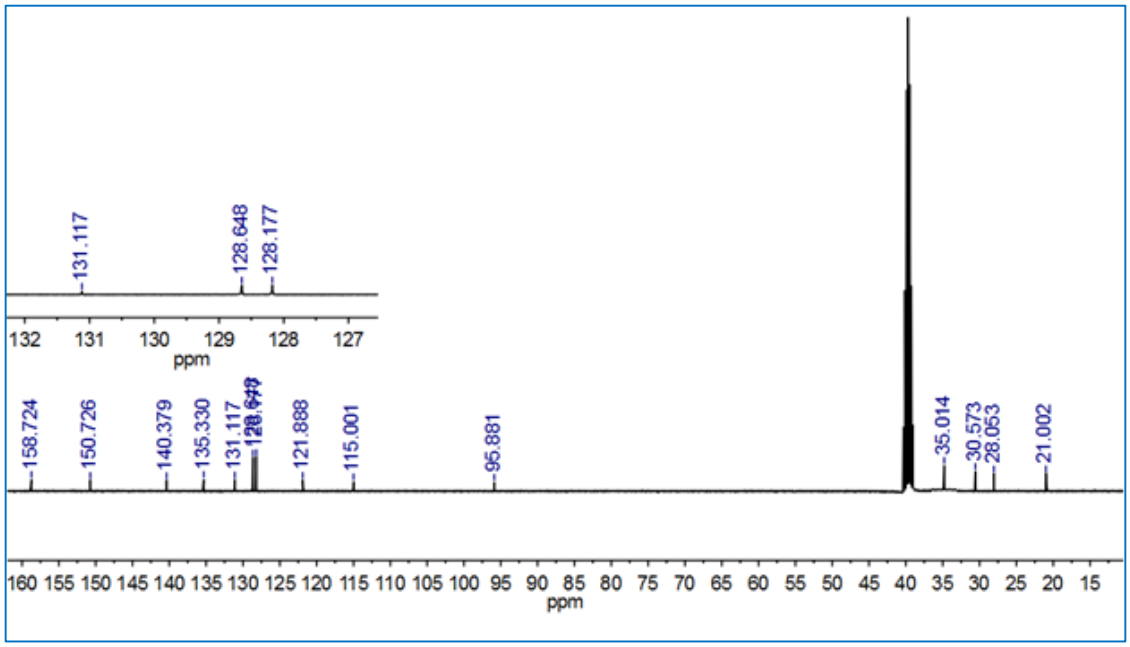


Fig. 19: ^{13}C NMR spectrum of **3k**

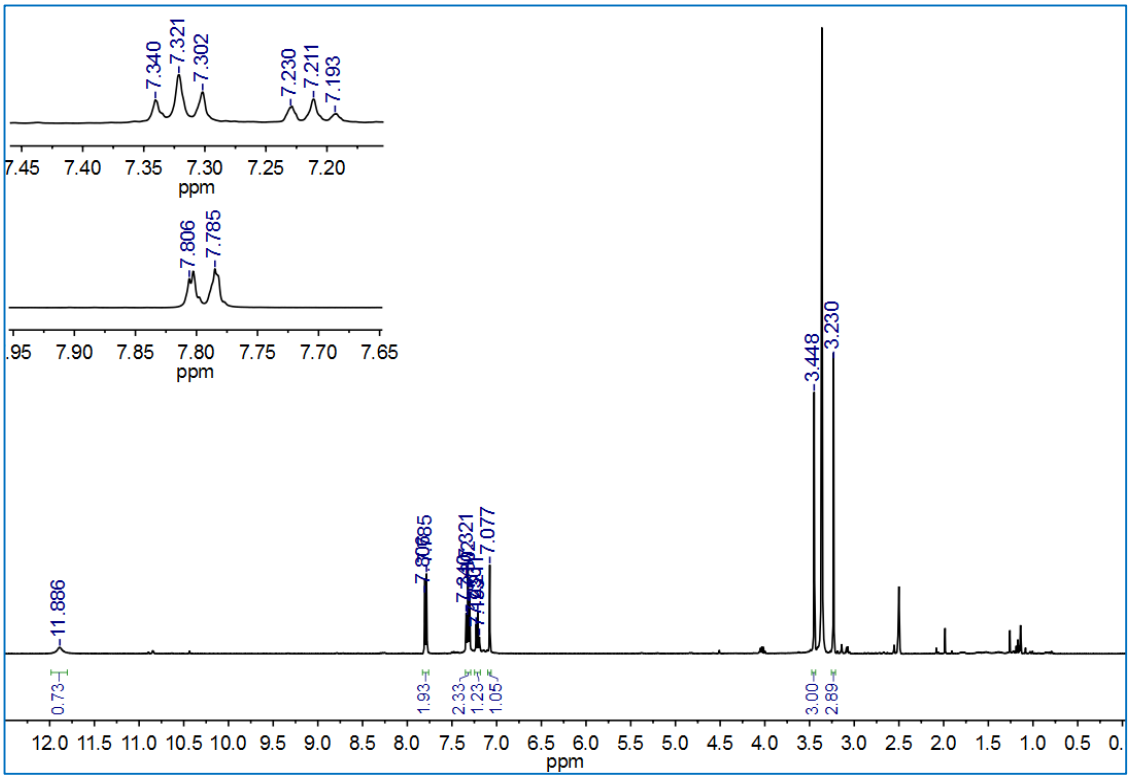


Fig. 20: ^{1}H NMR spectrum of **4a**

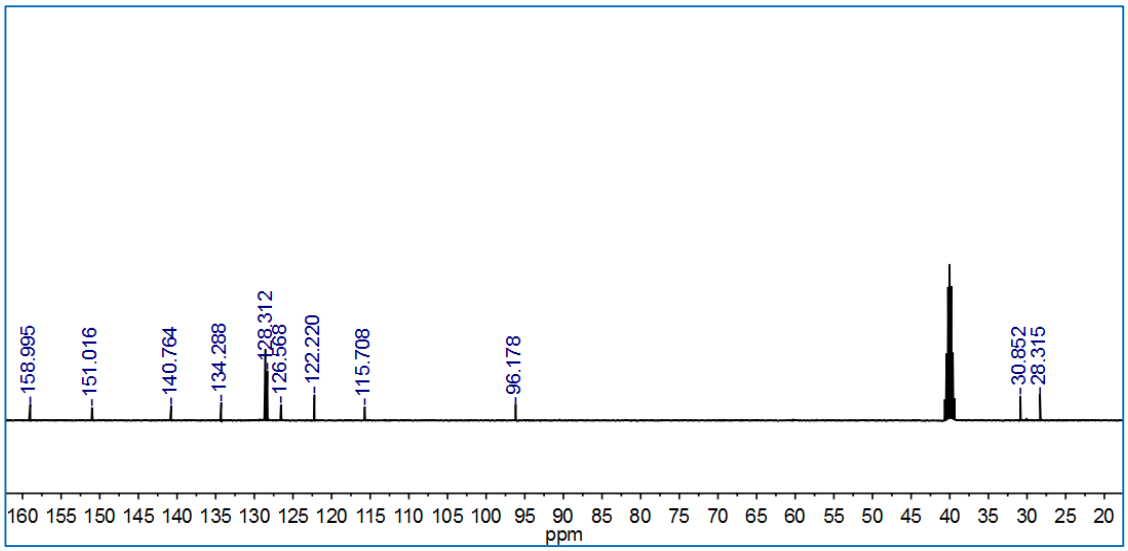


Fig. 21: ^{13}C NMR spectrum of **4a**

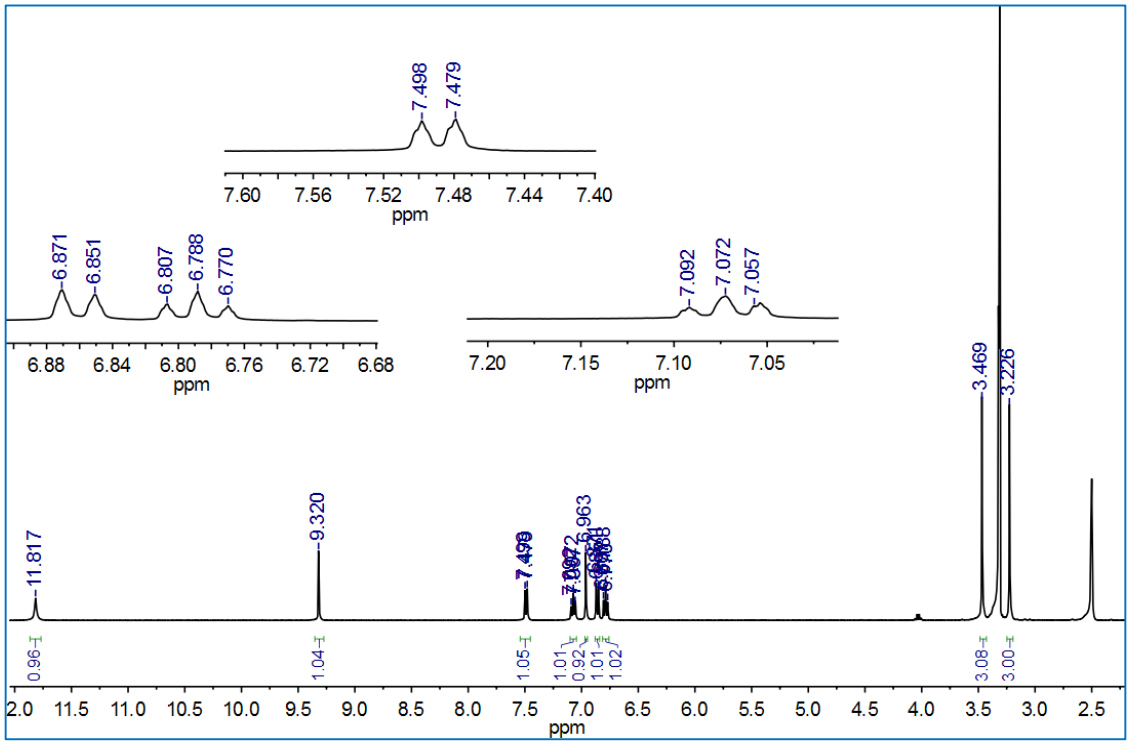


Fig. 22: ^{1}H NMR spectrum of **4c**

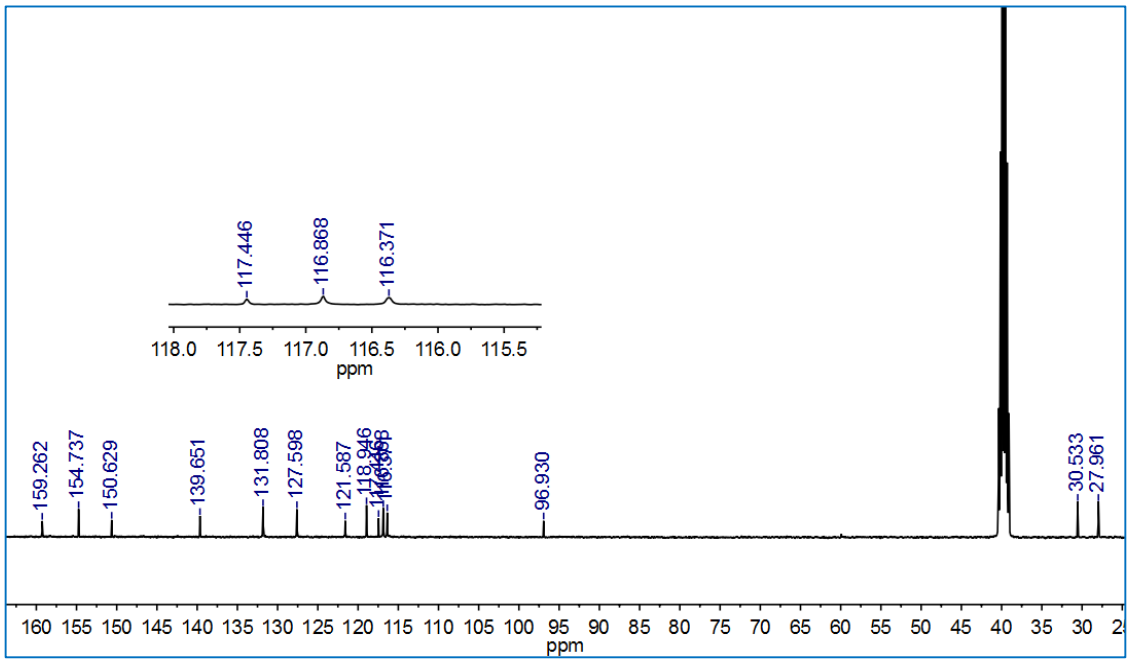


Fig. 23: ^{13}C NMR spectrum of **4c**

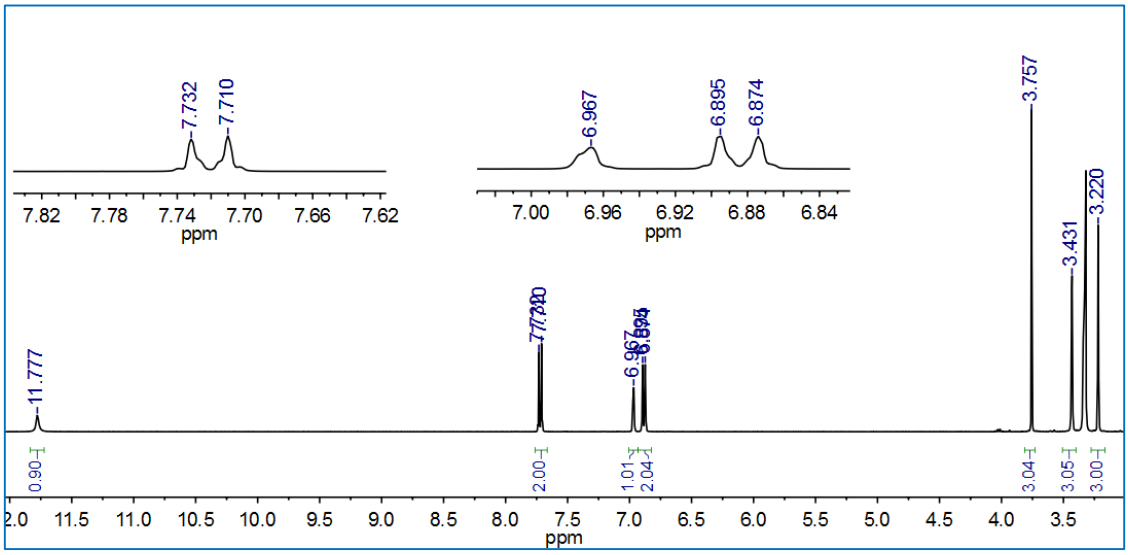


Fig. 24: ^{1}H NMR spectrum of **4d**

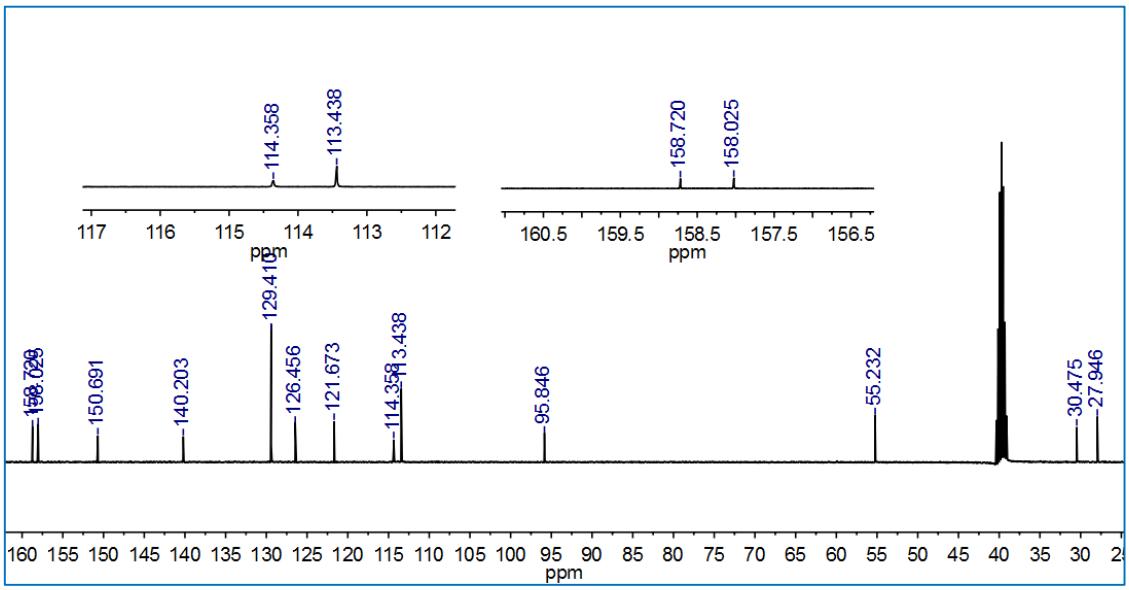


Fig. 25: ^{13}C NMR spectrum of **4d**

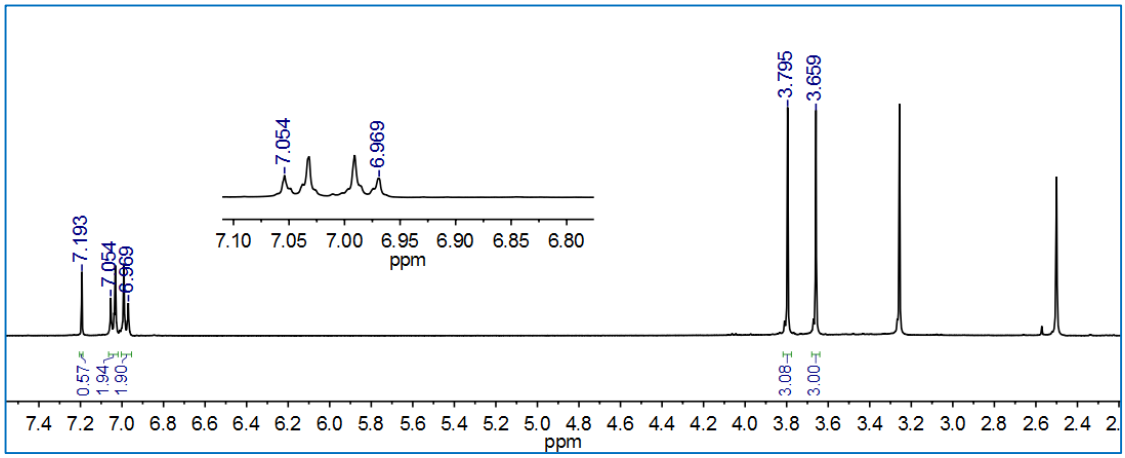


Fig. 26: ^{1}H NMR spectrum of **4e**

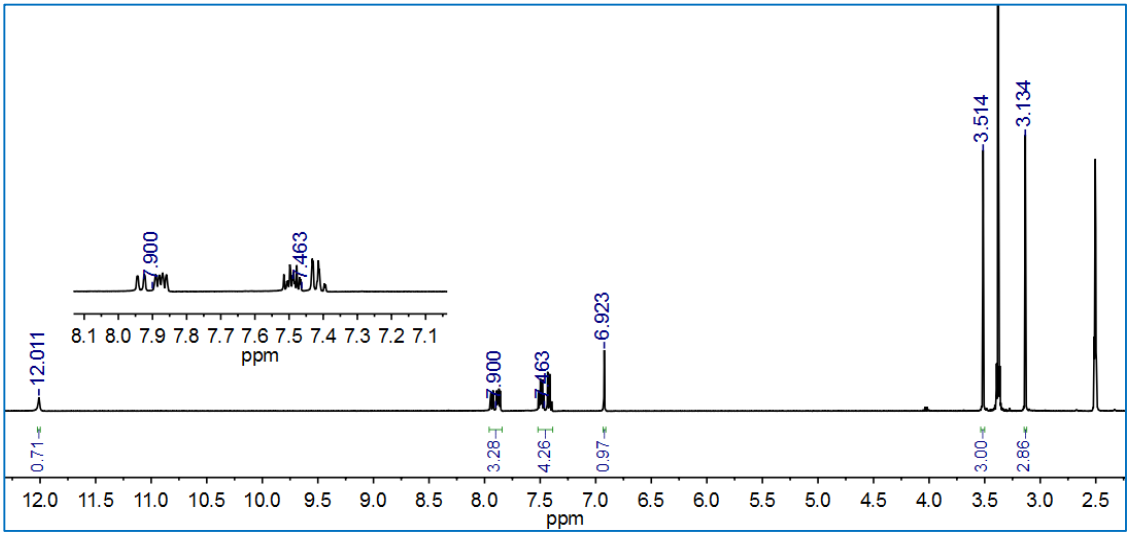


Fig. 27: ^{1}H NMR spectrum of **4g**

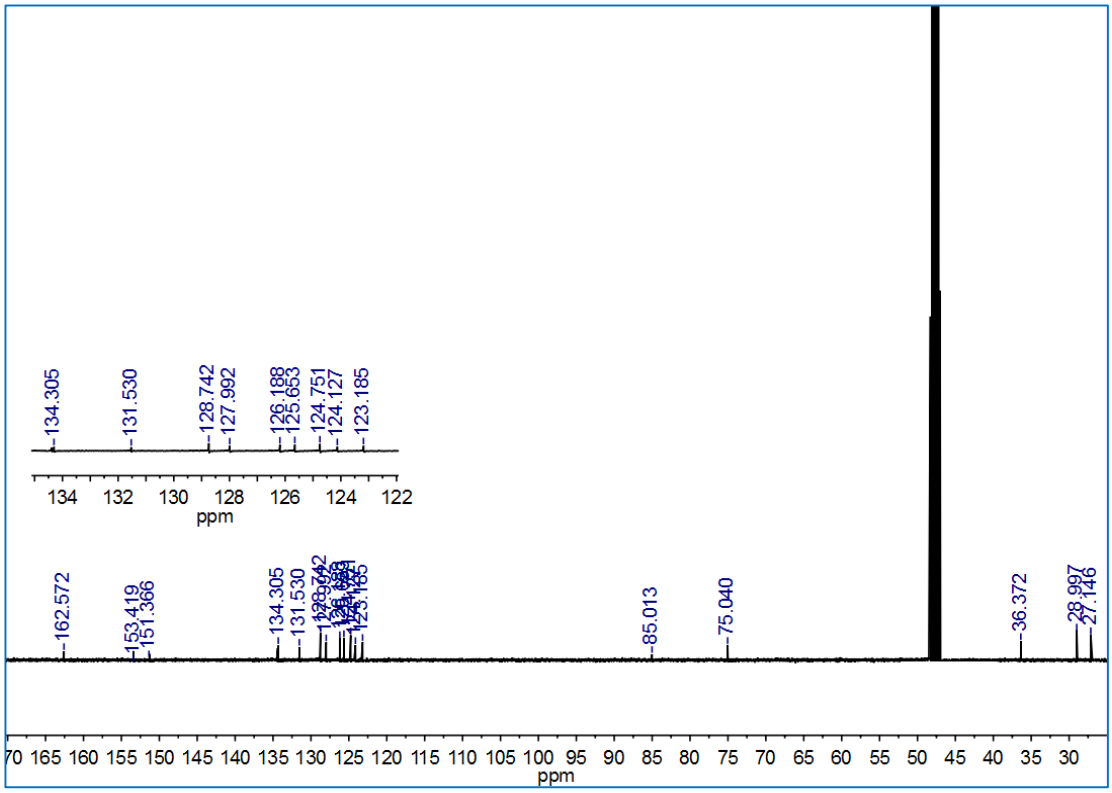


Fig. 28: ^{13}C NMR spectrum of **4g**

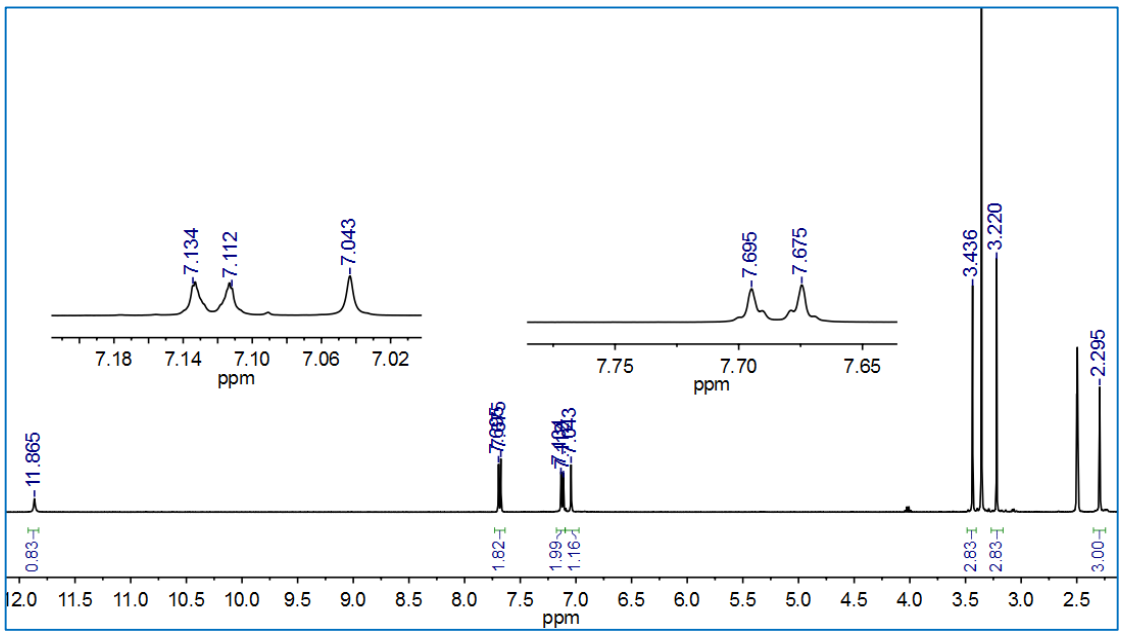


Fig. 29: ^{1}H NMR spectrum of **4h**

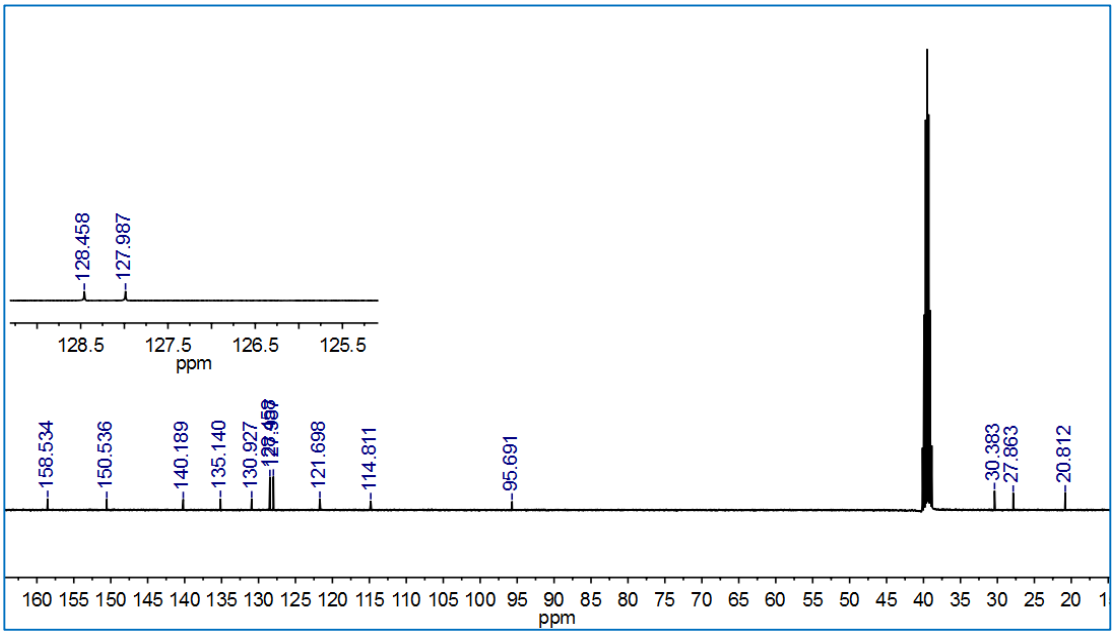


Fig. 30: ^{13}C NMR spectrum of **4h**

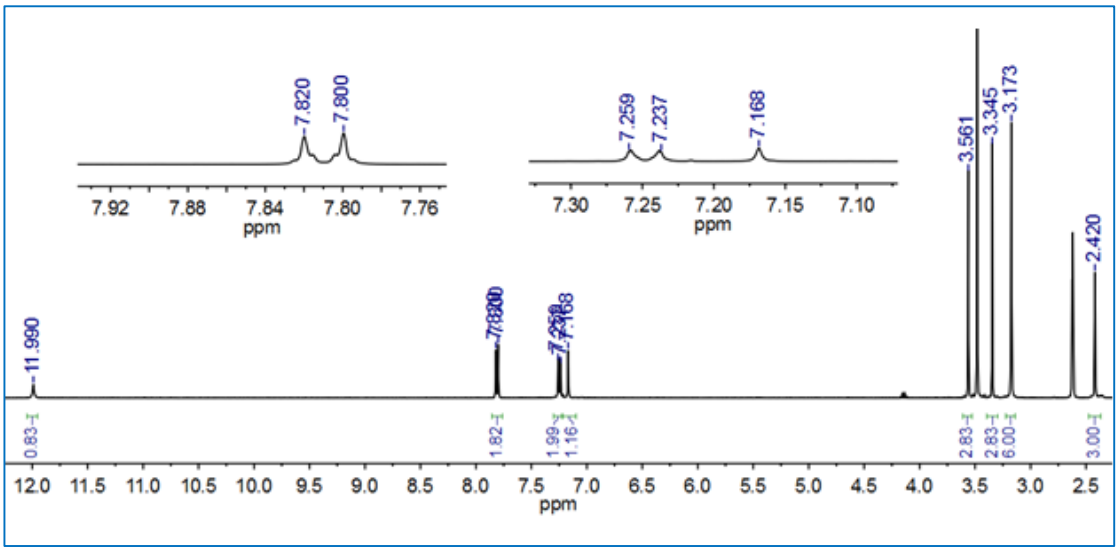


Fig. 31: ^{1}H NMR spectrum of **4k**

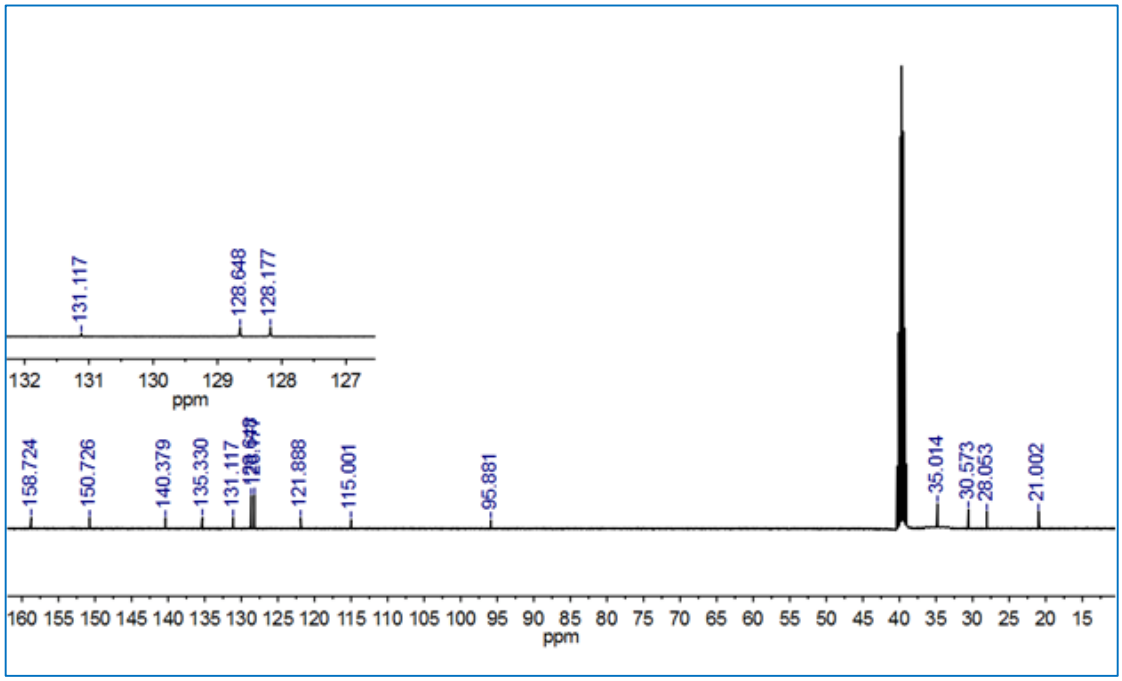


Fig. 32: ^{13}C NMR spectrum of **4k**

3.5 References

1. (a) Gangjee, A. et al. *J. Med. Chem.* **47**, 6893--6901, 2004. (b) Gangjee, A. et al. *J. Med. Chem.* **48**, 5329--5336, 2005. (c) Gangjee, A. et al. *J. Med. Chem.* **49**, 1055--1065, 2006.
2. (a) Desmoulin, S.K. et al. *Mol. Pharmacol.* **80(6)**, 1096--1107, 2011. (b) Wang, L. et al. *J. Med. Chem.* **54**, 7150--7164, 2011. (c) Wang, L. et al. *J. Med. Chem.* **55**, 1758--1770, 2012.
3. Hilmy, K. M. H. et al. *Eur. J. Med. Chem.* **45**, 5243--5250, 2010.
4. Rashad, A. E. et al. *Arch. Pharm. Chem. Life Sci.* **339**, 664--669, 2006.
5. (a) Mohamed, M. S. et al. *Eur. J. Med. Chem.* **45**, 2994--3004, 2010. (b) Mohamed, M. S. et al. *Eur. J. Med. Chem.* **46**, 3022--3029, 2011.
6. Varaprasad, C. V. N. S. et al. *Biorg. Chem.* **35**, 25--34. 2007.
7. Gangjee, A. et al. *Biorg. Med. Chem.* **20**, 2444--2454, 2012.
8. Liu, Y. et al. *Biorg. Med. Chem.* **20**, 5473--5482, 2012.
9. Gangjee, A. et al. *Biorg. Med. Chem.* **21**, 1180--1189, 2013.
10. Pittalà, V. et al. *Biorg. Med. Chem. Lett.* **16**, 150--153, 2006.
11. Taylor, E. C. & Liu, B. *J. Org. Chem.* **68**, 9938--9947, 2003.

12. Igarashi, K. et al. *J. Org. Chem.* **71**, 2690--2698, 2006.
13. (a) Skardžiūtė, L. et al. *Tetrahedron* **69**, 9566--9572, 2013. (b) Tumkevičius, S. & Dodonova, J. *Synlett.* **12**, 1705--1708, 2011.
14. Knorr, L. *Ber. Dtsch. Chem. Ges.* **17**, 1635, 1884.
15. Paal, C. *Ber. Dtsch. Chem. Ges.* **18**, 367, 1885.
16. (a) Watson, S. E. et al. *Synth. Commun.* **28(20)**, 3885--3894, 1998. (b) Fischer, R. W. & Misun, M. *Org. Pro. Res. Dev.*, **5(6)**, 581--586, 2001. (c) Kaïm, L. E. et al. *J. Org. Chem.* **75**, 5343--5346, 2010. (d) Prieur, V. et al. *Eur. J. Org. Chem.* 1514--1524, 2014. (e) Gorugantula, S. P. et al. *Tetrahedron* **66**, 1800--1805, 2010. (f) Tumkevicius, S. et al. *Tetrahedron Lett.* **51**, 3902-3906, 2010. (g) Dasari, R. & Kornienko, A. *Chem. Hetero. Compd.* **50(2)**, 139--144, 2014. (h) Lee, J. H. & Lim, H.-S. *Org. Biomol. Chem.* **10**, 4229--4235, 2012. (i) Matsumoto, N. & Takahashi, M. *Tetrhedron Lett.* **46**, 5551--5554, 2005.
17. Paul, S. et al. *RSC Adv.* **3**, 8637--8644, 2013.
18. (a) Papesch, V. and Schroeder, E. F., *J. Org. Chem.* **75**, 114--115. 1953. (b) Furniss, B. S.; Hannafold, A. J.; Smith, P. W. G.; Tatchell, A. R., *Vogel's Textbook of Practical Organic Chemistry*, 5th Ed., Pearson, India, 2005. (c) Rodríguez, J. M. &Pujol, M. D. *Tetrahedron Lett.* **52**, 2629--2632, 2011.