CHAPTER 2

Introduction and Review of Literature

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An essential transcription factor called p53 is responsible for either destroying or repairing cells with damaged DNA. p53 is responsible for coding for the protein MDM2, which prevents p53 from becoming hyperactive (which might result in premature ageing due to unneeded cell death.). Overexpression of MDM2 allows damaged DNA to replicate, which can result in the development of malignant cells.

The introduction will focus on p53-MDM2 interaction at different sites of p53 and MDM2, and small molecule inhibitors that bind to either MDM2 or p53 to prevent the p53-MDM2 interaction.

2.1. Cancer:

Cancer is a complex illness where cells are allowed to proliferate quickly and uncontrollably [27]. This often happens when the damaged DNA is capable of reproducing by evading cell cycle checkpoints.

In the same year, 14.1 million individuals had been identified as having the condition with cancer all over the world [28], while the disease was responsible for 8.2 million deaths worldwide. In the years 2010 and 2011, there was a 46% increase in the number of men as well as 54% increase in the number of female cancer patients who were predicted to live for ten years or more after their diagnosis [29]. These projections are based on the survival rates of those who were given a cancer diagnosis in 2010 and 2011. Breast cancer (which accounts for 15% of all cases), lung cancer (13%), prostate cancer (13%), and bowel cancer (13%), which together make up more than fifty percent of all cases of cancer in the globe, are the four most common types of malignancies [30]. Differences in the forms of cancer that occur with advancing age are noteworthy as well. Prostate cancer is more likely to occur in males over the age of 50, whereas breast cancer is more likely to develop in women over the age of 25 [31]. Leukemias are more likely to develop in infants, young children, and adolescents between the ages of 0 and 14 years old [32].

Cancer ranks among the most deadly illnesses in the world, and it has one of the highest fatality rates. According to the findings of GLOBOCAN (Global Cancer

Statistics) in 2020, which were prepared by the International Agency for Research for Research on Cancer, it was projected that 19.3 million new instances of cancer have been diagnosed, and roughly 10 million of cancer cases have resulted in fatalities [33]. Breast cancer is the kind of cancer that affects women the most often, with an incidence ratio of 11.7%. Lung cancer is the primary reason for mortality due to the disease, making it the top cause of cancer overall. Cases of lung cancer are expected to have an incidence of 11.4%, with 1.8 million fatalities. Following lung cancer in terms of high incidence rates are colorectal, prostate, and stomach cancers, each with respective rates of 10.0%, 7.3%, and 5.6%. It is estimated that there are 604,000 new instances of cervical cancer diagnosed in women each year, resulting in 342,000 deaths. After lung cancer, prostate cancer is the most frequent form of cancer among men, which accounts for around 1.4 million cases with 375,000 deaths annually [34].

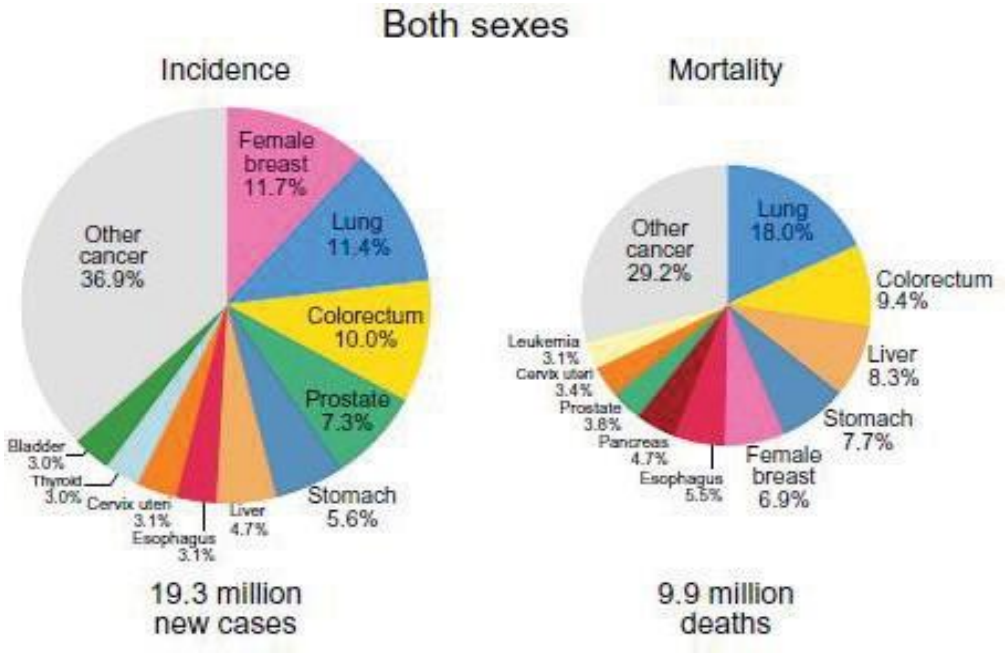


Figure 2.1. *Cancer incidences as well as mortality ratios in 10 common cancer types for both sexes in 2020. Taken from [34].*

Up to now, it has been known that there are more than 200 cancer types existing worldwide [33].

2.2. Causes of Cancer:

The epigenetic as well as genetic abnormalities in cells were responsible for the development of the illness known as cancer. Changes in genetic code and epigenetic

expression are both influenced by a complex web of physicochemical and other environmental variables.

There are many different substances that can cause cancer, either because they directly damage DNA or because they encourage unchecked cellular proliferation. Epigenetic alterations are induced by ageing and chronic inflammation, while genetic changes are caused by carcinogenic agents, ultraviolet radiation (UV), ionising radiation (-radiation), and ionising radiation, as well as viruses and ageing. [35].

One of the many chemicals that can harm DNA is: tobacco smoke (either from first-hand cigarette smoke or passive smoking). In places of social disadvantage, smoking is more common [36, 37].

Alcohol and its harmful byproducts, including byproducts of metabolism, can harm DNA. Alcohol-related upper digestive tract malignancies, such as those of the oral cavity and pharynx, are the most prevalent [38].

There has been a rise in the incidence of malignant melanoma throughout the course of the last three decades, which has been linked to greater sunbed use and vacation abroad [39]. 3.5% of all malignancies have been linked to ultraviolet radiation.[10] Radiation from ionizing sources including X-rays are also one of the causing factors [40]. The biggest number of instances were related to radiotherapy and radon, followed by background radiation and nuclear medicine respectively.

Despite the fact that infections are only a marginal contributor to the risk of developing cancer, it has nonetheless been connected some cancer cases. Females are more likely to contract it, and the leading infectious agents are the human papillomavirus, *Helicobacter pylori*, Epstein-Barr virus, hepatitis B and C, HIV/Kaposis sarcomavirus, and human herpes virus [41].

Genetic predisposition, also known as the presence of a mutated gene, as well as excessive body weight, sometimes known as obesity, are also additional variables that may contribute to the development of malignancies.

2.3. Cancer Cells:

Cancer is an illness that is triggered by the uncontrolled proliferation of cells in the

body. Instability in the genome, absence of a programmed cell death pathway, a specific energy metabolism, ability to invade, spread, and form new blood vessels are some of the defining characteristics of cancer cells. Cancer cells, in contrast to normal cells, lack the cellular degradation machinery necessary for programmed cell death in the event that they are aged or malformed. As a result, they are able to continue to proliferate and divide without limit. Because cancer cells have an invasive behavior, it is possible for the illness to spread from its original locations to other areas of the body. They have the potential to cause cell mass and tumours in various organs and regions of the body [42].

A self-sufficient supply of growth factor signals as well as a resistance to anti-growth factor stimulation are two further characteristics of cancer which contribute to the intricacy of the mechanisms by which cancer develops. Cancer cells acquire an infinite capacity for replicating as a result of this. In normal cells, the cell cycle is rigorously regulated relying on the growth signalling pathways for the purpose of maintaining cell proliferation. On the other hand, cancer cells having abnormalities in growth signalling pathways is appropriate for their survival. Reprogramming pathways in energy metabolism as well as avoiding immune response are indeed essential markers of cancer survival [43, 44].

2.4. Cancer Treatment Options:

Surgery is the most popular kind of cancer treatment, in which the patient's diseased tissue is removed along with some healthy tissue around it. However, this is not always feasible if the cancer is present in a hard-to-target place. Additionally, it's crucial to remember that depending on the afflicted tissue being removed, excision of the affected tissue may have additional effects. For instance, resection of the colon may result in malabsorption (The shorter the gut is, the less surface area there is for the body to absorb nutrients, which leads to less absorption).

Cost associated with the need of an overnight stay in the hospital (for instance, in the case of laparoscopic resection, which also increases the chance of venous thromboembolism as well as secondary infections like hospital-acquired pneumonia) is another aspect of surgical therapy to take into account [45].

The second most popular method of treating and diagnosing cancer is radiotherapy, which uses X-rays to kill tumor cells. Breast cancer, followed by lung cancer and prostate

cancer are the cancers most frequently treated with radiotherapy. Although this technique sometimes eliminates the need for surgery, the radiation it generates has the potential to cause cancer [46].

Chemotherapy frequently serves as an additional treatment and is based on the idea that it should be used to target cells that divide quickly [47]. The antitumor antibiotic doxorubicin as well as vinca alkaloid vincristine, both of which are still widely used today, are examples of treatments that are still the mainstay of care (both shown in **Figure 2.2**).

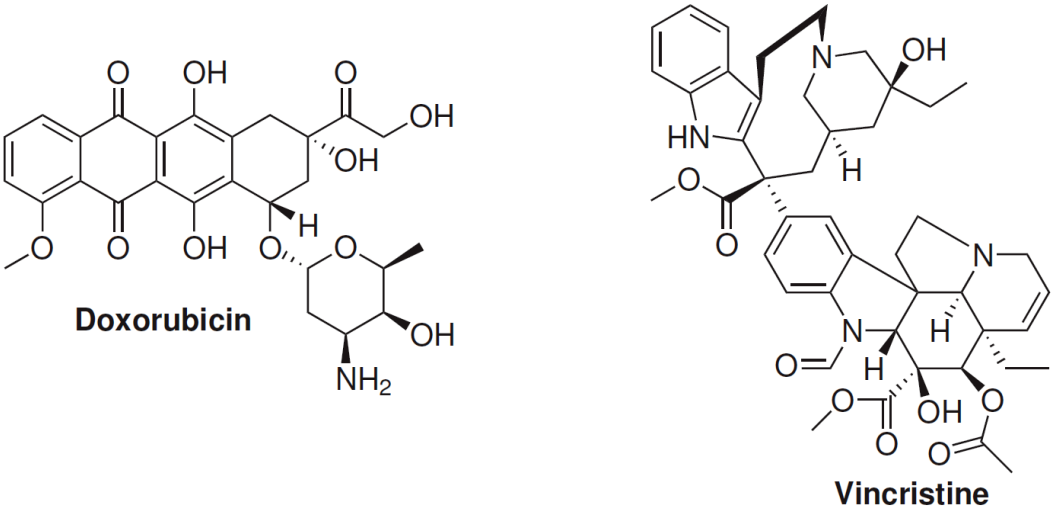


Figure 2.2. *Structures of doxorubicin and vincristine. Taken from [50].*

The greatest drawback of traditional chemotherapy drugs is the wide range of side effects they cause. For example, because they selectively target the cells dividing rapidly (rather than just tumor cells specifically), they also target other rapidly dividing cells like hair follicles, which leads to hair loss [48]. It is also conceivable that treatment with these agents, similar to radiotherapy, could result in the development of tumours in later life, necessitating the creation of novel, less harmful drugs while retaining potency [49]. Many of these cytotoxic treatments are combined in order to prevent resistance (such as PEGylated doxorubicin hydrochloride, paclitaxel and topotecan in order to treat advanced ovarian cancer) [50]. In order to increase the likelihood that combination therapy will result in complete cancer remission without recurrence, it is necessary to use a mixture of medicines. This decreases the possibility that cancer cells will develop an inherent resistance to the various treatments that are being used. Trials that have been reported in the literature typically involve the use of chemotherapy in conjunction with

another type of treatment like hormone therapy, because it is used as an adjuvant therapy. For instance, Gelmon *et al.*, conducted research that reviewed randomised controlled trials that were published between the years 1985 and 2000 and looked at the 15-year survival rate of breast cancer patients who already had treatmen with polychemotherapy and/or hormonal therapy all through the early stages of the illness. Introducing an anthracycline to either FAC or FEC for six months reduced the incidence of cancer in women under the age of 50 by 38%, while adding adjuvent treatment with tamoxifen over the course of 5 years decreased the incidence of cancer in a different patient sample consisting of female patients (despite the fact that this only applied to individuals whose result indicates that they have a positive oestrogen receptor). When these statistics were added to the data from meta-analyses, the final results showed that individuals who used adjuvent tamoxifen had a 57% lower occurrence of oestrogen-receptor positive breast cancer than those who did not take the medication had a 45% lower incidence of the disease.

Even while traditional chemotherapy drugs are still often employed in clinics, new substances are starting to be developed for the treatment of various malignancies [51]. Imatinib (**Figure 2.3**), the first of a revolutionary family of anticancer medications, operated by a unique mechanism of action that blocked an artificial tyrosine kinase. This was a significant step forward in the fight against cancer [52]. The Philadelphia chromosome mutation that caused this kinase to be formed, which combines the BCR-Abl genes to make said tyrosine kinase, was what made this method so intriguing and special. Other drugs, including as trastuzumab (an antagonist of the Her2/Neu receptor), have been created since imatinib, promising improved patient tolerability and cancer cell selectivity.

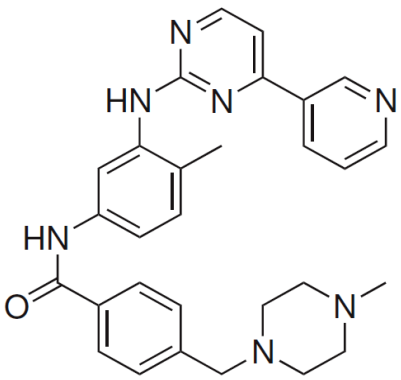


Figure 2.3. *Structure of imatinib. Taken from [54]*

The development of therapeutic drugs has shifted towards medications that modulate the pathways involved in cancer, using the knowledge gained from molecular therapies and the creation of imatinib. This represents a significant improvement in the perception of cancer therapy. Because of this, more recent cancer treatments have better side effect profiles and a more focused impact. Protein-protein interactions are currently the focus of a significant amount of research for the treatment of cancer as well as a wide range of other diseases. This is due to the fact that they perform specialized modulatory functions inside of cells and would cause effects that are specific to the situation if they were stimulated or inhibited.

2.5. Protein-Protein Interactions:

The medicinal chemistry community is becoming more and more interested in protein-protein interactions (PPIs), which provide more control than traditional drug targets like enzymes or receptors by modulating outcomes (such cellular death) within cells [53]. These interactions are widespread in the body and have the power to influence a variety of biological processes, including the control of inflammation [54] and the growth of new nerves [55].

Unfortunately, as protein interactions are variable and the state of their association is fleeting, problems might occur [56-62]. Additionally, interactions between proteins take place at vast, flat, hydrophobic surfaces (the usual interaction area is 1500 to 3000 $Å^2$, compared to 300 to 1000 $Å^2$ for interactions between proteins and small molecules) [63-70]. As a direct result of this, binding pockets may be concealed deep inside a protein and won't be revealed until a matching ligand is bound to one of them.

In spite of the apparent difficulties associated with targeting, researchers have continued to take use of these interactions [71-76]. We have studied here the protein-protein interactions of the p53 and MDM2 molecules, which is the main topic of this investigation.

2.5.1. The p53-MDM2 Interacting Partners:

An overview of the protein-protein interaction between p53 and MDM2 molecules is shown in **Figure 2.4**. The p53-MDM2 interaction has received the most attention to far,

and research by Lane and colleagues revealed that this kind of response is "druggable" for the first time [77].

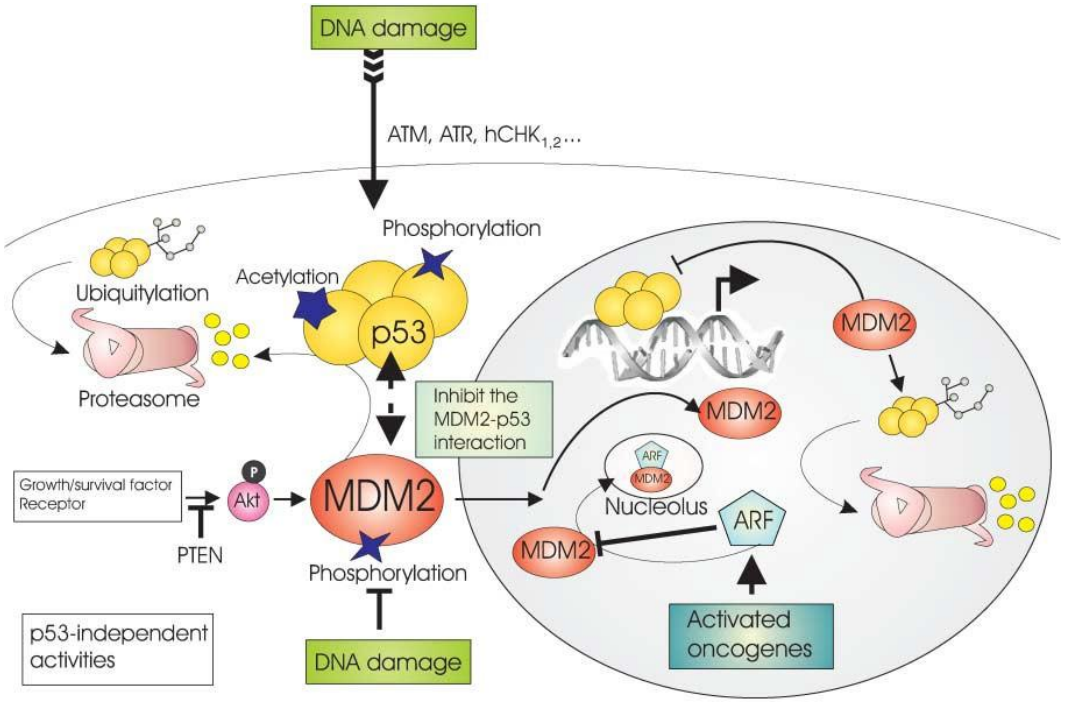


Figure 2.4. *Intracellular pathways affected by the interaction of the p53 molecules with MDM2. Taken from [78].*

The specifics of the proteins involved in this interaction are provided below, along with any potential downstream implications and inhibitors that have been shown to stop it.

2.6. p53 – An Introduction:

In 1992, Sir David P. Lane used the phrase "guardian of the genome" to describe p53 for the reason that it was responsible for causing damaged DNA to be either repaired or destroyed by apoptosis [78, 79]. The 393 amino acids that make up p53 are comprised of a TAD, a proline-rich domain (PRR) toward the NTD, a DBD in the core central region, and an oligomerization region where p53 is able to form a homotetramer [80]. When it is in its native condition, p53 may be found within the cell nucleus. There, it looks for broken DNA in order to direct the cell in the direction of either apoptosis or DNA repair.

Although it is anticipated that monomeric p53 would possess its unique pathway leading to degradation, the specific pathway behind this phenomenon is currently being researched. The p53 molecule in its monomeric state is expected to follow its own distinct

route to degradation, which differs from that of tetrameric p53, which is degraded by MDM2 once it has been released into the cytoplasm. It is hypothesised that the formation of tetrameric p53 will make the monomeric pathway difficult to discern [81].

An essential protein that acts as a tumour suppressor is known as p53. Its job is to make sure that some particular cells that have been damaged by cellular stress. In healthy cells, the amount of p53 present is rather modest. p53-induced apoptosis is caused by the downstream activation of pro-apoptotic proteins such as p53 upregulated modulator of apoptosis (PUMA) [82] and Phorbol-12-myristate-13-acetate-induced protein 1 (NOXA) [83]. These signalling proteins bind the anti-apoptotic proteins Bcl-2A1 and Mcl-1 when they translocate to the mitochondria (in case of NOXA) [84], and Bcl-X, Bcl-2, and Bcl-W (in case of PUMA) [85]. Apoptosis may be caused by p53's translocation to the mitochondria, where it can directly activate pro-apoptotic Bcl-2 family members, in addition to the downstream NOXA and PUMA binding to anti-apoptotic proteins. The last way cells may die is by a process known as downstream signalling of p21. This process is triggered by increasing levels of p53 in the cell. An increased level of p21 is what leads to senescence as well as the opsonization of damaged cells by macrophages [86]. Additionally, the p21cif1/waf1 complex is responsible for inhibiting the activity of cyclin-dependent kinases (Cdks) [87].

Other proteins assist p53 to attach to apoptosis-promoting partners and increase the likelihood of apoptosis occurring. Both the ASPP1 and the ASPP2 operate as cofactors to enhance the intercation of p53 with BAX [88]. Overexpression of the RNAi iASPP1 in breast cancer has been discovered to decrease apoptosis, and a connection has been made between overexpression of this gene and treatment resistance in AML and ovarian cancer [89]. There is evidence that melanomas harbour iASPP1, despite the fact that it is not overexpressed in these tumours [90].

The protein ARF is necessary for the synthesis of p53. Additionally, MDM2 can be bound by ARF, which may help to boost p53 stability inside the nucleus by preventing MDM2 shuttling [91]. It has been demonstrated *in vitro* that ARF binding to MDM2 does not interfere with MDM2's capacity to bind p53, resulting in the formation of both ARF-MDM2 and ARF-MDM2-p53 complexes [92]. MDM2 is likewise localised in the nucleolus by ARF.

The link between p53 and ubiquitin-specific protein 1 (USP1) is more complicated: Deubiquitination of DNA-binding inhibitors is caused by USP1 (IDs) [93]. Following the

interaction of E2A with bHLH (which are transcription factors), these IDs then promote osteosarcoma model uncontrolled proliferation by preventing p53-mediated cell cycle arrest and activating p21 in the downstream pathway.

In addition to being a p53 modulator, C-Abl also inhibits MDM2 in a non-oncogenic manner, which promotes p53 stability [94]. The p53/MDM2 negative feedback loop is used by oncogenic Bcr-Abl to inhibit p53 from performing its tumour suppressor functions, despite the fact that it can also lead to p53 accumulation.

Over fifty percent of malignancies contain p53 mutations (typically in the DNA binding area), and E177R and S46A are two of the most common ones [95, 96].

2.6.1. The *TP53* Family:

One of the most extensively researched proteins in cancer biology, p53 has several roles comprising cell-cycle arrest, senescence, and apoptosis in response to different stress stimuli and DNA damage. *TP53* gene is often altered in human cancers, leaving it non-functional [97-101].

Li-Fraumeni syndrome is a rare hereditary autosomal dominant condition that has been linked to germline abnormalities in the p53 gene [102, 103]. Such individuals have a significant chance of developing sarcomas, breast cancer, adrenocortical carcinoma (ACC), leukemia, and brain malignancies [104, 105]. Research on p53-deficient mice revealed that while they developed normally as embryos, they were more prone to developing spontaneous malignancies, such as lymphomas and sarcomas [106].

Two p53 homologues, p63 and p73, have overlapping but different roles. [107, 108] Similar to p53, p73 may be triggered by DNA damage, which can cause cells to undergo apoptosis and die [109]. By influencing FoxJ1, the main regulator of ciliogenesis, p73 has recently been shown to modify the development of airway multicilia in mice [110]. Mice lacking TP73 displayed abnormalities in neurogenesis, reproductive, social, and fluid dynamics of the central nervous system [111]. Additionally, it was discovered that when p53 was inactivated, p73 was crucial for preserving genomic stability [112]. However, as seen in TP63 knockout mice, p63 is crucial for the formation of squamous epithelial cells. These mice had abnormalities in the development of their limbs and craniofacial structures, as well as problems in epithelial tissues like the lack of hair, skin, and sweat glands [113, 114].

2.6.2. Transcriptional role of p53:

p53 identifies the DNA's responsive element, which is made up of two repetitions of the consensus sequence 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′ [115, 116]. Though, p53 recognises a pentanucleotide microsatellite sequence (TGYCC)n on the PIG3 promoter (where n=15) [117]. By attaching to these sensitive components, it functions as a transcription factor and speeds up transcription [118]. Numerous genes have been shown to be under the direct control of p53 (such as BAX, PUMA, NOXA, p21) [119, 120]. The ability of p21 to induce senescence through cell-cycle arrest has also been noted [121]. This is accomplished by decreasing the elements that encourage cellular growth.

2.6.3. Structure of p53:

The 393 amino acids that make up the p53 protein are subjected to a variety of post-translational changes, including as phosphorylation, ubiquitination, acetylation, neddylation , and sumoylation. (**Figure 2.5**) [122].

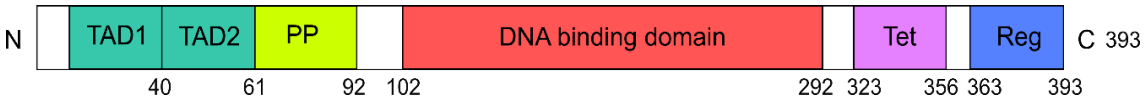


Figure 2.5. *p53 comprises of two transactivation domains (TADs), a proline-rich domain (PP), a central DNA binding domain (DBD), a tetramerization domain (Tet) and a C-terminal regulatory domain (Reg/CTD). Taken from [122].*

The TADs (TAD1 and TAD2) of p53 are intrinsically disordered regions, which means these domains lack a stable three-dimensional structure [123, 124].In response to stress stimuli, p53 protein undergoes substantial post-translational changes [125, 126]. The sequence PXXP is repeated five times in the PRR, which spans residues from 61 to 92. This area participates in p53-mediated apoptosis induction [127-129]. The p53 protein's DBD is what binds to the DNA's responsive regions, allowing its target genes to be transcriptionally activated. This area contains the majority of the hotspot mutations [130]. These mutations stop p53 from attaching to the target genes' promoters in a sequence-specific manner. 10% of these hotspot mutations are caused by either frameshift or nonsense mutations that result in the absence of a functional protein, and the remaining 90% are caused by missense mutations. Two homodimers can be combined to generate an active p53 tetramer via the tetramerization domain [131], which provides the proper

conformation for DNA binding. Additionally, it has a Nuclear Export Signal (NES) that permits the export of p53 to the cytoplasm between the residues 340 and 351 [132]. The numerous post-translational changes that the p53 CTD experiences are crucial for the protein's activity and stability. Due to the abundance of lysine residues in this terminal region, MDM2-mediated ubiquitination that results in the destruction of the p53 protein occurs there [133-135].

2.6.4. The Quaternary Structure of p53:

Since p53 has a poor solubility, a propensity to aggregate, and a mix of ordered as well as disordered domains, determining its structure has been challenging. The following quaternary p53 structural model was developed as a result of the combining of data from X-ray, SAXS, NMR, and electron microscopy. In solution, the free p53 protein folds into an extended cross-shaped tetramer (**Figure 2.6a**). Since the dimers of the core domain are only weakly linked, they can interact with the regulatory proteins. The long and adaptable N- and C-termini remain compatible with their biological activities. When it binds to DNA, p53 encircles the DNA helix (**Figure 2.6b**). The flexible linker between the core DBD and the Tetramerization Domain (TET) facilitates this process, and the overall structure becomes more stiff. [136]. One symmetrical core DBD dimer of the four p53 core domains is linked to every DNA half site (superdomain I). The distal location of the core domain DNA complex contains the p53(TET) (superdomain II). The two superdomains are only weakly coupled in the Joerger and Fershts model [136], allowing for a change in relative orientation in response to the introduction of additional protein binding partners. A basic nuclear localization signal (NLS) that can be accessed in both the DNA-free and DNA-bound forms is present in the flexible linker region that connects the two folded domains. Contrarily, one of the two leucine-rich nuclear export signals (NESs) is obscured inside the tetramerization domain, necessitating the dissociation of the tetramer as part of the identification procedure involved in nuclear export. Both the free as well as DNA-bound p53 models have prolonged N-termini, which is consistent with the fact that these regions are targeted by a wide variety of signalling proteins and are prone to a great deal of posttranslational modification. The naturally unfolded areas in p53 not only offer binding promiscuity but also flexible linkers to permit structural reconfiguration upon creation of higher-order complexes. The core domain-DNA complex's one face is pointed away by all four of the N-termini.

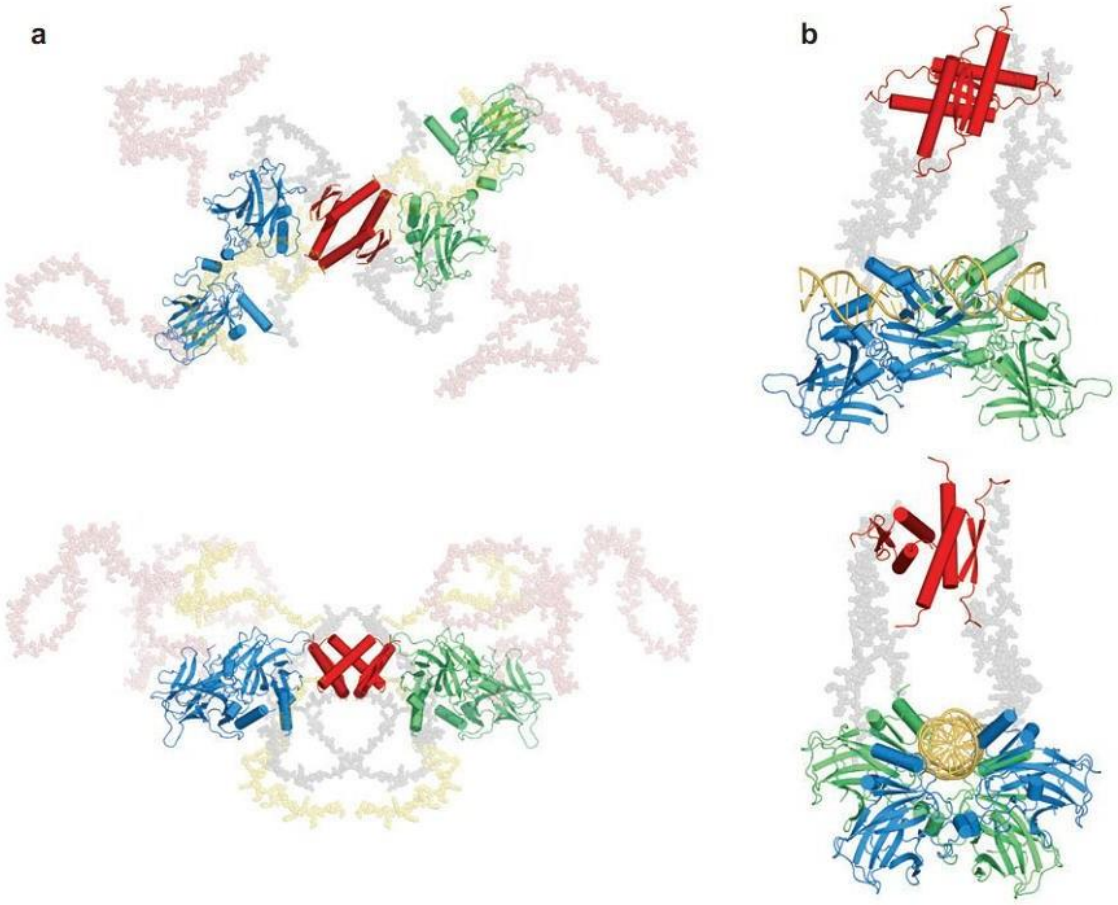


Figure 2.6. *Rigid body models of* ***(a)*** *the free (apo) and* ***(b)*** *the DNA-bound p53 in solution from small-angle X-ray scattering data. Both models are represented in two different orientations. Taken from [136].*

2.6.5. The cell cycle and the role of p53:

The crucial process that controls cell growth in all living things is the cell cycle. Although the specifics of the procedure differ amongst different organisms, the fundamental method by which genetic information is transmitted on to the following generation does not. **Figure 2.7** depicts the progression of the cell cycle's phases. The chromosomes are duplicated during the S-phase. In the M-phase, chromosome segregation as well as cell division take place. The G1- and G2-phases, respectively, are found between M- and S-phases as well as between S- and M-phases. These two Gap-phases provide the cell extra time for cell development, but more crucially, they give it time to check its internal and external environments and make sure that everything is ready before committing to the massive convulsions of the S-phase and mitosis. In this regard, the G1-phase is

particularly significant. The switches often initiate events completely and irreversibly and are binary in nature. Additionally, following procedures can only start if the preceding one was successful. The control mechanisms keep track of the proper advancement (both chronologically and statically), as well as the synchronisation of the cell cycle and the repair of faults. Checkpoints are the phases during which the cell cycle is regulated [137]. If the extracellular environment is adverse, the cells can postpone entering the G1 phase or they can stop and enter the specialised resting state G0, where they can stay for years before starting to proliferate again. However, cells in the early G1- or G0-phases will pass the first checkpoint, that is the so-called restriction point near the end of the G1- phase, provided extracellular circumstances are suitable and growth- and division-signals are present. When the cell reaches this stage, DNA replication is its only goal. The G1 checkpoint would cause cell cycle arrest in the event of DNA damage, preventing the DNA damage from being reproduced during the S-phase. The G2 checkpoint causes cell cycle stop in regard to damaged and/or unreplicated DNA, which ensures that S-phase is properly completed. Chromosome segregation is halted by the M-checkpoint as a result of the mitotic spindle's misalignment.

At these checkpoints, the cell cycle is halted, allowing the cell to I repair any cellular damage that has occurred, (ii) eliminate external stress signals, or (iii) enable the appearance of vital growth substances, hormones, or nutrients. The checkpoint will indicate apoptosis if the damage caused cannot be adequately healed. Cell cycle checkpoint defects can cause aneuploidy, chromosomal damage, and gene alterations, all of which can aid in the development of tumours [138].

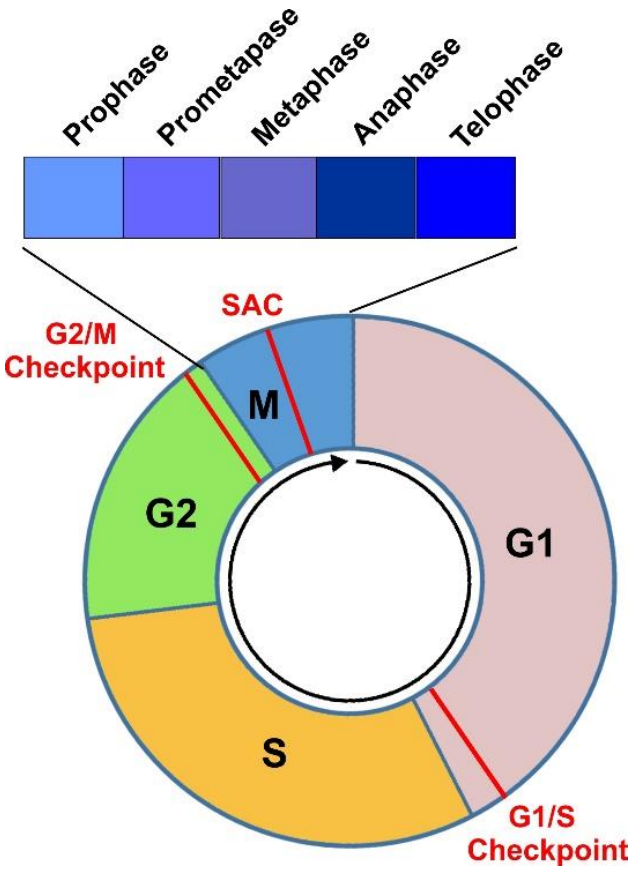


Figure 2.7. *Diagram to illustrate a complete cell cycle progression through four cell cycle phases (G1, S, G2, and M) and three major checkpoints (G1/S, G2/M, and SAC). M phase is further divided into Prophase, Prometaphase, Metaphase, Anaphase, and Telophase. Taken from [137].*

The cyclin dependent kinases closely watch the cell cycle (Cdks) [139]. Cyclins, regulatory proteins with cyclical protein level variations, in turn regulate the activity of the Cdks. Events related to the cell cycle can only be started by the cyclin-Cdk complex. The phosphorylation as well as dephosphorylation of Cdks as well as two inhibitor families, p16ink4a and p21, also control Cdk activity. Additionally, p53 is the principal regulator and the "so-called" keeper of the genome. This transcription factor is essential for sustaining a G2 arrest and regulates the G1/S cell cycle checkpoint: Genotoxic chemicals activate p53 in G1-phase cells, which then transcriptionally upregulates the Cdk inhibitor p21 [140]. p21 interacts with the proliferating cell nuclear antigen (PCNA), inactivating the cyclin-Cdk complexes and inhibiting the DNA replication's elongation phase. [141, 142].

Before entering the M-phase, cells will halt at the G2 checkpoint if they have already sustained DNA damage or have not successfully completed the S-phase. ATR or ATM

are two kinases that are activated by DNA damage. These respectively phosphorylate and activate Chk1 and Chk2 kinases, which inactivate Cdc25, which is de charge of activating Cdks. Also directly phosphorylating human p53 are ATM and ATR [143-146]. Also demonstrated was the role of Chk1 and Chk2 in the phosphorylation and stability of p53 following DNA damage [147-149]. It is currently unclear how p53 signalling affects the G2 checkpoint in further detail.

2.6.6. Cellular functions of p53:

In order to ensure homeostasis in normal cells, p53 is always kept at low levels since it has a comparatively short half-life, which may vary anywhere from 5 to 20 minutes (Figure 2.19). Negative regulators of p53 are also present. These include the E3 ubiquitin ligases (mainly MDM2) [150], PACT [151], COP1 [152], and Pirh2 [153], as well as phosphatases (WIP1/PPM1D) [154, 155].

p53 is maintained and activated in response to a variety of genotoxic stressors, including as DNA-damaging substances, hypoxia, and nucleotide deficiency, which causes its levels to steadily rise (**Figure 2.8**).

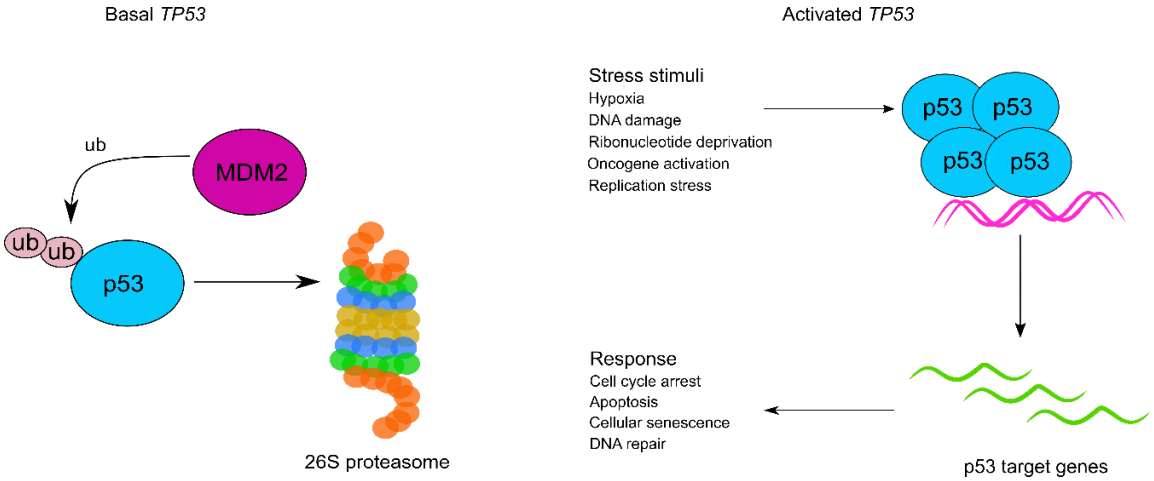


Figure 2.8. Activation of p53. *MDM2 keeps the amounts of p53 in normal cells in a homeostatic state. Following MDM2's ubiquitination of p53, p53 is degraded by proteasomes using the 26S proteasomal machinery. When under stress from the outside, p53 builds up inside the cell and then forms a tetramer. It then binds to the responsive regions of its target genes and activates transcription of the several genes involved in senescence, cell-cycle arrest, apoptosis, and repair. Taken from [156].*

Whether the cell experiences temporary cell-cycle arrest, irreversible cell-cycle arrest or apoptosis depends strictly on the circumstances. Strong p53 activating signals may lead to senescence or apoptosis whereas little DNA damage may activate DNA repair or cell-

cycle arrest pathways. ChIP-seq, RNA-seq, and GRO-Seq studies have been extensively used to recognize the consequential cell fate upon various stress stimuli, and they have helped to paint a more compound depiction of the regulation of the p53 gene. [156-158]. Below is a summary of p53's crucial functions and the genes that give rise to them.

2.6.6.1. Cell-cycle arrest:

Before the start of DNA replication in S-phase, broken DNA is found and repaired when cells are momentarily arrested during the G1-S interphase. Before entering mitosis, cells are examined for damaged or unreplicated DNA at the G2-M transition/shift in the cell cycle (**Figure 2.9**). Incorrect or damaged DNA that enters the M-phase has the potential to cause cell death brought on by aneuploidy, if it is not corrected [159].

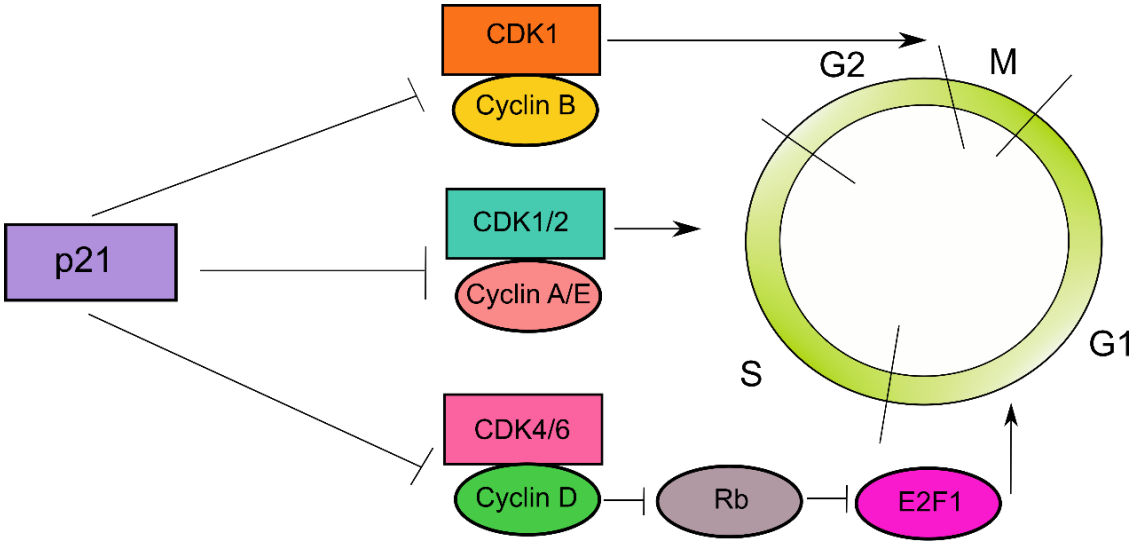


Figure 2.9. p21-mediated cell-cycle arrest. *One of p53's main target genes, p21, a CDK inhibitor, is activated upon p53 activation. This gene produces CDK4/ Cyclin D, Rb, and E2F1 proteins, which arrest cells at the G1-S phase; S-phase by CDK1/2 and Cyclin A/E complexes; and G2-M phase by CDK1/ Cyclin B complexes, which arrest cells at the S-phase. Taken from [159].*

The main gene that p53 targets to cause cell-cycle arrest is CDKN1A. Although CDKN1A-deficient animals were resistant to developing cancers on their own [160], they had abnormal G1 checkpoint activity in response to DNA damage as well as p53 activation [161, 162].

2.6.6.2. DNA repair:

DNA damage may be effectively repaired before synthesis by inducing cell-cycle arrest

by activating p53 during the G1-S transition [163-166]. When DNA is damaged, p53 activates proteins called DDB2 [167] as well as XPC proteins, which in turn activate Nucleotide Excision Repair (NER) and other repair pathways [168-170]. DNA repair pathways are started by the recruitment of repair proteins such as RAD6 and RAD18 by the ubiquitination of PCNA [171]. Additionally, by activating Pol η in cells exposed to mild amounts of UV light, p53 can start translesion DNA synthesis (POLH) [172, 173]. This turns on the ATM/CHK2 pathway, enabling cells to avoid the damage and aiding in the successful restart of the broken fork. **Figure 2.10 and 2.11** represent in detail the role of p53 in damaged DNA repair process [174, 175].

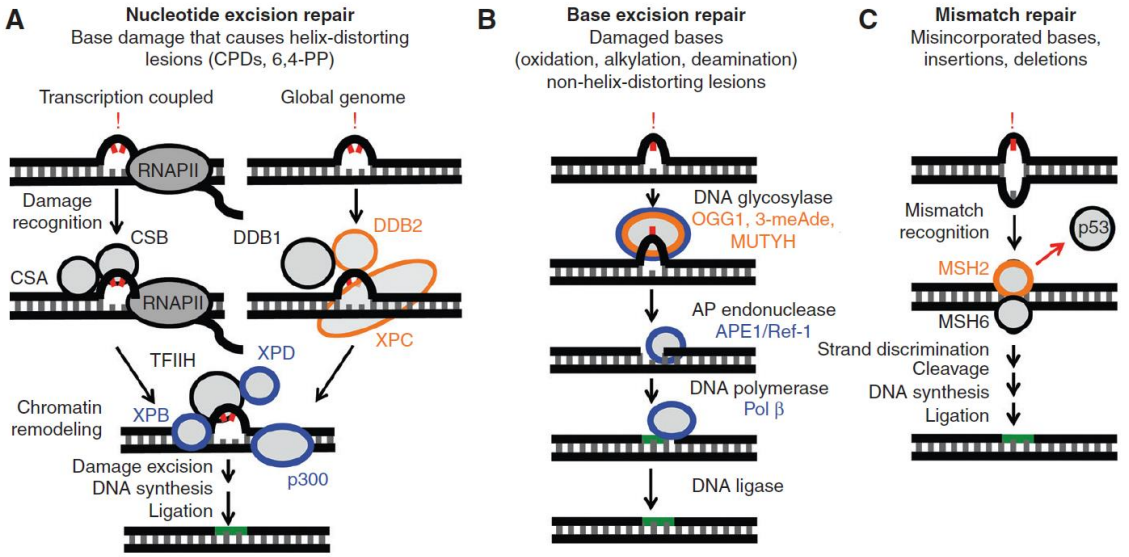


Figure 2.10. *Examples of p53 relations with DNA-repair pathways. Taken from [174].*

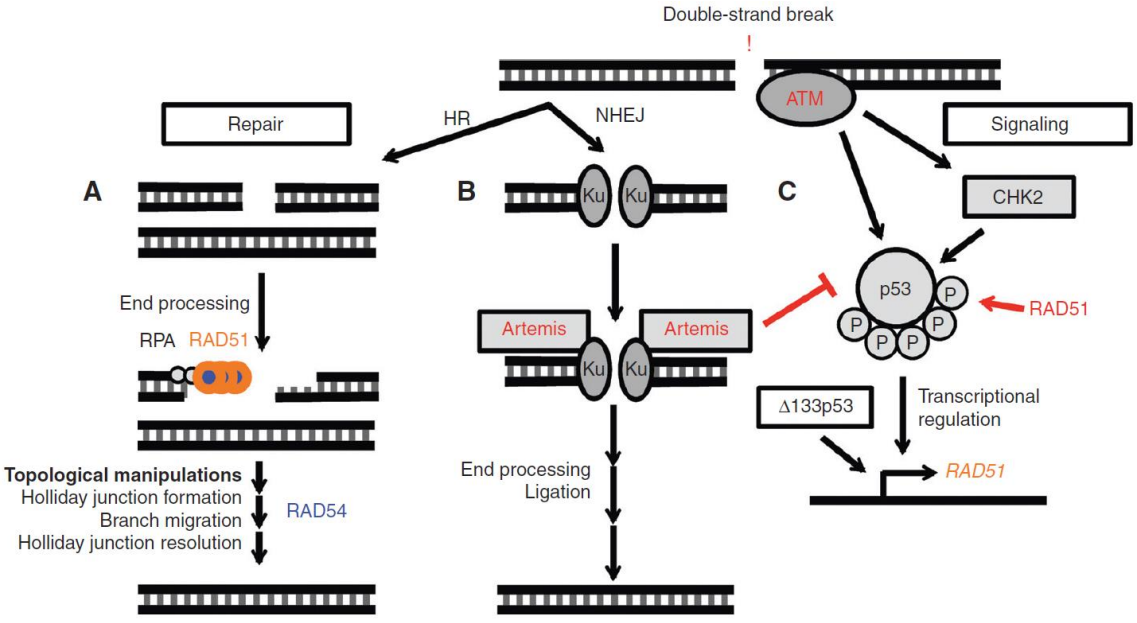


Figure 2.11. *Examples of p53 relations with double-strand break repair pathways (left) and p53 checkpoint signaling (right). Taken from [174].*

2.6.6.3. Apoptosis:

If the numerous processes described in section 2.6.6.2 are unable to effectively repair DNA damage caused by extreme stress, the cells will initiate a predetermined series of events that will eventually result in apoptosis. Both external and internal stimuli have the potential to cause this. Extrinsic stimuli include death ligands which bind to cell-surface receptors, like the Fas-L and TNF [176]. Contrarily, intrinsic stimuli are centred around the mitochondria in response to DNA damage, and several various factors, and are controlled by Bcl-2 family members [177-182]. ICAD and PARP play a crucial part in apoptosis. The DNAse-CAD is released by this proteolytic cleavage, which causes DNA fragmentation as well as nuclear blebbing, which in turn causes apoptosis [183-188]. Furthermore, both the intrinsic and the extrinsic apoptotic pathways' genes can be activated by p53.

2.6.6.4. Senescence:

Senescence is a cell-cycle arrest condition in which cells continue to function metabolically but stop dividing. The inhibition of malignant tumours is thought to be the cause of senescence induction. Stress inside the cell, either acute or chronic, or DNA Double-Strand Breaks (DSBs) are the causes of its onset. Plasminogen Activator Inhibitor-1 (PAI1), E2F7 [188], Promyelocytic Leukemia protein (PML) [189], and p21 are all activated by p53, which also activates p21, PML protein, and PML. In addition to p53 and Rb [190-192], kinases like p38 and the PI3K/AKT/mTOR pathways can also play a role in oncogene-induced senescence [193, 194]. Senescence prevents the metamorphosis of cells by inhibiting cellular proliferation.

2.6.7. Post-translational modifications (PTMs) on p53:

Several PTMs are carried out on the NTD as well as CTD of p53 (**Figure 2.12**). Several DNA Damage Response (DDR) kinases are capable of phosphorylating p53 in response to diverse stressors like Ataxia Telangiectasia Mutated (ATM) [195-197], DNA-dependent Protein Kinase (DNA-PK) [198, 199], Ataxia Telangiectasia and Rad-3 related

(ATR) [200], Jun NH2-terminal Kinase (JNK) [201-203], checkpoint kinases CHK1 and CHK2 [204], p38 [205] and others.

ATM specifically phosphorylates p53 at Ser15 (Ser18 in mice) in reaction to DNA double-strand breaks [206, 207].

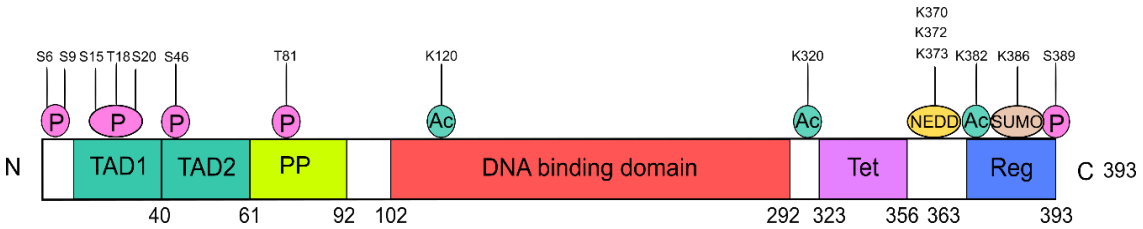


Figure 2.12. Post-translational modifications on the p53 protein. *Important post-translational modifications on p53 have been specified at the respective domains. P denotes phosphorylation, Ac denotes acetylation, SUMO denotes Sumoylation, and NEDD denotes neddylation. Taken from [125].*

When exposed to UV light, JNK [208] and p38 kinase [209] phosphorylate p53 at Thr81 and Ser389, respectively. When exposed to UV radiation, the homeodomain-interacting protein kinase 2 (HIPK2) is able to drive the phosphorylation of p53 on Ser46, which has been linked to apoptosis in cells [210, 211]. The aforementioned mentioned changes are what make p53 a more stable and active transcription factor.

Tip60 can acetylate Lys120 in the DNA-binding region of p53 in response to DNA damage. This is crucial for mediating BAX- and PUMA-mediated p53-dependent apoptosis [212-214].

MDM2 and F-box protein 11 both have the ability to neddylate p53 at the Lys370, Lys372, and Lys373 positions (Fbx11). The aforementioned changes prevent p53-mediated transcription. [215, 216]. When exposed to UV radiation, SUMO-1 performs sumoylation, another post-translational alteration that promotes the activation of p53 target genes at Lys386 [217]. The precise result of these changes is yet mostly unknown.

While p53 is vulnerable to a number of post-translational changes that make the protein active, these modifications are also altered by a number of proteins weakening the signalling axis, hampering p53 activity. It is possible for DNA damage to activate PP2C family members (WIP1 or PPM1D), dephosphorylating p53 and subsequently causing its downregulation [218, 219]. Because of this, they control p53 activity negatively.

The removal of acetyl groups from p53 by HDACs (HDAC1 as well as SIRT1), can reduce transcriptional activity and cell viability in response to stressors [220, 221]. Set9, which is a histone lysine methyltransferase, was shown to methylate p53 at Lys372 in 2004, and this finding was associated with enhanced p53 stability [222].

Finally, p53 is subjected to dynamic and extremely reversible ubiquitination. It can go through polyubiquitination or monoubiquitination, and each has a different effect. p53's transcriptional target MDM2, an E3 ubiquitin ligase, ubiquitinates it the C-Terminal lysine residues [223]. This helps p53 export nuclear material [224, 225]. A mechanism for polyubiquitinating all the p53 molecules and ultimately causing their destruction is provided by the enhanced expression of MDM2 which is brought on by p53 activation, and is controlled by the autoregulatory feedback loop [226].

Deubiquitinating enzymes (DUBs), mainly HAUSP or USP7 can intensely stabilize p53 and cause cell-cycle arrest and death even in the presence of MDM2 [227].

Thus, in reaction to stress signals, p53 is controlled by a variety of post translational changes that cause its target genes to either be activated or repressed.

2.7. MDM2 – An Introduction:

The gene *MDM2* was reported to be overexpressed in a murine cell line that underwent spontaneous transformation [228], and its product was shown to interact with and downregulate p53 [229, 230]. Mice without p53 were alive and showed normal development, but mice lacking *MDM2* were embryonically fatal [231, 232]. MDM2 is an E3 ligase, which ubiquitinates p53. The 26S proteasome is then used to degrade p53 as a result of this. MDM2, an oncogene with a bad prognosis, is typically overexpressed in sarcomas, leukemia, and lymphomas [230, 233].

2.7.1. Structure of MDM2:

The NTD of the MDM2, which has 491 amino acid residues, interacts with p53's TAD1 through the protein's N-terminus. (**Figure 2.13**) [234-238].

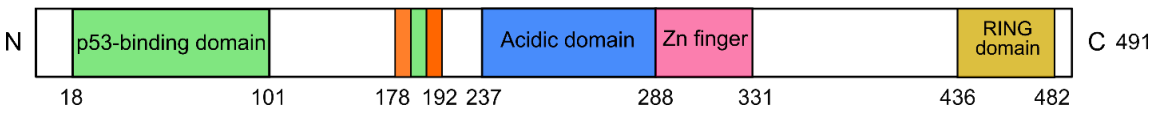


Figure 2.13. *Domain structure of MDM2 protein. Taken from [235].*

MDM2's association with the RPL5, RPL11, and RPL23 (which are ribosomal proteins) is disrupted by mutations in its zinc finger domain, which inhibits the activity of its ubiquitin ligase and, consequently, its capacity to destroy the p53 molecules [239-242].

The p53 binding domain at the protein's N-terminus and the RING finger domain at the C-terminus are the two crucial domains. According to biochemical as well as crystallographic studies, the MDM2 NTD creates a deep hydrophobic gap that p53's N-terminal TAD1 can bind into [243]. The most important p53 residues for binding to MDM2 are Phe19, Trp23, and Leu26 [244], whereas the most important MDM2 residues for binding to p53 are Gly58, Gly68, Val75, and Cys77 [245]. It was discovered that the p53 Thr18 residue is crucial for the integrity of the p53 α-helix [234]. The interaction between p53 and MDM2 is over 10-fold less effective when this residue is phosphorylated than when Ser15 and Ser20 are phosphorylated in p53 [246].

When directed at p53 and itself, MDM2 can act as an E3 ubiquitin ligase [247, 248]. MDM2 mediates the monoubiquitination of p53, while p300's ubiquitin ligase activity is required for polyubiquitination. [249, 250]. While the E3 ubiquitin ligase function was eliminated by mutations in RING domain, its interaction with p53 was left unaffected [251].

Additionally, according to Oren and colleagues, MDM2 degradation can cause p300 to acetylate MDM2 at its RING domain, which enhances p53's transcriptional activity. [252].

In addition, MDM2 has a nuclear export signal (NES) (residues 181–185) as well as a nuclear localization sequence (NLS) (residues 190-200), which facilitates the movement of MDM2 into as well as out of the nucleus. [253].

MDMX is an MDM2 homologue. Through their shared RING finger domains, MDMX and MDM2 can heterodimerize. But in contrast to MDM2, MDMX lacks inherent E3 ubiquitin ligase function. [254, 255]. Nevertheless, MDMX can attach to p53 via its p53-binding domain, masking the TAD of p53 that causes the transcriptional function of p53 to be reduced in a way similar to MDM2. [256, 257].

2.7.2. Transcriptional regulation of MDM2:

Two separate promoters, P1 and P2, regulate the transcription of MDM2. [258] (**Figure 2.14**). Due to translation beginning at two different AUG start codons, the two isoforms move at a respective 90 kDa and 75 kDa. The promoter P1, which is located upstream of exon 1, regulates MDM2's basal expression, while the promoter P2, which is located inside the first intron, is tightly controlled and is also inducible [259]. Since the P2 promoter contains p53 responsive regions upstream, MDM2 can be expressed when p53 is activated [260, 261].

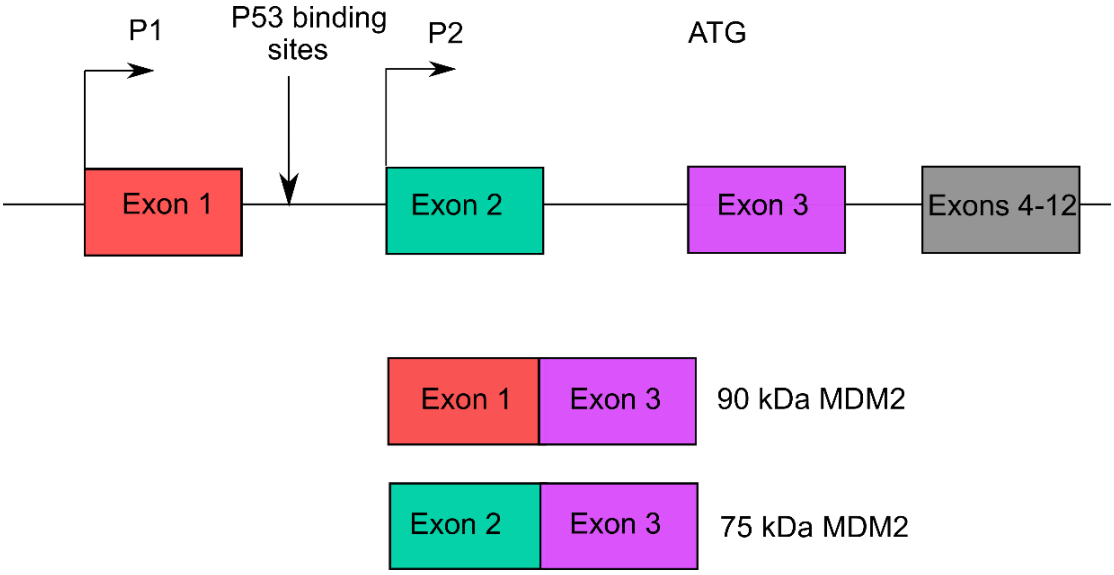


Figure 2.14. P1 and P2 promoters of MDM2. *Schematic representation of 5' end of the MDM2 gene. Exons 1 and 3 are present in the MDM2 protein's long isoform, whereas exons 2 and 3 are present in the protein's short isoform, P2. Taken from [261].*

2.7.3. Post-translational regulation of MDM2:

MDM2 is phosphorylated at different domains (**Figure 2.15**).

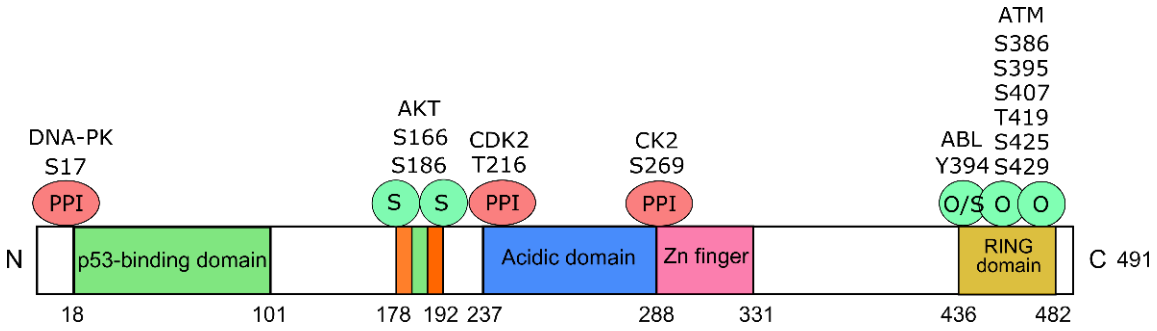


Figure 2.15. *Phosphorylation sites on MDM2. Taken from [262].*

When DNA double strand breaks occur, ATM may phosphorylate MDM2 that inhibits MDM2's ability to ubiquitin ligase p53 and activates p53 [262, 263]. However, WIP1 phosphatase preserves this equilibrium by dephosphorylating MDM2 when the stimulus is stopped [264]. AKT is one of the additional kinases that has the ability to phosphorylate MDM2, stabilising it and inhibiting p53 in the process [265]. The MDM2 protein is phosphorylated at Ser17 by DNA-PK, which separates p53 from its inhibitory regulators and enables p53 stability. [266-274]. In response to doxorubicin, S6K1 kinase, a component of the mTOR pathway, phosphorylates MDM2, which prevents MDM2 from ubiquitinating p53. [274-280]. CDK1 and CDK2 phosphorylate p53 at Thr216, which may impact MDM2's ability to interact to other proteins [281, 282]. By phosphorylating MDM2 at Tyr394, c- Abl can prevent p53's ubiquitination and proteasomal destruction [269].

By inhibiting its ubiquitination function [270] and so increasing p53 responsiveness, P14-ARF was found to promote sumoylation of the MDM2 molecules. MDM2 has been shown to have p53-independent functions in addition to maintaining p53 levels.

2.8. The p53-MDM2 Sites of Interaction:

The interaction between p53 and MDM2 has been recognised as a promising target for the creation of cancer-treatment medications [283]. Due to its participation in cell cycle arrest, DNA repair, cellular apoptosis, and cell senescence, the transcription factor p53 is crucial in preventing the growth of tumours.

Cell cycle arrest or apoptosis are the final results of post-translational alterations to p53 brought on by stress signals, which are predominantly produced by DNA damage but can also include hypoxia and heat shock [284, 285]. The p53 protein is affected by these alterations in two ways: first, the half-life is extended from minutes to hours, and the amount of p53 in the cell is multiplied by ten. Furthermore, because these alterations make it easier for certain DNA sequences to bind to p53, transcriptional activation is enhanced [286].

The majority of human malignancies, if not all of them, include dysfunctional p53. Mutations in *TP53* gene can result in p53 inactivation (the gene coding for p53) [287]. Numerous positive and negative feedback loops also control the p53 activity, with MDM2 acting as the most significant negative regulator (**Figure 2.16**) [286, 288]. The main way

that MDM2 controls p53 activity is by maintaining low level of p53 in cells that are not under stress and turning p53 off after a stress-induced activation. But excessive MDM2 expression causes p53 in its natural state to degrade quickly.

When MDM2 binds to p53, three things happen: (1) P53 is ubiquitinated by MDM2, an E3 ligase, and is thereafter degraded by proteasomes. (2) By attaching to p53's N-terminal TAD, MDM2 stops p53 from directly interacting with DNA and acting as a transcription factor. (3) MDM2 promotes p53's export from the nucleus of the cell.

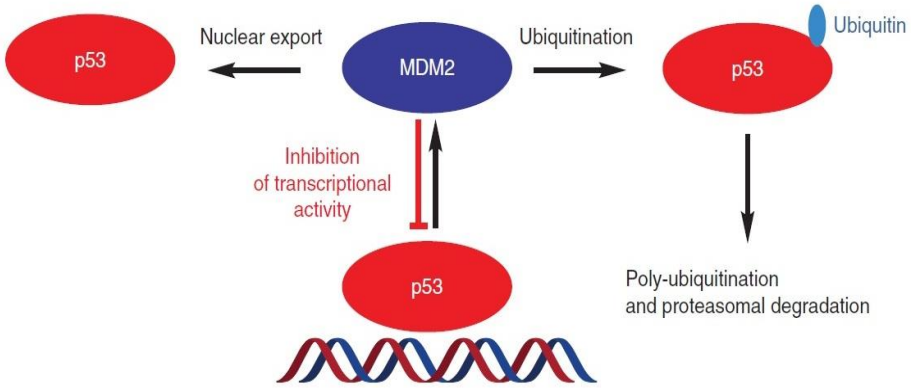


Figure 2.16. *Autoregulatory loop of p53 and MDM2. [Taken from 374].*

Therefore, by reactivating wild type p53's tumour suppressor function, suppression of the MDM2/p53 connection has the potential to cure human malignancies that still contain this gene. Because of the poor responsiveness to therapeutic therapy, higher recurrence, and increased metastasis, an MDM2 overexpression is occasionally linked to a worse survival rate [289, 290]. Inhibiting the connection between MDM2 and mutant p53 would not have any therapeutic benefit in these circumstances since mutant p53 is frequently resistant to the degradation mediated by MDM2 [291].

2.8.1. The Primary Site of Interaction – Between the NTD of MDM2 and TAD1 of p53:

The p53(NTD)-p53(TAD1) protein-protein interaction is depicted diagrammatically by two distinct techniques in **Figure 2.17** [292], which shows the X-ray crystal structure of the structure. p53 levels increase when damaged DNA is found by p53 in the nucleus, and depending on the level of damage and variables influencing the downstream pathway selected, such as the cell's p53 threshold levels, the damaged DNA is either repaired or the cell is apoptosed.

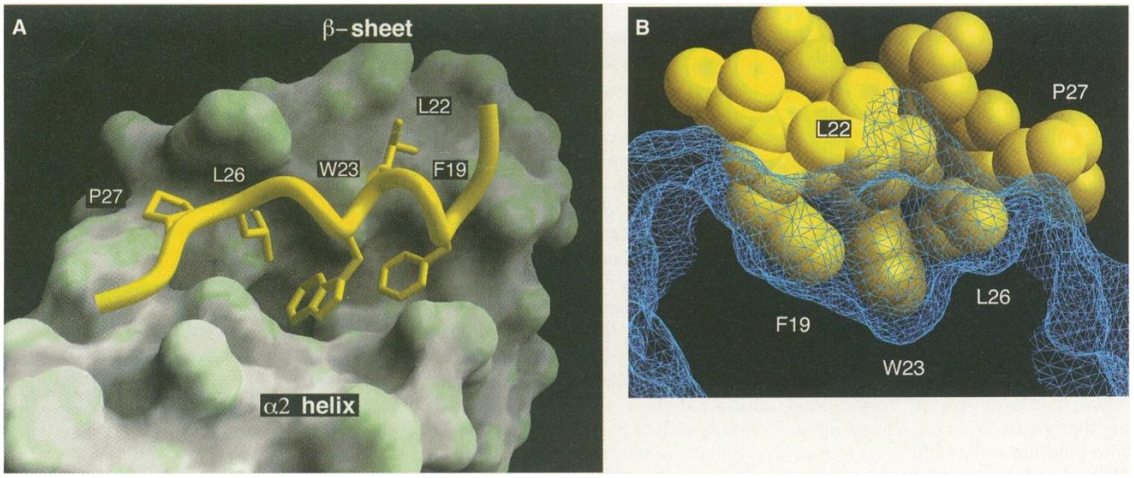


Figure 2.17. *X-ray crystal structure of the p53 transactivation domain 1 (TAD1) bound to N-Terminal Domain of MDM2, indicating the crucial binding amino acids Phe[19], Trp[23] and Leu[26]. Taken from [292].*

In order to stop p53 from overworking and producing excessive quantities of apoptosis, p53 also transcribes MDM2, a negative modulator of p53. The activity of p53 within the nucleus is stopped when MDM2(NTD) attaches to the p53(TAD1) located in the p53(NTD) of the -helical tetrameric p53 through the hydrophobic pocket present in MDM2(NTD) (including Phe19-, Trp23-, and Leu26-binding areas, as illustrated in figure 1.17).

Overexpression of MDM2 causes p53 to be rapidly degraded and hinders p53 from performing its tumour suppressor activities within the cell, which causes malignancy. In 7-8% of all malignancies, this mechanism is present. As a result, substances that try to either (re)activate p53 or inhibit MDM2 activity are now being looked at as potential cancer therapy.

2.8.2. Between the central AD of MDM2 and core DBD of p53:

There exists a secondary site of interaction between the DBD of p53 and the central AD of MDM2. And this interaction has been found to be important for the proper ubiquitination of p53 by MDM2 via its E3 ligase activity [293-296].

2.8.3. Between the NTD of MDM2 and CTD of p53:

In a study conducted by Poyurovsky *et al.*, in 2010, it was found that the p53 CTD

interacts directly with the N-Terminal Lid present in the MDM2(NTD) [297]. They also proposed that there might be a distinct conformational change in the p53-MDM2 complex when the p53(CTD) binds to MDM2(NTD).

2.8.4. Between the central AD of MDM2 and CTD of p53:

In a study conducted by Wang *et al.,* in 2017, they found that there exists an interaction between the unacetylated p53(CTD) and the MDM2(AD) [298]. They also found that the interaction is affected by many factors, including charge effect between p53(CTD) and the MDM2(AD), length of the MDM2(AD), and the amino acid composition of MDM2(AD).

2.8.5. Between the NTD of MDM2 and Oligomerization Domain (OD) of p53:

Oligomerization Domain (OD) (also called Tetramerization Domain; TET) is the site where the p53 molecule forms a tetramer conformer. In a study conducted by Katz *et al.,* in 2018, they discovered a fourth site on p53 that is interacted by MDM2. This interaction was found between the 33-43 residues present in MDM2(NTD) and p53(TET/OD) [299]. The necessity for p53 to be in a dimer form makes this p53-MDM2 interaction distinct.

2.8.6. Between the Really Interesting New Gene (RING) Domain of MDM2 and DBD of p53:

In a study conducted by Yang *et al.,* in 2019, it was found that not only the central AD of MDM2 binds to the p53(DBD), but also the RING domain of MDM2 binds to the p53(DBD). Additionally, they discovered that the mutant form of p53 (R175H) had stronger avidity than wild-type p53 when it came to binding to the MDM2 AD as well as RING domain [300].

2.9. p53-MDM2 Interaction Inhibitors:

2.9.1. Potent Small Molecule Inhibitors of the p53/MDM2 Interaction and their Development:

Nutlins, spiro-oxindoles, pyrrolidines, and piperidones are the four primary types of small molecules [301-308] that block the p53-MDM2 protein-protein interaction [309-321]. The top four small-molecule p53/MDM2 inhibitor classes' chemical structures, biological information, and companies involved in their discovery and development are summarised in **Figure 2.18** [322].

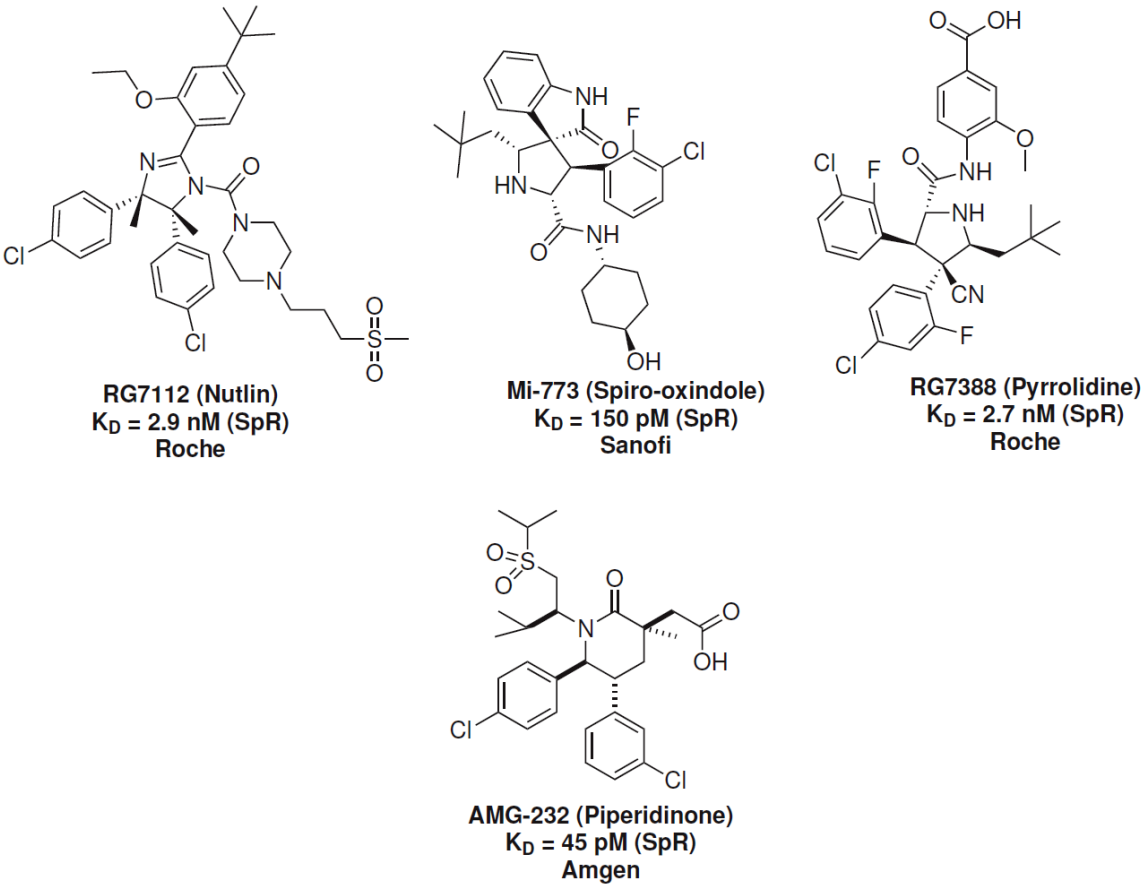


Figure 2.18. *Top four inhibitors (antagonists) of p53-MDM2 interaction (SpR: surface plasmon resonance) Taken from [322].*

2.9.2. Discovery of Nutlins: an Imidazoline-Based Library:

The first family of inhibitors for the protein-protein interaction between p53 and MDM2 was synthesised, and it was called nutlins [323-325]. High throughput screening was used by Pazgier and colleagues to examine the nutlin library of chemicals in the beginning [326]. They have a 4,5-dihydroimidazoline structure as its foundation [327].

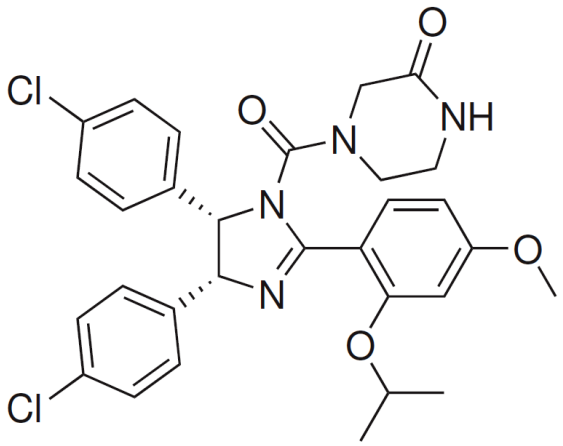


Figure 2.19. *Structure of Nutlin-3a. Taken from [326].*

After that, Nutlin-3a (**Figure 2.19)** was examined to see how it affected the cell cycle in the cell lines HCT116 (wt p53, crucial binding sequence SQETFSDLWKLLP), and SJSA-1 (overexpressed MDM2). With a considerable decrease in S-phase activity, the stage of the cell cycle where DNA replication takes place, there were higher levels of both G1 as well as G2/M activity in both instances 24 hours after 4 M nutlin-3a therapy. When nutlin-3a was used to study gene expression, p53 and p21 transcription levels significantly increased (p53's downstream pathway includes a connected tumour suppressor protein).

To examine the impact of nutlin-3a on apoptosis using transferase-mediated deoxyuridine triphosphate nick end labelling, additional cytotoxicity experiments were conducted (TUNEL). Fluorescence microscopy and flow cytometry may be used to measure how extensive the labelling is when cells suffer damage and death [328]. 48 hours after being incubated with nutlin-3a, it was discovered using TUNEL that 45% of the SJSA-1 cells were positive for the enzyme (At 24 hours, the number of TUNEL-positive cells was negligible).

Nude mice xenografts seeded with the SJSA-1 cells showed a tumour growth inhibition of 81% at 10 mg/kg inhibitor during the progression from the single cells to the animal systems in preclinical tests with nutlin-3a (the maximum tolerated dose).

The disubstituted benzene ring filled the Phe19 pocket, whereas the two chlorophenyl rings inhibited the Trp23 and Leu26 pockets, according to solution-phase NMR investigations and X-ray crystal structures [329]. Although the molecule is otherwise exceedingly lipophilic, the piperidone-like moiety serves to increase water solubility while having no effect on binding.

Despite nutlin-3a's considerable potential as an inhibitor, its subpar pharmacokinetic

qualities prevented it from becoming an option for clinical studies (since it has a high lipophilicity, it was difficult to formulate a medication for drug administration and to penetrate the required tissues) and therefore analogs of nutlin-3a that kept or enhanced efficacy while simultaneously enhancing water solubility were studied.

2.9.3. Optimisation of Nutlins - RG7112:

Further SAR experiments on nutlin-3a were conducted in 2011 in order to increase the nutlins' potency and their ability to bind to MDMX, which is a secondary binding partner of the both p53 and MDM2 [330]. The creation of RG7112 was made possible by this optimization (**Figure 2.18**). The first p53-MDM2 interaction inhibitor to enter Phase I clinical trials for early-stage solid tumours, advanced solid tumours, haematological malignancies, liposarcomas, and acute myeloid leukaemia (AML) in conjunction with cytarabine was RG7112 [331].

The development of RG7112, the most powerful nutlin produced to date (shown in **Figure 2.18**), began with the optimization of nutlin-3a, a nutlin in which the chlorophenyl groups were projected into the Trp23 and Leu26 made binding pockets of MDM2 as the primary binding mode [331].

The methoxy group on nutlin-3a was susceptible to degradation when RG7112 was being developed, and as a byproduct of metabolism, phenol was formed. A hazardous metabolite called phenol was not produced when the methoxy was replaced with a t-butyl activity [332]. Another significant change was the addition of methyl functionalities that stop the imidazoline core from degrading into imidazole, which was totally ineffective against MDM2. To make the molecule more "druggable" (that is, a substance that allows the drug to be formulated into a medication and has robust pharmacodynamics and good pharmacokinetics.), the isopropoxy activity was changed to an ethoxy. This reduced the molecular weight. A range of various polar substitutents were investigated since it was hypothesised that the solvent-exposed amide side chain might be exploited for solubilization in aqueous conditions. The most effective substance, RG7112, comprised a propyl linker that connected a piperidine side chain to a methylsulfoxy. SpR competition results showed that RG7112's IC50 was 2.9 nM. (according to competition SpR, nutlin-3a has a concentration of 90 nM).

Despite the great efficacy of the nutlin molecules (class), RG7112 as well as other

nutlins are resistant to them due to mutations in MDM2's binding pocket as well as lid region. As a result, more inhibitors need to be developed [333-339]. It's interesting to note that a recent paper investigated how nutlin-3a interacts with both the p53/MDM2 interaction [334] and the anti-apoptotic Bcl family, which might lessen resistance and boost cancer treatment efficiency. The groundwork was laid for the creation of new small molecules that could block this interaction using the X-ray crystal structure of p53-MDM2 complex (PDB ID: 1YCR) and nutlin-3 as small molecule templates.

2.9.4. The Pyrrolidines:

The most powerful pyrrolidine that has been reported in the literature with a known structure is RG7388, which is seen in **Figure 2.20**. A five-membered ring system rather than a six-membered ring system was the initial concept for the creation of pyrrolidine family by Graves *et al*., in 2013, as the 5-membered ring allowed for more flexibility and was theorised to better fit into the hydrophobic pocket [340]. The creation of RG7112, which likewise has a 5-membered ring core, served as a major source of motivation for the pyrrolidine library. The pyrrolidine library had been created using non-stereoselective techniques as a racemic mixture, separated by chiral HPLC, and evaluated using HTRF binding assays as well as MTT proliferation in cell lines with wild-type (SJSA-1) and mutant (SW480) p53 [340]. RG7388 was 344 times more powerful against wt p53 cells than cells with mutant p53.

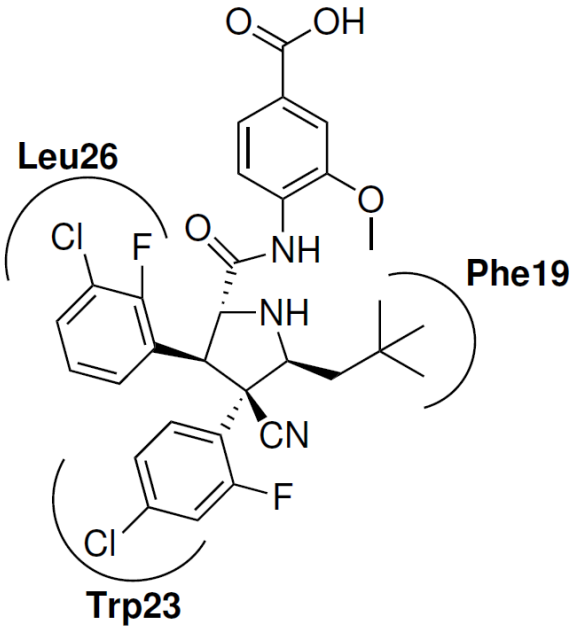


Figure 2.20. *Structure of RG7388. Taken from [340].*

By using its 2-fluoro-4-chlorophenyl ring, RG7388 interacted with the Trp23 in a π-π stacking manner, as revealed by a study of the pyrrolidines' binding motif. The neopentyl group was discovered to inhabit the Phe19 pocket, whereas the 2-fluoro-3-chlorophenyl ring paired with the Leu26 pocket (**Figure 2.20**). Additionally, the pyrrolidine's carbonyl might establish hydrogen connections with His96 (PDB entry 4JRG). These substances are currently undergoing clinical testing, but the results have not yet been released [341].

2.9.5. Key Features of the optimum p53-MDM2 Small Molecule Inhibitors and their Further Development:

Numerous small molecule inhibitors of p53-MDM2 interaction share specific important characteristics. Most small compounds have fused bicyclic cores or monocyclic aromatic cores (**Figure 2.21**). The central core of the monocyclics, which are typically 5-membered aromatic rings with an imidazole, furan, or thiophene activity, is inflexible due to this functionality [342-347]. A 5-membered core is present in three of the major inhibitors, although they are not aromatic (**Figure 2.18**, the spiro-oxindole, a 5-membered ring united to a 6-membered aromatic ring, being the sole exception.). RITA (**Figure 2.21**) is a linear molecule without a central core that possesses both thiophene and furan activities.

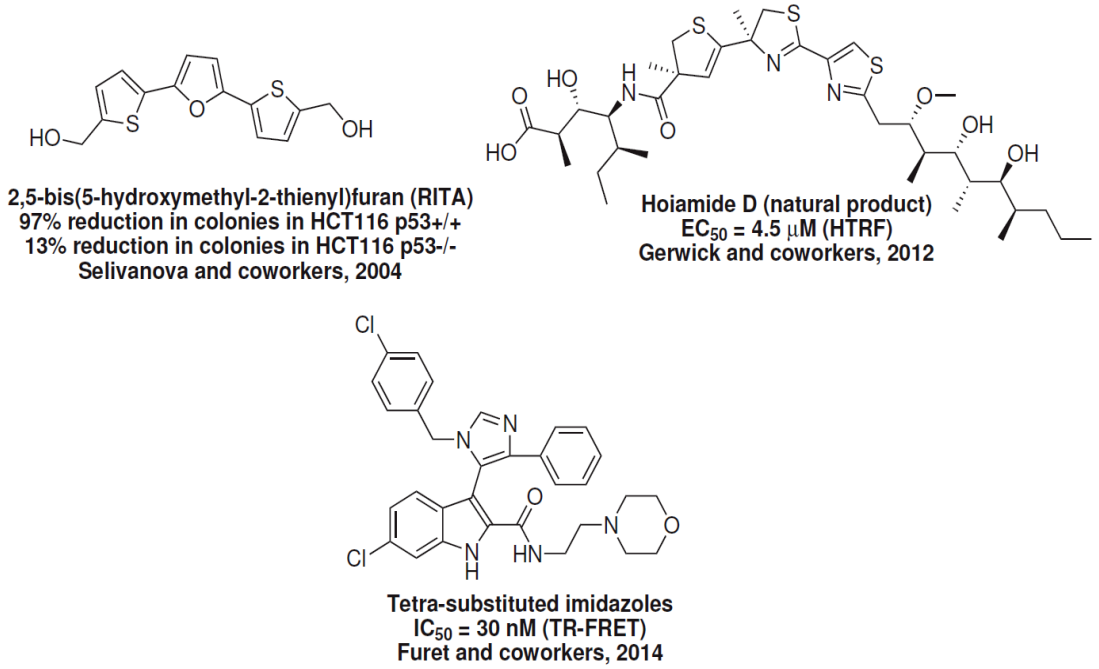


Figure 2.21. *Small molecule p53-MDM2 interaction inhibitors comprising of a 5-membered aromatic core Taken from [345-347].*

Numerous monocyclic and bicyclic small molecule inhibitors have a kind of lactam present in their central core (**Figure 2.22**), which gives the compounds some stiffness and makes them reasonably resistant to degradation since the lactam may reverberate its charge via its bonds. Piperidinones have a six-membered lactam ring, whereas benzodiazepinediones and thiobenzodiazepinediones have a seven-membered lactam ring. Isoindolinones have a five-membered ring [348-351]. Several of these small molecule inhibitors share the lactam functionality, which may be synthesised by a number of processes (including the Castagnoli reaction) [352].

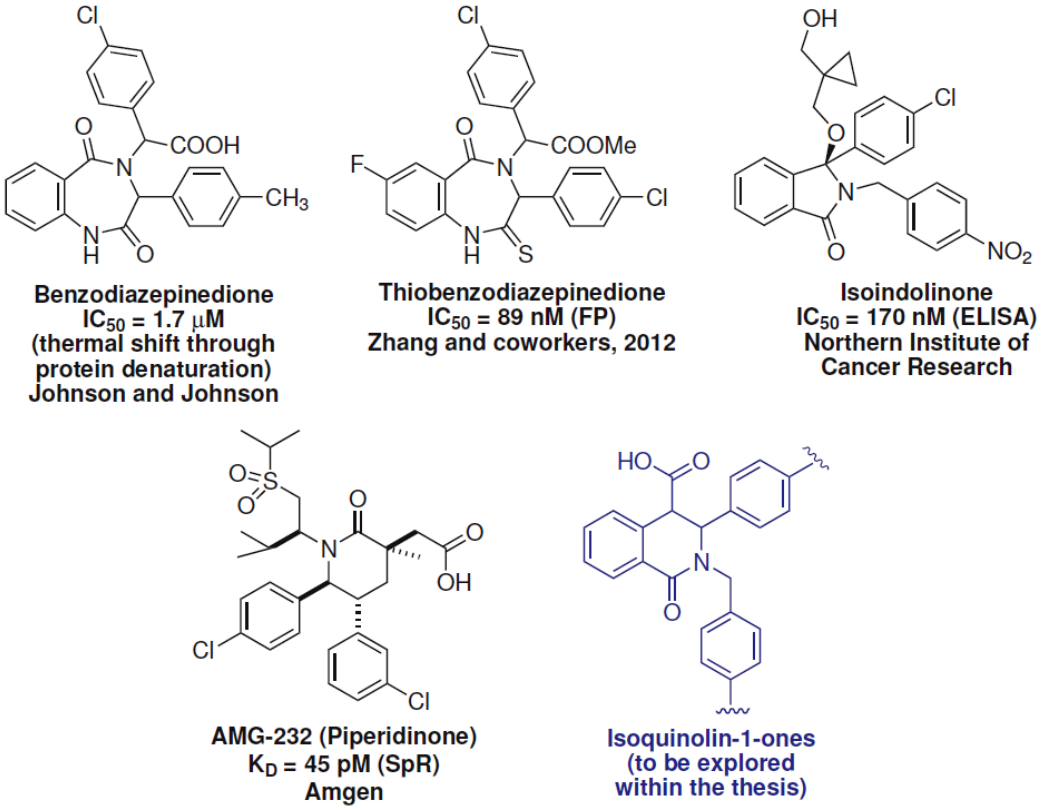


Figure 2.22. *Small molecule p53-MDM2 interaction inhibitors comprising of a lactam component. Taken from [348].*

After the monocyclic as well as bicyclic cores, the polycyclic compounds that have been demonstrated to stymie the interaction between p53 and MDM2 come next (**Figure 2.23**). Using a yeast hybrid screen, it was determined that α-mangostin as well as gambogic acid hinder the interaction between p53 and MDM2 [353]. The presence or absence of p53 in MCF-7 cell lines affected these drugs' ability to function. High levels of interaction between the two substances were seen at the MDM2 pocket's Gly58, Asp68, Val75, and Cys77, however only gambogic acid was capable of forming hydrogen bonds

with Gln72 as well as Phe55.

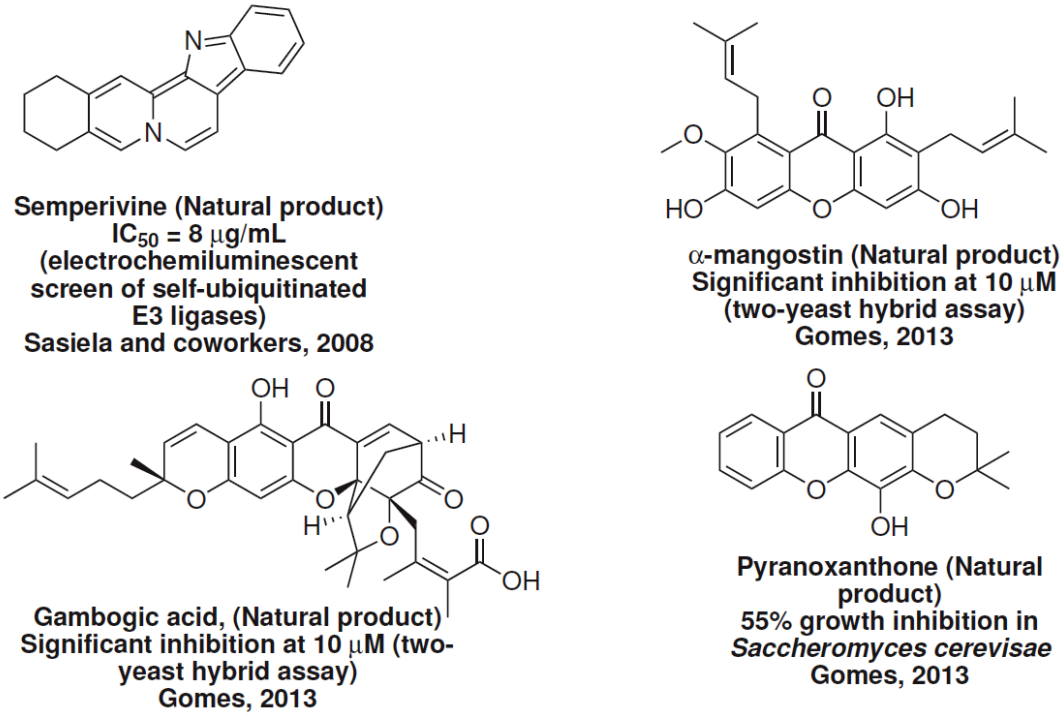


Figure 2.23. *Small molecule p53/MDM2 interaction inhibitors comprising of a poly-cyclic planar core. Taken from [353, 354].*

The existence of halogenated aromatics, particularly chlorophenyl groups, is perhaps the key factor in binding to the hydrophobic pocket. This idea was proved by Hardcastle and his coworkers using his strongest isodolinone (**Figure 2.24**). Hardcastle and colleagues demonstrated that activity was lost when methoxy or ethoxy groups were replaced for the most active isoindolinone chloro groups.

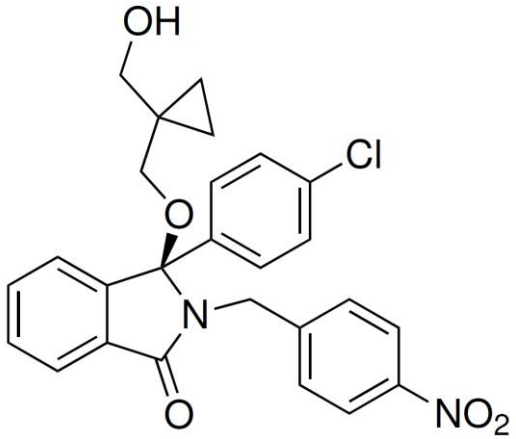


Figure 2.24. *Structure of the most potent isoindolinone. Taken form [348].*

The major four classes of p53-MDM2 interaction inhibitors' binding epitopes are

depicted in **Figure 2.25**. While further research has been done to generate hydrogen bonds with His96 as well as hydrophobic interactions with Val93 located within the MDM2(NTD) hydrophobic pocket, the literature demonstrates that the Leu26 pocket is big enough to accommodate an aromatic ring [354-356].

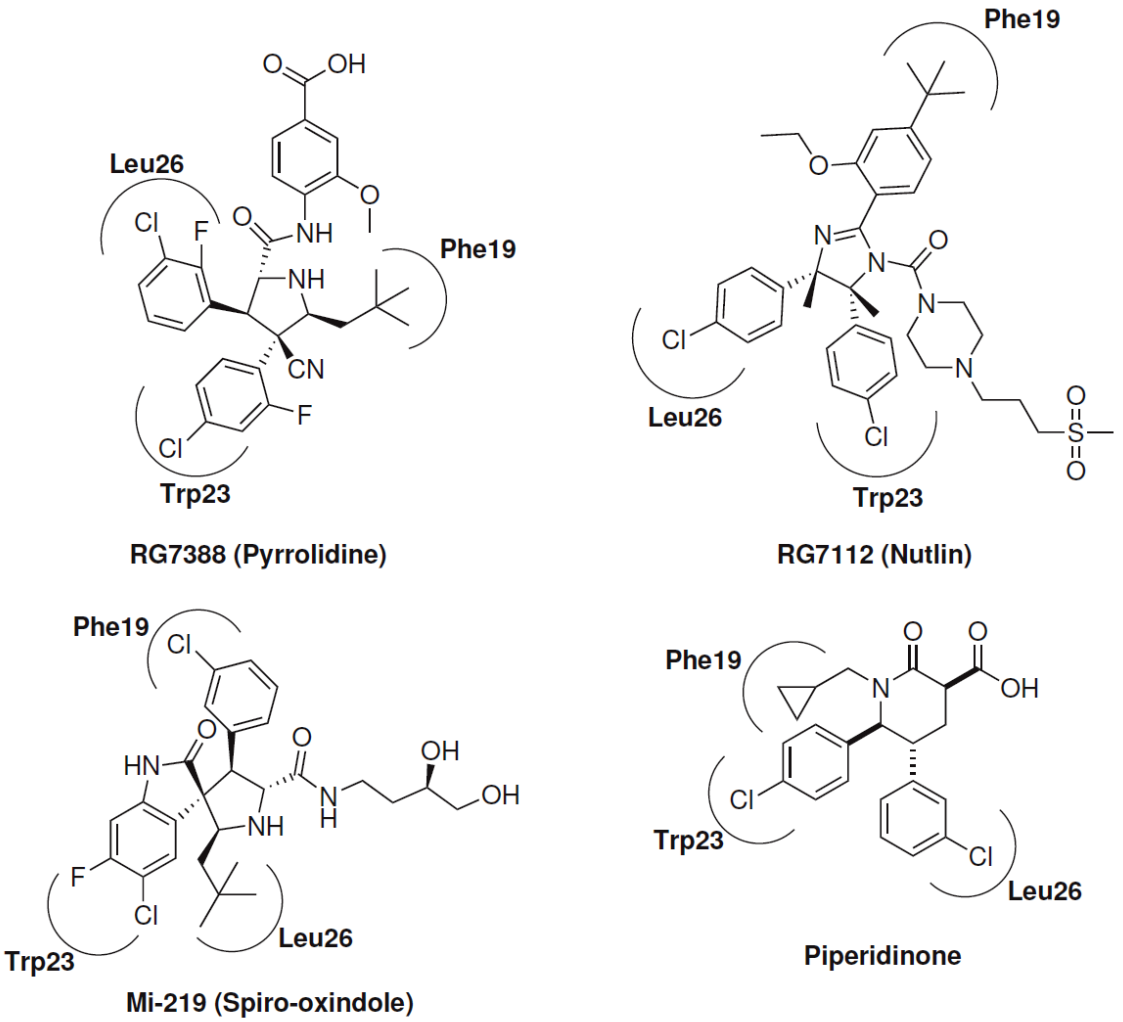


Figure 2.25. *Binding epitopes present in the top four groups of p53-MDM2 interaction inhibitors. Taken from [355, 356].*

2.9.6. Peptide-Based Inhibitors:

Numerous studies have been conducted on the usage of peptide-based inhibitors, such as stapled peptide as well as helical mimetics, in addition to studies on small molecule inhibitors. High selectivity, efficacy, and little toxicity are advantages of peptide-based inhibitors [357]. Furet *et al.*, began their initial research on peptide-based inhibitors in 2000 [358]. Initial research focused identifying the critical binding sequence by attaching monoclonal antibodies to certain p53 sites. Sequencing was done on peptides having an

acetylated N-terminus to allow entrance into cells and structural similarity towards the p53 sequence. Although it was demonstrated that the original hexapeptide TFSALW could bind to HDM2, the binding affinity was incredibly low (IC50 = 700 M). A powerful octapeptide with an IC50 of 8.95 M was found after more phage display library screening and ELISA analysis. Its name is Ac-FMDYWEGL-CONH2. Aspartic acid was replaced with R-aminoisobutyric acid (Aib), tyrosine was changed to 6-Cl- β,β-pentamenthylene-β-mercaptopropionic acid (Pmp-6-Cl) (Pmp-6-Cl) [359], as well as glycine was changed to 1-aminocyclopropanecarboxylic acid (Ac3c) [358], that led to an IC50 by ELISA of 5 nM.

Using phage display, Lu et al. continued their investigation into the application of peptide-based inhibitors in 2009. It was discovered that PMI (TSAFAEYWNLLSP) is a high affinity binder using phage display targeting biotinylated GST-tagged MDM2 as well as MDMX. According to analyses of PMI using surface plasmon resonance and isothermal titration calorimetry, its Kd was 3.3 nM (MDM2) & 8.9 nM (MDMX), respectively. In the binding experiments, the sections of the p53 amino acid sequence employed in each assay are denoted by the numbers in brackets: p53(15-29) and p53(17-28).

Inhibitors made of peptides are quickly degraded by enzymes. D-amino acids provide a different approach to solving this issue. Due to the stereospecificity of the body's enzymes, which can only handle L-amino acids, they are far more resistant to proteolytic destruction. Chemical ligation as well as mirror image phage display were used to find DPMI-α(TNWYANLEKLLR) and DPMI-γ(DWWPLAFEALLR). SpR analysis revealed Kd values of 219 nM and 53 nM for DPMI-α and DPMI-γ. However, these peptides weren't able to enter cells and as a result, neither HCT116 p53 +/+ nor HCT116 p53 -/- displayed any cytotoxicity. A cell-penetrating peptide (TAT) containing arginine was added, and both p53 +/+ and p53 -/- cells experienced nonspecific cytotoxicity. The same result was obtained when tumour necrosis was produced without p53 activation using different cell-penetrating peptides linked to p53-like sequences. However, the DPMI-alpha sequence was effective in human glioblastoma and naked mice xenographs when the peptide was encapsulated in liposomes with cyclic-RGD peptide (a fluorescent and cell-permeable peptide that demonstrates whether the liposomes release into the cell or not).

By conducting ELISA against the proteins GST-MDM2 and GST-MDMX, an

additional peptide, pDI (LTFEHAWAQLTS), also was identified as a high affinity binder [308, 360-365]. The peptide based inhibitors can be classified mainly into three categories: Helical *β*-peptide Inhibitors, Stapled Peptides (**Table 2.1**), and Peptide Helical Mimetics.

Table 2.1. *Stapled Peptides Inhibitors of MDM2. Taken from [308].*

Peptide	Sequence[a]	α helicity	K_d (nM)
WT p53	LSQETFSDLWKLLPEN	11%	410
SAH-p53-1	LSQETFSD*WKLLPE*	25%	100
SAH-p53-2	LSQE*FSDLWK*LPEN	10%	400
SAH-p53-3	LSQ*TFSDLWKLL*EN	12%	1200
SAH-p53-4	LSQETF*DLWKLL*EN	59%	0.92
SAH-p53-5	LSQETF*NLWKLL*QN	20%	0.8
SAH-p53-6	LSQQTF*NLWRLL*QN	14%	56
SAH-p53-7	QSQQTF*NLWKLL*QN	36%	50
SAH-p53-8	QSQQTF*NLWRLL*QN	85%	55

where, [a] Staple attachment positions are indicated by *.

2.9.7. Circular RNA (circRNA):

In a recent study conducted and then published by Lou *et al*., in 2020, it was found that the circular RNA (circRNAs): CDR1 directly binds to p53 protein. They also found that CDR1 binds to the DBD of p53. The central AD usually binds to p53(DBD), which is necessary for proper ubiquitination of p53 molecules. They also proved that binding of CDR1 to p53(DBD) prevents p53-MDM2 interaction completely, preventing the MDM2-mediated ubiquitination of p53, and hence stabilizes the p53 molecules [366].

Two other circRNAs have also been discovered that bind to p53 molecules directly. One is FOXO3 [367], and the other is CNM1 [368]. Both of the circRNAs bind to the CTD of p53.

2.9.8. MicroRNA (miRNA):

Recently, microRNAs (miRNAs) have also been found to bind directly to p53 molecules and inhibit the p53-MDM2 interaction [369]. Three examples are miR4749 (that binds to the DBD of p53) [370], miR21 (that binds to the DBD of p53) [371], and miR23 (that binds to the CTD of p53) [372]. **Figure 2.26.** shows a summary of the circRNAs interacting with the p53 molecule, and **Figure 2.27.** shows a summary of the miRNAs interacting with the p53 molecule.

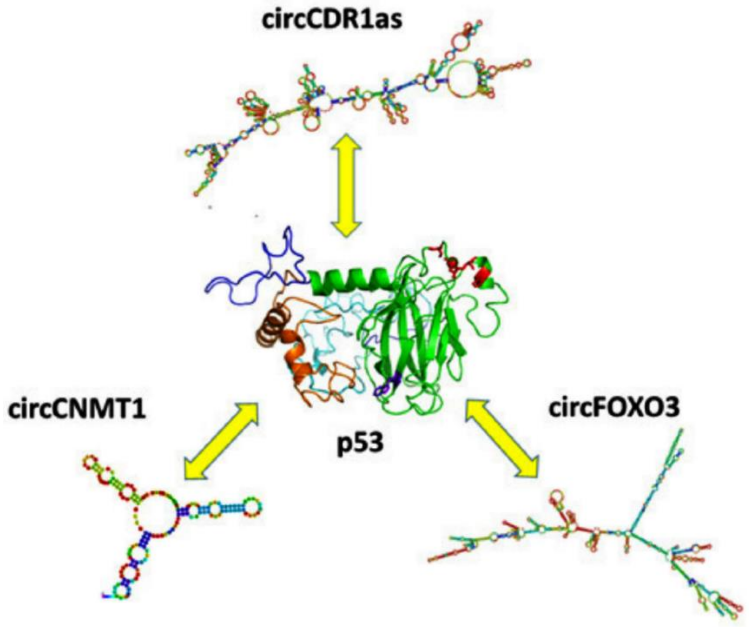


Figure 2.26. *Summary of the circRNAs interacting with the p53 molecule. Taken from [369].*

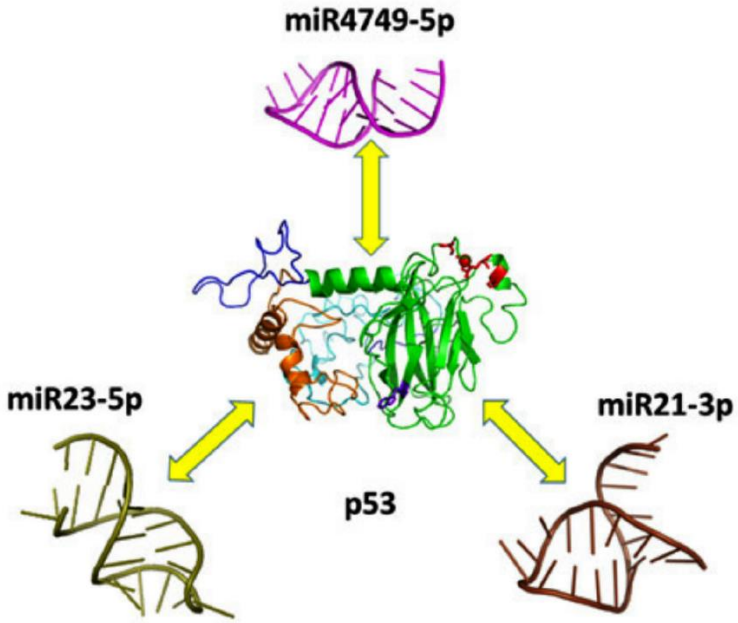


Figure 2.27. *Summary of the miRNAs interacting with the p53 molecule. Taken from [369].*

2.9.9. Small-molecule MDM2 inhibitors in clinical trials:

Table 2.2 represents the list of nine MDM2 inhibitors belonging to various types of structural patterns that have entered clinical trials [373]:

Table 2.2. *Lists of small-molecule inhibitors of MDM2 under clinical trials. Taken from [373].*

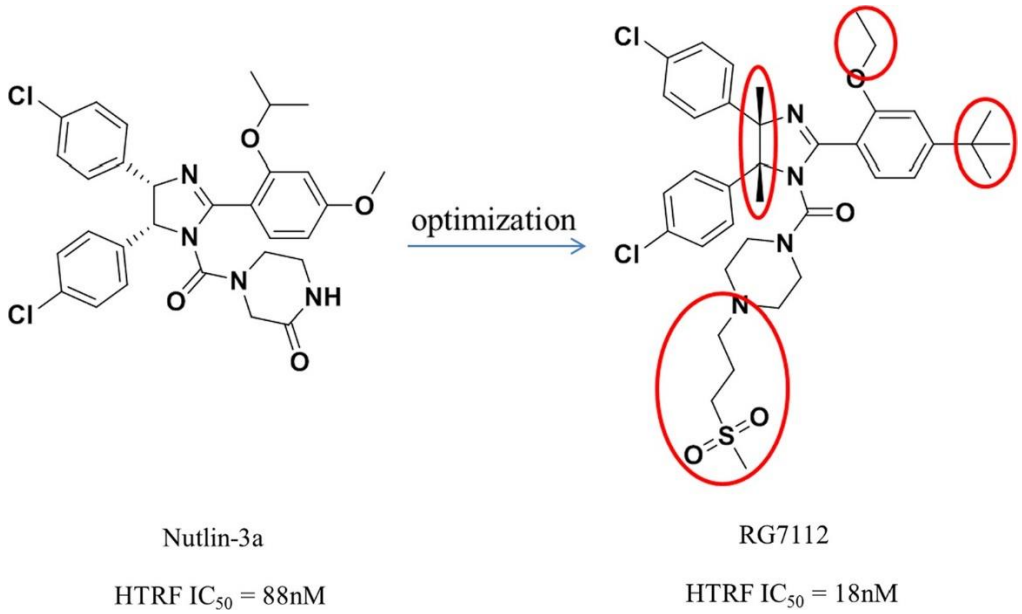
Drug	Disease	Combination	Phase	Status	Trial No
RG7112 (RO5045337)	Advanced solid tumors		I	Completed	NCT00559533
	Hematologic neoplasm		I	Completed	NCT00623870
	Solid tumors		I	Completed	NCT01164033
	Sarcoma	Doxorubicin	Ib	Completed	NCT01605526
	AML	Cytarabine	Ib	Completed	NCT01635296
	Sarcoma		I	Completed	NCT01143740
	CML, neoplasms, AML		I	Completed	NCT01677780
RG7388 (Idasanutlin)	Advanced malignancies, except leukemia		I	Completed	NCT01462175
	Solid tumors		I	Completed	NCT03362723
	AML	Idarubicin Daunorubicin Cytarabine	I/Ib	Completed	NCT01773408
	Relapsed and refractory AML	Cytarabine	III	Terminated	NCT02545283
	Non-Hodgkin's lymphoma	Obinutuzumab Rituximab	I/Ib	Terminated	NCT02624986
	Relapsed and refractory AML	venetoclax	Ib	Completed	NCT02670044
	Relapsed and refractory follicular lymphoma,relapsed and refractory diffuse large B-cell lymphoma	Obinutuzumab Venetoclax Rituximab	Ib/II	Terminated	NCT03135262
	AML	Cytarabine Daunorubicin	Ib/II	Completed	NCT03850535
	Breast cancer	Atezolizumab	I/II	Terminated	NCT03566485
	Solid tumors		I	Completed	NCT02828930
	Polycythemia vera, essential thrombocythemia	Pegasys	I	Completed	NCT02407080
	Neoplasms	Posaconazole	I	Completed	NCT01901172
	AML, acute lymphocytic leukemia, neuroblas-toma, solid tumors	Cyclophosphamide Topotecan Fludarabine Cytarabine	I/II	Recruiting	NCT04029688
	Relapsed multiple myeloma	Ixazomib Dexamethasone venetoclax	I/II	Active, not recruiting	NCT02633059
	Solid tumors	Entrectinib Alectinib Atezolizumab Ipatasertib Trastuzumab emtansine Inavolisib Belvarafenib Pralsetinib	II	Recruiting	NCT04589845
	Colorectal cancer	Regorafenib Atezolizumab Imprime PGG Bevacizumab Isatuximab Selicrelumab AB928 Genetic: LOAd703	I/II	Recruiting	NCT03555149
	Glioblastoma	APG101 Alectinib Atezolizumab Vismodegib Temsirolimus Palbociclib	I/II	Recruiting	NCT03158389

Drug	Disease	Combination	Phase	Status	Trial No
AMG232 (KRT-232)	Advanced solid tumors, multiple myeloma		I	Completed	NCT01723020
	AML	Trametinib	I	Completed	NCT02016729
	Metastatic melanoma	Trametinib Dabrafenib	Ib/IIa	Completed	NCT02110355
	AML, relapsed and refractory AML	Decitabine	I	Suspended	NCT03041688
	Soft tissue sarcoma	Radiation therapy	Ib	Recruiting	NCT03217266
	Polycythemia vera	Ruxolitinib	II	Active, not recruiting	NCT03669965
	Relapsed multiple myeloma	Carfilzomib Dexamethasone Lenalidomide	I	Recruiting	NCT03031730
	Brain cancer	Radiation therapy	I	Suspended	NCT03107780
	AML	Cytarabine Idarubicin HCI	Ib	Recruiting	NCT04190550
APG-115 (AA-115)	Advanced solid tumors, lymphomas		I	Completed	NCT02935907
	Metastatic melanomas, advanced solid tumors	Pembrolizumab	Ib/II	Recruiting	NCT03611868
	Salivary gland carcinoma	Carboplatin	I/II	Recruiting	NCT03781986
	AML, acute lymphocytic leukemia, neuroblas-toma	Azacitidine Cytarabine	Ib	Recruiting	NCT04275518
	AML	5-Azacitidine	Ib/II	Recruiting	NCT04358393
	Liposarcoma, advanced solid tumors	Toripalimab	Ib/II	Recruiting	NCT04785196
	T-prolymphocytic leukemia	APG-2575	IIa	Recruiting	NCT04496349
CGM-097	Advanced solid tumors with TP53wt		I	Completed	NCT01760525
HDM201	Liposarcoma	Ribociclib	Ib/II	Completed	NCT02343172
	Uveal melanoma	LXS196	I	Completed	NCT02601378
	Advanced solid and hematological TP53wt tumors	Ancillary treatment	I	Completed	NCT02143635
	AML		I/II	Withdrawn	NCT03760445
	Advanced/metastatic colorectal cancer	Trametinib	I	Recruiting	NCT03714958
	Myelofibrosis	Ruxolitinib	I/II	Recruiting	NCT04097821
	Colorectal cancer, nonsmall cell lung carci- noma, triple negative breast cancer, renal cellcarcinoma	Spartalizumab	I	Completed	NCT02890069
	Malignant solid tumors	Ribociclib	II	Recruiting	NCT04116541
	AML	Midostaurin	I	Recruiting	NCT04496999
	AML, myelodysplastic syndromes	MBG453	Ib	Recruiting	NCT03940352
DS-3032b (Milademetan)	Advanced solid tumors, lymphomas		I	Completed	NCT01877382
	Relapsed and refractory AML		I	Completed	NCT03671564
	AML	Quizartinib	I	Terminated	NCT03552029
	AML, myelodysplastic syndromes	5-Azacitidine	I	Terminated	NCT023199369
	AML, relapsed and refractory AML	Cytarabine Venetoclax	I/II	Completed	NCT03634228
	Myeloma		I	Terminated	NCT02579824
SAR405838	Neoplasm malignant	Pimasertib	I	Completed	NCT01985191
	Neoplasm malignant		I	Completed	NCT016636479
MK-8242	AML	Cytarabine	I	Terminated	NCT01451437
	Solid tumors		I	Terminated	NCT01463696

2.9.10. Synthesis of some of the MDM2 inhibitors:

A number of structural types of inhibitors of MDM2 have been discovered, and nine of them have entered clinical trials (RG7112, RG7388/RO6839921, MK-8242, AMG232, SAR405838, DS-3032b, HDM201, NVP-CGM097 and APG-115) (**Table 2.2**). This is because of the three unambiguous binding locations, present in the NTD of MDM2, with a diversity of scaffold types [374-377]. The lead compounds' structural modifications for six of them have been documented, along with the specifics of their discovery. RG7388's inactive prodrug is called RO6839921. In contrast to MK-8242 and DS-3032b, which lack particular literature to describe the sources of this molecule, the discovery procedure of HDM201 has been widely publicized. Therefore, here the focus is on the synthesis of the following seven compounds.

(i) RG7112:



Nutlin-3a

HTRF IC_{50} = 88nM

RG7112

HTRF IC_{50} = 18nM

Figure 2.28. *Discovery of RG7112. Taken from [373].*

***(ii)* RG7388:**

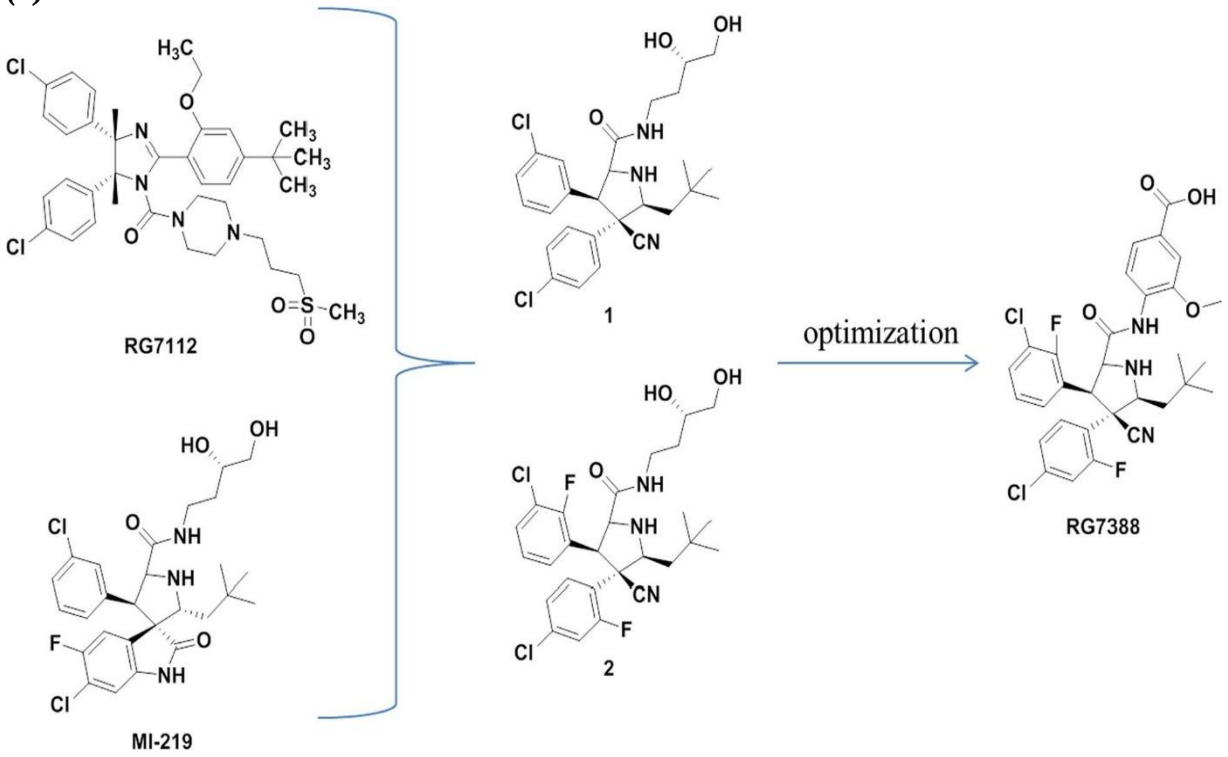


Figure 2.29. *Discovery of RG7388. Taken from [373].*

(iii) MI77301:

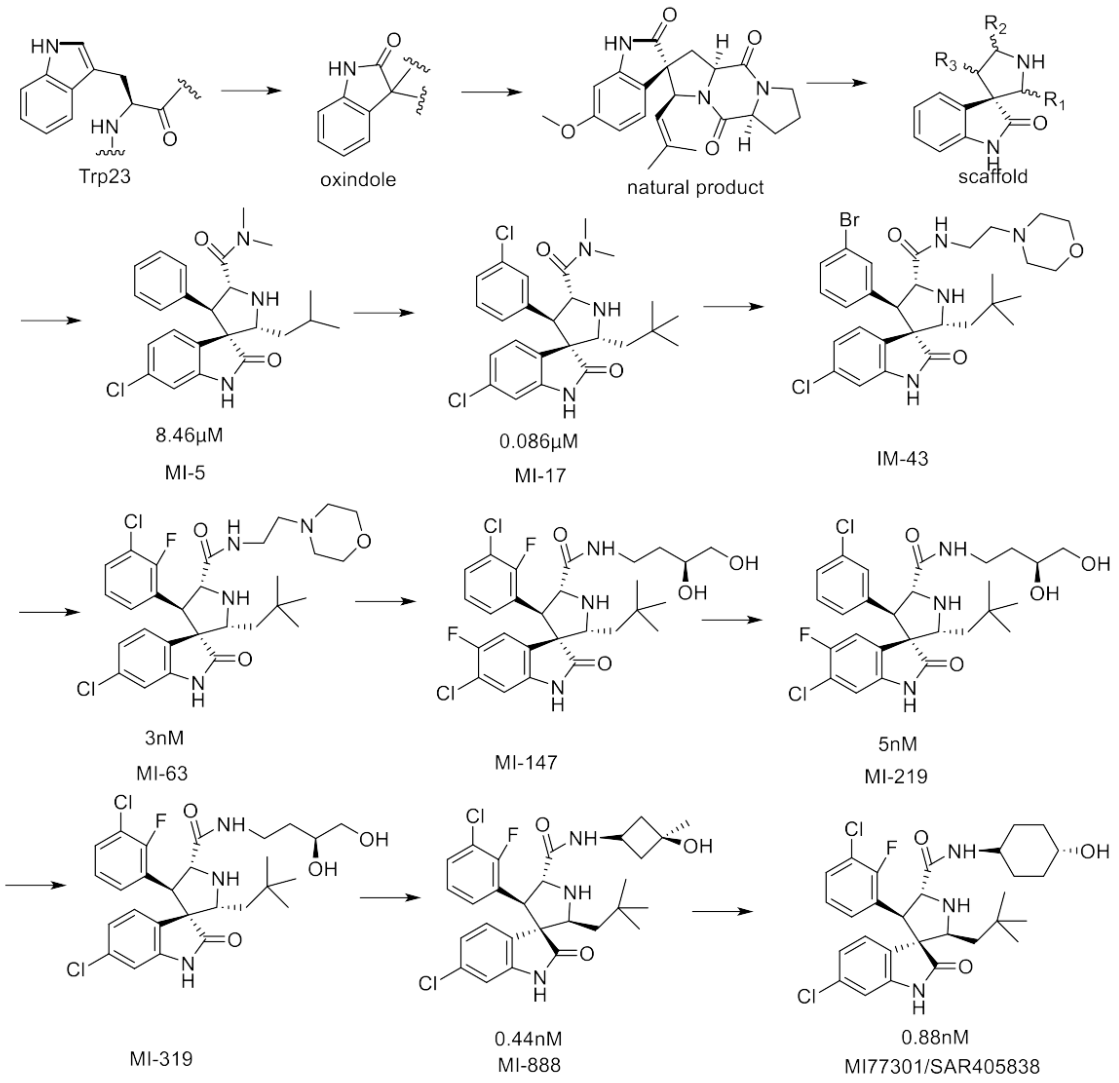


Figure 2.30. *Discovery of MI77301. Taken from [373].*

(iv) APG-115:

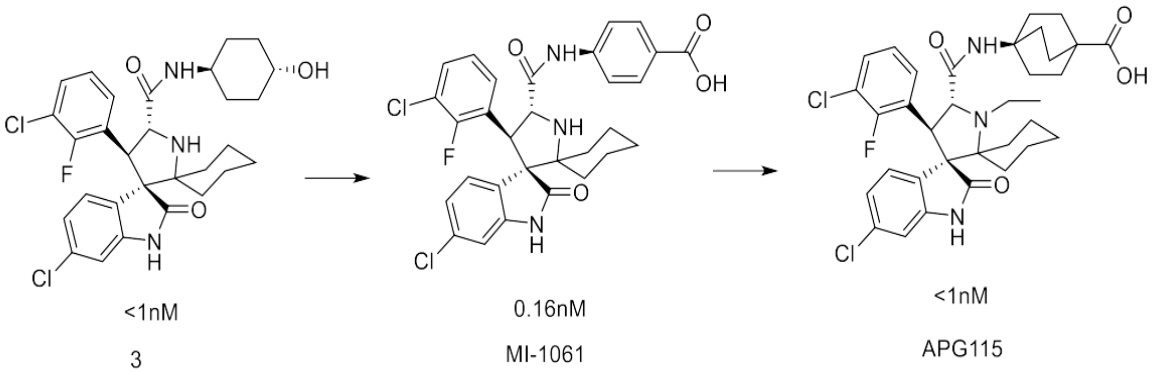


Figure 2.31. *Discovery of APG-115. Taken from [373].*

(v) AMG232:

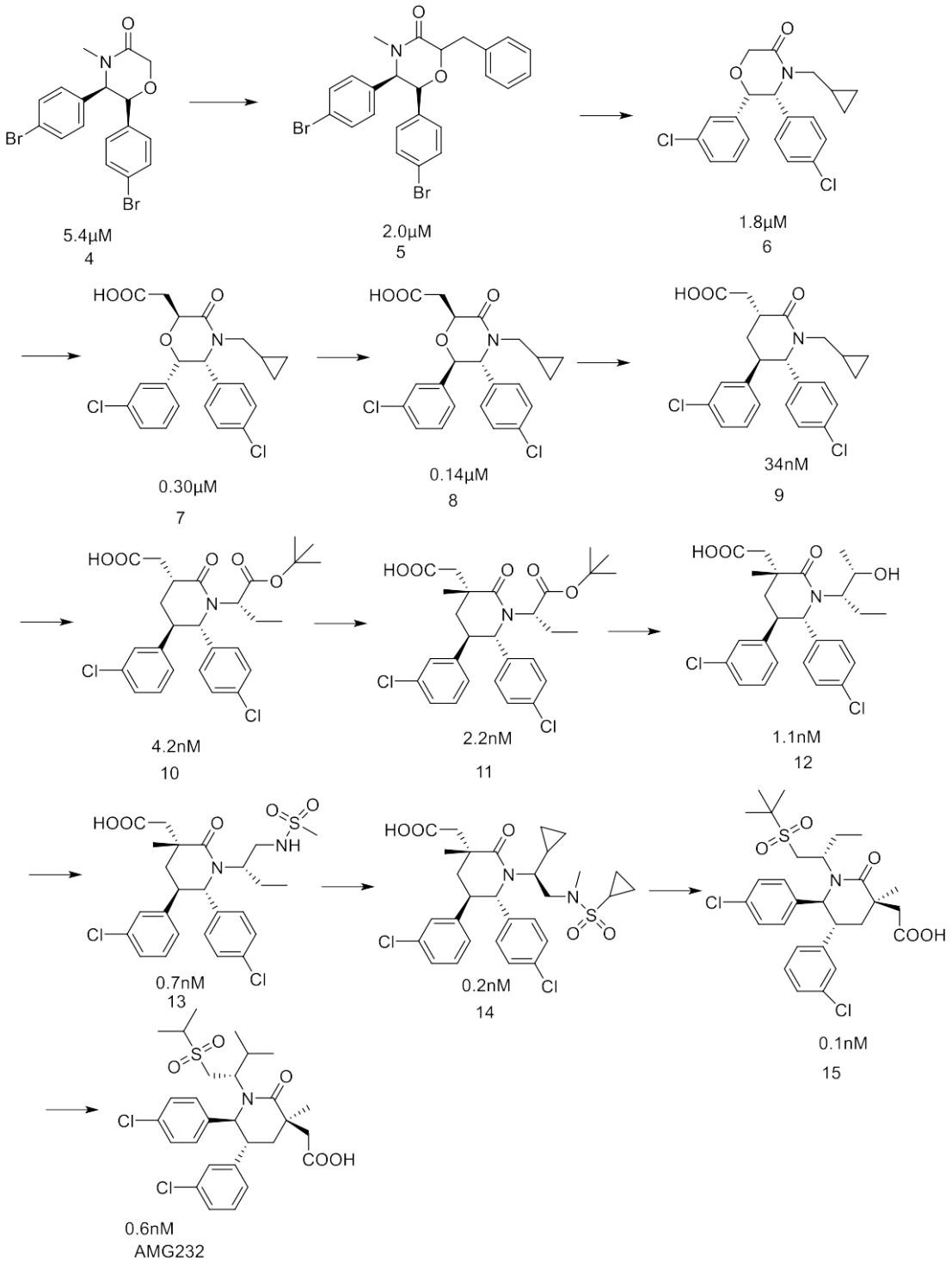


Figure 2.32. *Discovery of AMG232. Taken from [373].*

(vi) NVP-CGM097:

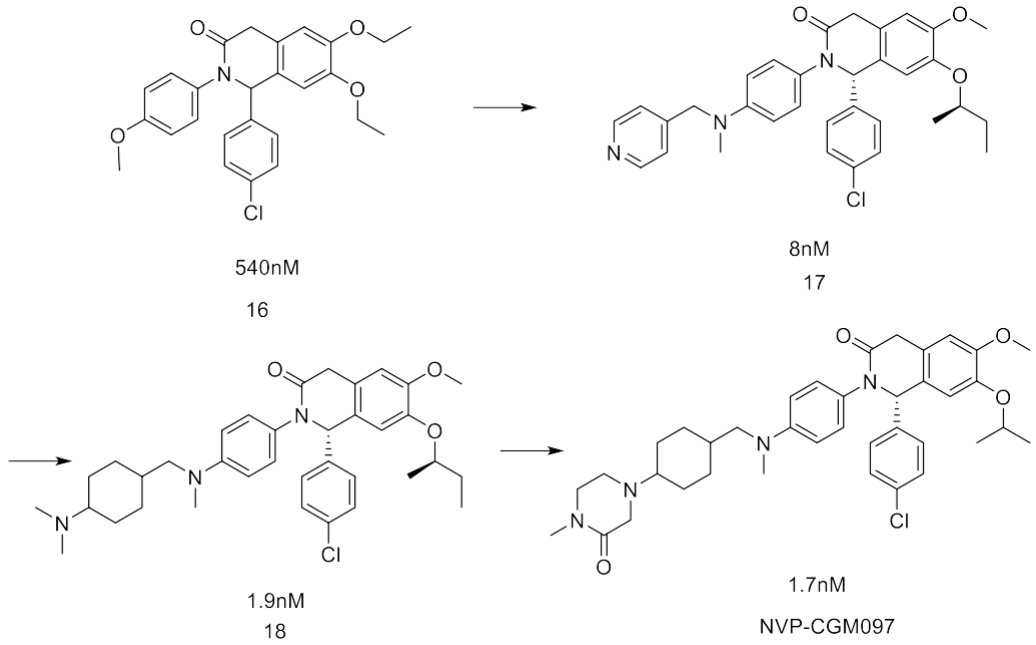


Figure 2.33. *Discovery of NVP-CGM097. Taken from [373].*

(vii) HDM201:

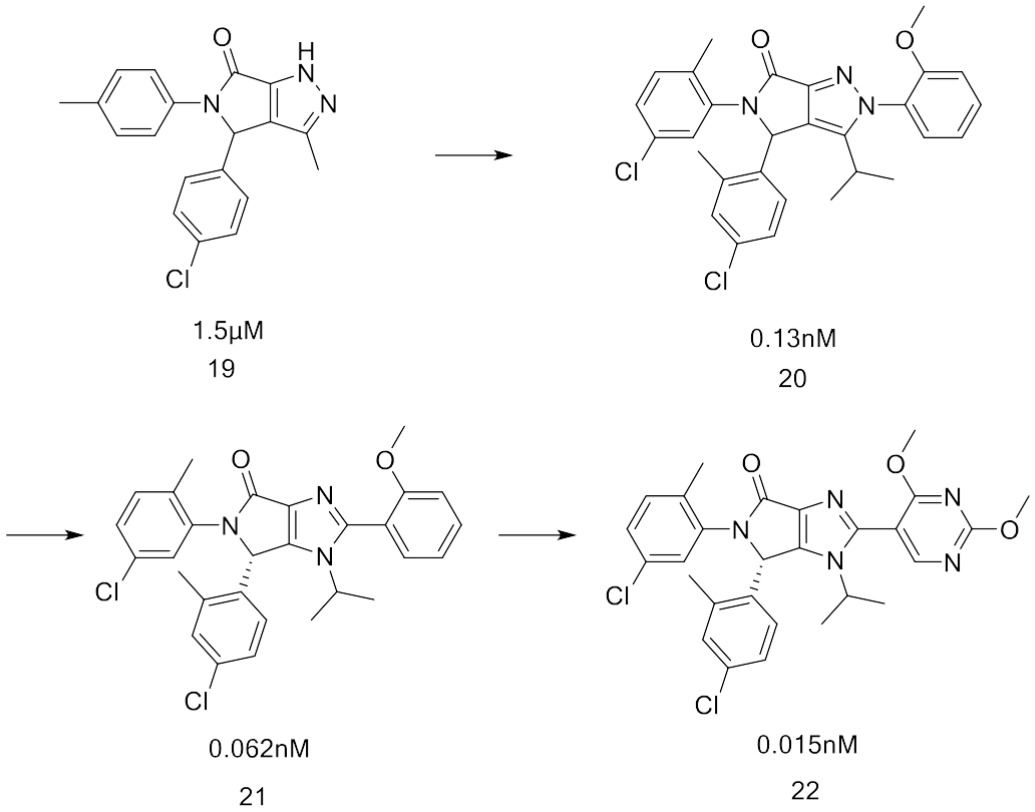


Figure 2.34. *Discovery of HDM201. Taken from [373].*

2.10. Inhibitors of Interest:

(i) Idasanutlin:

Idasanutlin (RG7388), a pyrrolidine-containing compound, has similar MDM2 inhibitory action to that of RG7112 but is more powerful and focused. Idasanutlin has demonstrated anticancer effects in preclinical studies using nude mouse models of SJSA1 osterosarcoma xenografts [378, 379]. It causes cancer cells with functioning p53 to stabilize p53 and activate p53-dependent pathways, such as apoptosis as well as cell cycle arrest [380].

In 2018, Hansen *et al.*, designed and synthesized a photoactivatable version of Idasanutlin by adding a photoremovable protecting group (PPG) to it, which acts as a photoswitch, which allows its selective activation upon irradiated by light (400 nm) (**Figure 2.35**) [381-383].

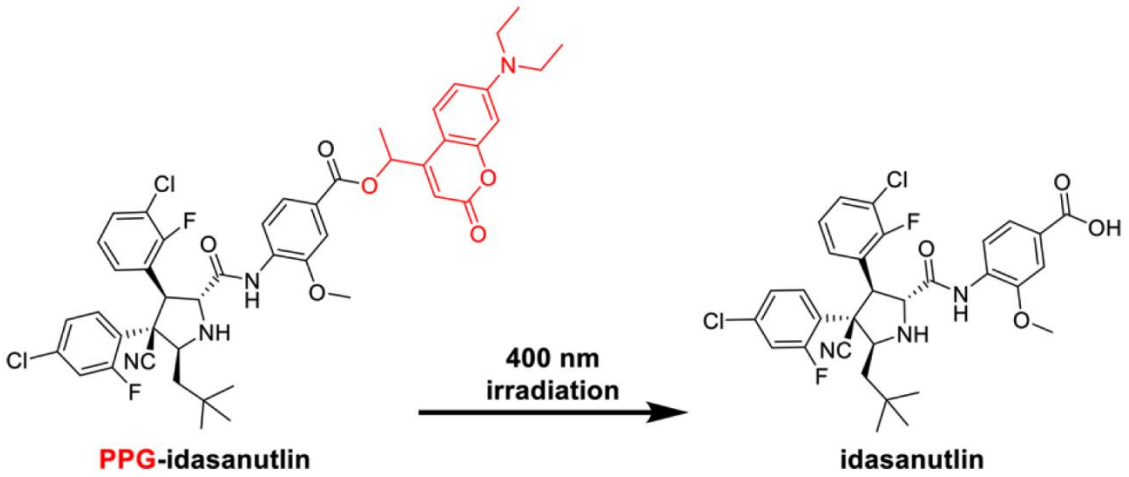


Figure 2.35. *Idasanutlin, photo-regulated by a Photoremovable Protecting Group (PPG). Taken from [381].*

(ii) XR-2:

XR-2 is chemically known as 2-(2-(2-methoxyethoxy)ethoxy)ethyl-(29S,3R,49S,59R)-5'-((4-carbamoyl-2-methoxyphenyl)carbamoyl)-6-chloro-4'-(3-chloro-2-fluorophenyl)-29-neopentyl-2-oxospiro[indoline-3,39-pyrrolidine]-19-carboxylate (**Figure 2.36**). In a recent study conducted by *Wu et al.*, in 2022, it was found that XR-2 could bind to MDM2 and inhibit the interaction between p53 and MDM2. Castration-resistant prostate cancer (CRPC) cells' ability to proliferate could be inhibited in a p53-dependent way. In addition,

XR-2 causes apoptosis and cell cycle arrest in CRPC cell lines that express wild-type p53 [384].

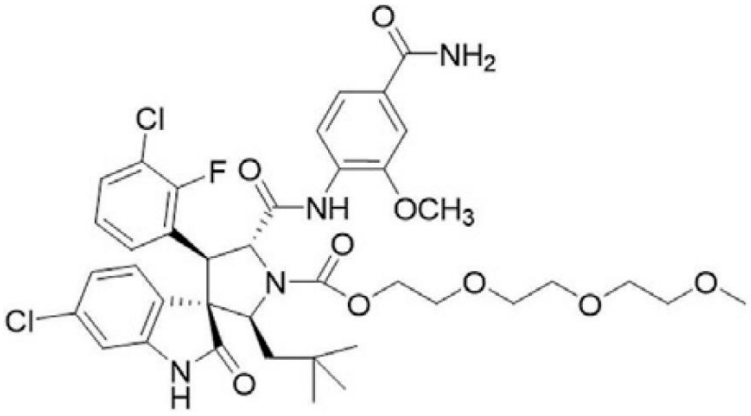


Figure 2.36. *Structure of XR-2. Taken from [384].*

(iii) Epigallocatechin gallate (EGCG):

A common beverage enjoyed by people all over the world, green tea has been shown to have inhibitory effects against a number of cancers, including breast, prostate, lung, and colon cancer. Green tea's ability to prevent cancer is mostly related to its polyphenol components, of which epigallocatechin-3-gallate (EGCG) (**Figure 2.37**) is the most significant. Between 50 and 80 percent of the catechins in green tea are made up of EGCG. Clinical trials, statistical, cell culture, animal investigations, as well as animal models, have all shown that EGCG has an anti-cancer impact. At the molecular level, EGCG has been shown to interact with cancer-related proteins with affinities of around μM, including Ras-GTPase-activating protein SH3 domain binding protein 1 (G3BP1) and glucose-regulated protein 78 (GRP78). Moreover, p53 was discovered to play a significant role in EGCG-induced cell growth arrest and apoptosis [385].

In a recent study conducted by Zhao *et al.*, in 2021, they discovered that there exists a direct interaction between p53 and EGCG, mediated by the p53(NTD). They also found that the interaction between EGCG and p53 impairs p53's connection with MDM2 and prevents p53's ubiquitination, likely stabilizing p53 for antitumor activity and supplying a structural explanation for EGCG's anticancer effect [386].

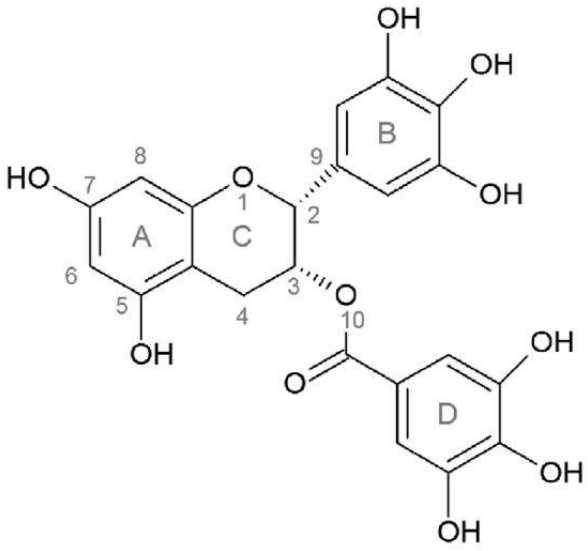


Figure 2.37. *Structure of EGCG. Taken from [385].*

(iv)Reactivating p53 and Inducing Tumor Apoptosis (RITA):

In a cell-based search for p53 reactivating substances, Issaeva *et al.*, discovered a small molecule RITA (**Figure 2.38**) in 2004. Through an allosteric change in the intrinsically disordered N-terminus of p53, RITA prevents p53/MDM2 interaction and modifies p53's binding to MDM4 in tumour cells, restoring wild-type p53. In a recent study performed by Grinkevich *et al.*, in 2022, they found that in addition to identifying important structural components in the RITA molecule and contact residues in p53 that are essential for the interaction, RITA targets p53(NTD) residues which are not present in the p53(TAD1), which interact with the N-Terminal binding cavity of MDM2. And binding of RITA to the portion of human p53 between residues 33 and 37 causes p53 to reactivate [387, 388].

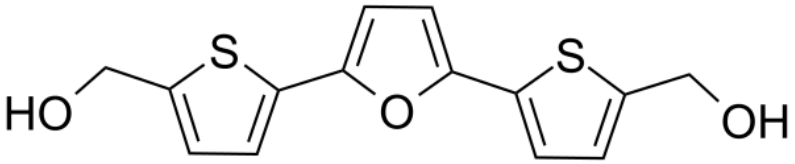


Figure 2.38. *Structure of RITA. Taken from [388].*

2.11. Scope of the Thesis:

(i) The binding and unbinding mechanism of the p53(TAD1)-MDM2(NTD) complex is not known yet. In **Chapter 4**, we have studied the dissociation(association) pathway of p53(TAD1)-MDM2(NTD) complex using Potential of Mean Force (PMF) study.

(ii) The secondary site of interaction between p53(DBD) and MDM2(AD) has been found to be crucial for the ubiquitination of p53. But the interaction profile as well as the structural characteristics of the p53(DBD)-MDM2(AD) complex has not been studied at the molecular level. In **Chapter 5**, we have studied the interaction profile as well as the structural characteristics of the p53(DBD)-MDM2(AD) complex using Molecular Dynamics (MD) simulation.

(iii) The p53(CTD) has been found to bind to the N-Terminal Lid of MDM2. But whether this interaction is causing any structural conformational change in the p53(CTD)-MDM2(NTD) complex is not known yet. In **Chapter 6**, we have studied the effect of p53(CTD) on the conformational dynamics of MDM2(NTD) when bound to the N-Terminal Lid of MDM2.

(iv) The small molecule inhibitor of MDM2: Idasanutlin was found to be a potent inhibitor of p53-MDM2 interaction. But how the structural conformation of the MDM2-Idasanutlin complex changes on adding a PPG to Idasanutlin has not been studied at the molecular level. In **Chapter 7**, we have studied the interactions at the molecular level between MDM2 and its photoactivatable inhibitor Idasanutlin, in presence as well as absence of a PPG.

(v) The binding and unbinding mechanism of the MDM2(NTD)-Idasanutlin complex is not known yet. In **Chapter 8**, we have studied the dissociation (association) pathway of MDM2(NTD)-Idasanutlin complex using PMF study.

(vi) XR-2 is a recently found small molecule inhibitor of p53-MDM2 interaction that binds to MDM2(NTD). But the structural dynamics, conformational changes and the interaction profile at the molecular level of the MDM2(NTD)-XR-2 complex has not been studied in detail yet. In **Chapter 9**, we have studied the structural dynamics, conformational changes and the interaction profile of the MDM2(NTD)-XR-2 complex using Molecular Dynamics (MD) simulation.

(vii) EGCG is a recently found small molecule inhibitor of p53-MDM2 interaction that

binds to p53(NTD). But the structural dynamics, conformational changes and the interaction profile at the molecular level of the p53(NTD)-EGCG complex has not been studied in detail yet. In **Chapter 10**, we have studied the structural dynamics, conformational changes and the interaction profile of the p53(NTD)-EGCG complex using Molecular Dynamics (MD) simulation.

(viii) The small molecule inhibitor of p53: RITA was found to be a potent inhibitor of p53-MDM2 interaction. But the structural dynamics, conformational changes and the interaction profile at the molecular level of the p53(NTD)-RITA complex has not been studied in detail yet. In **Chapter 11**, we have studied the structural dynamics, conformational changes and the interaction profile of the p53(NTD)-RITA complex using Molecular Dynamics (MD) simulation.

2.12. Main Objectives of the Thesis:

(i) Computational Investigation on the p53(TAD1)-MDM2(NTD) Interaction Using the Potential of Mean Force Study.

(ii) Computational Investigation on Molecular Interactions between the DNA Binding Domain (DBD) of p53 and Acidic Domain (AD) of MDM2.

(iii) *In silico* Investigation on the Conformational Dynamics of N-Terminal Lid of MDM2 in the presence and absence of p53 C-Terminal Domain (CTD).

(iv) Computational investigation on the molecular interactions between MDM2 and its photoactivatable inhibitor.

(v) Computational Investigation on the MDM2-Idasanutlin Interaction Using the Potential of Mean Force Method.

(vi) Computational investigation on the molecular interactions between MDM2 and its inhibitor XR-2.

(vii) *In silico* investigation on the effect of Epigallocatechin gallate (EGCG) on the interaction between p53 (NTD) and MDM2 (NTD).

(viii) Computational investigation on the molecular interactions of the p53(NTD)-RITA complex.