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Targeting MDM2-p53 Interaction for Cancer Therapy: Are We There Yet?

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Abstract

Inactivation of the tumor suppressor p53 and/or overexpression of the oncogene MDM2 frequently occur in human cancers, and are associated with poor prognosis, advanced forms of the disease, and chemoresistance. MDM2, the major negative regulator of p53, induces p53 degradation and inactivates its tumor suppressing activity. In turn, p53 regulates MDM2 expression. This MDM2-p53 negative feedback loop has been widely studied and presents an attractive target for cancer therapy, with a few of the inhibitors of this interaction already having advanced into clinical trials. Additionally, there is an increasing interest in understanding MDM2's p53-independent activities in carcinogenesis and cancer progression, which may also have implications for cancer therapy. This review aims to highlight the various roles that the MDM2-p53 interaction plays in cancer, the p53 independent oncogenic activities of MDM2 and the various strategies that may be used to target MDM2 and the MDM2-p53 interaction. We will summarize the major preclinical and clinical evidences of MDM2 inhibitors for human cancer treatment and make suggestions to further improve efficacy and safety of this interesting class of cancer therapeutics.

Keywords

Drug target; MDM2; p53; MDM2-p53 interaction; small molecule inhibitor

1. INTRODUCTION

Human malignancies remain a leading cause of death in both women and men worldwide; there are still major challenges in understanding cancer etiology, pathogenesis, treatment and prevention. It is widely accepted that carcinogenesis and cancer progression are complex and

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CONFLICT OF INTEREST

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multi-factorial processes with the emergence of cells possessing features such as sustained proliferation, evasion of growth suppressors, resistance to cell death and replicative mortality, increased angiogenesis, and activation of invasion and metastasis [1]. The cellular changes at genetic and epigenetic levels during carcinogenesis and cancer progression have been the center of cancer research for several decades. These genetic changes involve deletions, mutations, and rearrangements in a set of specific genes (typically oncogenes and tumor suppressors) that may affect their protein products. Oncogenes generally encode cell proliferation and apoptosis-controlling proteins; and their amplification or activation leads to development of the malignant phenotype [2–4]. In contrast, tumor suppressor genes activate anti-proliferative and pro-apoptotic pathways, thus halting cell cycle progression; and inactivation or loss of tumor suppressor genes also leads to the malignant phenotype.

Since its discovery more than thirty years ago, p53 has become the most widely studied tumor suppressor gene [5]. This short-lived protein has multiple functions in normal cells and is essential in preventing cancer onset and development. There are several outstanding reviews which document the functions and regulations of p53 [6–17]. As a transcription factor, p53 is responsible for maintaining genomic integrity and is activated in response to diverse stress signals, leading to DNA repair, cell cycle arrest, apoptosis, and senescence [9, 16]. The importance of p53 in cancer biology and etiology can be gauged by the fact that this protein is deleted or mutated in more than half of all human malignancies [15]; with the loss of its wild-type function negatively impacting prognosis and response to cancer therapy. These observations render the restoration of p53 functions in the cancerous cell as an attractive anticancer approach. However, after more than two-decades of intensive preclinical and clinical research, activation of p53, including p53 gene therapy, has not been proven to be a practical clinical approach to cancer therapy. One of the reasons for ineffective consequence of p53 therapy may be the inactivation of endogenous and exogenous p53 by its negative regulators such as MDM2 (murine double minute 2) [18].

MDM2 is a cellular phosphoprotein that forms a complex with p53 [18–22]. MDM2 has E3 ubiquitin ligase activity which plays a critical role in the degradation of p53 [23]. MDM2 is overexpressed in many human malignancies, indicating that it is a major mechanism utilized by cancer cells to escape p53 surveillance [24]. Indeed, the MDM2 gene is frequently amplified in most cancers; and alterations in the p53 and MDM2 genes and/or their protein products, separately or concomitantly, lead to poor prognosis and treatment failure in cancer patients [25, 26]. Therefore, considering the obstacles faced in p53-based cancer therapy [27–31], we [32, 33] and others [34] have proposed that MDM2 is a valuable molecular target for cancer therapy. In the last 15 years or so, the MDM2-p53 interaction has become a focal point of research in both academia and the industry to develop better targeted cancer therapeutics [28, 30, 33, 34]. In this review, we aim to critically evaluate the rationale and status of targeting MDM2 and advances made in the development of MDM2 inhibitors as novel cancer therapeutics and what the future holds for this class of novel cancer therapeutics.

2. MDM2, p53, AND CANCER

2.1. MDM2 As A Negative Regulator of p53

The p53 protein was first discovered in 1979 as a cellular partner of simian virus 40 large T-antigen; it was found to migrate as a 53-kD band in gel electrophoresis, thus imparting its name [6]. Subsequent studies showed that p53 maintains genomic integrity of the cell, prevents malignant transformation, induces cell cycle arrest and apoptosis in response to stress signals [16]. Disruption of this gene facilitates the oncogenic process leading to increased cancer risk [12, 13]. Indeed, p53 mutations are seen in more than 50% of all human cancers, being highly prevalent in both solid cancers as well as in leukemias and lymphomas; thus providing an insight into the genetic machinery that controls the carcinogenic process [15]. With the discovery of MDM2, a more complete picture of the p53 pathway emerged a decade after p53 discovery.

The *mdm2* gene is located on chromosome 12q13–14 and encodes for a 491 amino acid protein [19]. Under normal conditions, MDM2 is expressed in the nucleus, but it translocates to the cytoplasm to mediate the degradation of its substrates. The first indication that MDM2 acts as an oncogene came from the observation that it could cause spontaneous transformation of an immortalized murine cell line, BALB/c 3T3 and that *mdm2* overexpression rendered rodent fibroblasts tumorigenic in nude mice [20]. The direct evidence for the crucial role of MDM2 in negatively regulating p53 comes from the fact that targeted deletion of the *mdm2* gene in mice is embryonic lethal due to p53-mediated apoptosis, whereas simultaneous deletion of the *TP53* gene rescues the lethality [35, 36].

The auto-regulatory feedback loop between MDM2 and p53 may be the most important finding in this field. As illustrated in (Fig. 1), the MDM2 protein binds to the N-terminal transactivation domain of p53, inhibiting its transcriptional activity [37–39], promotes p53 export out of the nucleus [40, 41], prevents p53 from interacting with transcriptional co-activators [42], and targets p53 for ubiquitination and degradation by the proteasome [41, 43, 44]. On the other hand, p53 regulates the transcription of the *mdm2* gene, thus forming a negative feedback loop that maintains cellular p53 at low level [45]. The MDM2-p53 interaction was initially thought to result from the mutual binding of MDM2 and p53 via their N-terminal domains, but recently the p53 C-terminus has also been shown to be involved in the MDM2-p53 interaction [46, 47]. The mechanisms responsible for the MDM2-induced p53 degradation have been intensively investigated. MDM2 serves as an E3 ubiquitin ligase via its C-terminal RING finger domain and ubiquitinates p53 at several lysine residues [23]. While low levels of MDM2 induce p53 mono ubiquitination and nuclear export, high levels of MDM2 cause polyubiquitination and degradation of p53 in the nucleus [48, 49]. MDM2 can also auto ubiquitinate itself, leading to self-degradation [23].

Several studies demonstrate that the MDM2-p53 interaction is more complicated than previously thought. There have now been increasing evidences indicating that the MDM2-p53 feedback loop is regulated or modulated by a host of factors (as summarized in Table 1), including ribosomal proteins [50–63], proteasome activator (PA28γ) [64], polycomb protein RNF2 [65, 66] and RYBP [67], the ARF tumor suppressor [68–71], DNA damage response elements (14-3-3-σ, PML, and JMY) [72–74], and MDMX [75–77], among others. These

molecules may bind to MDM2 and/or p53, altering their conformation, modification, interaction, and modulating the E3 ligase activity of MDM2 towards itself and p53. This complex interplay of cellular players adds multiple layers of regulation to the MDM2-p53 loop. The MDMX-MDM2-p53 interaction can serve as an excellent example of the complexity of p53 signaling pathways. MDMX (also referred as MDM4) possesses a high degree of homology to MDM2, especially in its N-terminal p53 binding domain [75, 77]. Similar to MDM2, it possesses, at its N-terminus, a p53 binding domain and at its C-terminus, a RING finger domain through which it forms hetero dimers with MDM2. MDMX is highly and/or abnormally expressed in human tumors and seems to promote carcinogenesis [77–79]. MDM2 and MDMX perform non-redundant functions to inactivate p53 during embryogenesis and throughout development [79–81]. MDM2 and MDMX are proposed to work independently to inhibit p53 activity, or alternatively, MDM2 and MDMX may form a complex that is more effective at inhibiting p53 transactivation or enhancing p53 turnover. MDM2 and MDMX are also postulated to form heterooligomers through their RING domains, whereby MDMX increases MDM2's E3 ligase activity (as shown in Fig. 1). MDM2 can also directly ubiquitinate and degrade MDMX in response to DNA-damage stimuli [82–85].

2.2. MDM2 As An Oncogene, Independent of p53

In addition to its role in the MDM2-p53 loop, recent findings indicate that MDM2 also has critical roles in carcinogenesis, independent of p53. For example, bladder cancer and sarcoma patients with increased MDM2 levels and mutant p53 present a worse prognosis than those with a single defect [26]. In animal studies, 33% lymphomas that arise in *Eμ*-myc transgenic mice with deleted or mutated p53 show overexpression of MDM2 [86, 118]. Studies with genetically engineered mice support the hypothesis that MDM2 has p53-independent functions that contribute to carcinogenesis. $p53^{-/-}mdm2^{+/-}$mice and $p53^{-/-}$mice expressing an MDM2 trans-gene develop more sarcomas than $p53^{-/-}$mice [119]. Many investigations have now identified numerous MDM2 interacting molecules that are involved in cell proliferation, apoptosis, and tumor invasion and metastasis [120–123].

The representative p53-indpendent activities of MDM2 in carcinogenesis and cancer progression are depicted in (Fig. 2). In addition to the inhibition of p53, MDM2 overexpression in cancer cells influences various cellular signaling pathways and contributes to tumor development and progression. MDM2 binds and regulates multiple proteins that are involved in cell cycle control, apoptosis, DNA repair, cell migration and invasion, angiogenesis, and chemo resistance. These signaling pathways work together to ensure the integrity of genetic information, and as such MDM2 may be considered as a central meeting point regulating genome stability, cell transformation and ultimately tumor formation and progression.

The MDM2's p53-independent activities have been intensively investigated, but remain not fully understood. MDM2 overexpression may contribute to genomic instability seen in cancer cells. For instance, MDM2 contributes to chromosomal breakage and failure of genomic integrity by direct interaction with proteins such as dihydrofolate reductase (DHFR), a key enzyme in folate metabolism [124]. MDM2 also plays an important role in

DNA repair mechanisms by binding directly to the DNA repair protein Nbs1 [125]. MDM2 overexpression inhibits p21, the cyclin-dependent kinase inhibitor [126]. MDM2 directly interacts with p21, inducing its conformational change and ubiquitin-independent proteasomal degradation [126–128]. This may at least, in part, explain the MDM2's p53-independent activity in promoting cell proliferation.

Furthermore, MDM2 overexpression drives cell cycle progression in the S-phase by binding with retinoblastoma (Rb) protein and E2F-1, members of the Rb-E2F tumor suppressor pathway [129–131]. MDM2 stabilizes E2F-1, inducing E2F-1 transcriptional activation and ultimately cell cycle progression, while inhibiting the ability of Rb to induce G1 arrest. MDM2 regulates Rb protein stability through both its ubiquitin ligase activity as well as by directly interacting with the C8 subunit of the 20S proteasome, thereby facilitating its destruction [129]. Interestingly, MDM2-dependent degradation of Rb increases DNA methyltransferase DNMT3A activity which is associated with silencing of tumor suppressor genes [132]. MDM2 overexpression also inhibits apoptosis induced by co-overexpression of E2F-1 and DP-1(E2F dimerization partner 1), in osteosarcoma cells lacking p53 and Rb [133].

Other evidence supporting MDM2's anti-apoptotic roles includes its interaction with the pro-apoptotic proteins p73 and FOXO3a [134–136]. MDM2 mediates the NED Dylation of the pro-apoptotic form of p73-TAp73, promoting its cytoplasmic translocation and mitigating its p53-transactivational activity [134]. MDM2 negatively regulates the stability of FOXO3a by mediating its ubiquitination and degradation [135]. FOXO3a is also responsible for regulating p27, which leads to MDM2-mediated control of cell cycle progression via oncogenic growth factor or Ras activation [137]. In addition, MDM2 positively regulates XIAP (X-linked inhibitor of apoptosis protein) by enhancing its translation and subsequent expression [138].

Apart from its roles in cell cycle and apoptosis control, MDM2 also regulates angiogenesis by increasing expression levels of transcription factors such as hypoxia inducible factor (HIF-1α), subsequently increasing the expression of vascular endothelial growth factor (VEGF) [139–141]. In addition, MDM2 has been identified in the epithelial-tomesenchymal transition process via its capability to target E-cadherin for proteasomal degradation [142], implicating its role in cancer metastasis. MDM2 also interacts with MTBP (MDM2 binding protein), a metastasis suppressor, and over-expression of MDM2 can reverse MTBP's function, indicating a potential role for MDM2 in cancer metastasis [143]. MDM2 binds to and stabilizes Slug mRNA, increasing its expression and cellular invasiveness in p53-null cells [144]. MDM2 contributes to chemo resistance by degrading HUWE-1 which ubiquitinates the antiapoptotic protein Mcl-1 [145]. Further, MDM2 enhances p65-mediated transcriptional activity of NFκB in ALL cells, leading to resistance towards doxorubicin-induced apoptosis [146].

In brief, as discussed above, the p53-independent activities of MDM2 are important because more than half of all human cancers exhibit mutated and non-functional forms of p53. If MDM2 is to be considered as a valid and viable therapeutic target, the oncogenic activity of

MDM2, independent of its role as a negative regulator of p53, must be understood in greater detail [147].

2.3. Clinical Relevance of Targeting MDM2 for Cancer Therapy and Prevention

Multiple lines of preclinical and clinical evidences suggest the existence of a direct relationship between MDM2 and cancer development and progression [26] (Table 2). MDM2 overexpression has been observed in many cancer types [148, 151, 156, 165, 179], with the overall frequency of gene amplification being 7% and the highest frequency being observed in soft tissue tumors [24]. These tumors often show a higher incidence of MDM2 amplification than p53 mutation. Overall, a negative correlation is seen between occurrence of p53 mutations and MDM2 amplification, thus supporting the hypothesis that MDM2 negatively regulates p53. In many cases, a higher frequency of MDM2 protein overexpression is observed in tumors with wild-type p53.

A well-documented mechanism for MDM2 overexpression is the single nucleotide polymorphism at nucleotide 309 (SNP309) in its promoter [180, 181]. The clinical significance of SNP309 remains to be clarified. Although the MDM2 SNP309 variant increases MDM2 expression and is associated with tumor formation [182–184], the SNP is not associated with increased risk or a prognostic factor in certain cancers such as glioblastoma [185]. MDM2 overexpression induced by TGF-β is observed in late stage metastatic breast cancer and correlates with poor prognosis [186]. Other mechanisms apart from gene amplification, such as increased transcription and translation also contribute to MDM2 over-expression [109]. High levels of both MDM2 and MDMX proteins without increased copy number have been observed in some cancer types, such as melanoma, Ewing's sarcoma, colon carcinoma, and retinoblastoma [109].

In breast cancer cell lines, it appears that MDM2 expression is linked to the ER (estrogen receptor) status of the cells [187]. Higher MDM2 expression is observed in ER^+ cells. Interestingly, estrogen-mediated increase in cell proliferation correlates with increased MDM2 levels, without a concomitant decrease in the p53 protein level [188]. These findings may have an implication in breast cancer diagnosis and treatment.

MDM2 is also linked to chemo resistance in several cancer types. Overexpression of MDM2 is an important event in regulating sensitivity to chemotherapy in childhood acute lymphoblastic leukemia (ALL) [189]. Functional inactivation of p53 by mutation or other mechanisms is common in relapsed neuroblastoma and associated with MDM2 gene amplification, contributing to chemo resistance [190]. Several studies have reported that either increased MDM2 activity or the *SNP309*G allele is associated with cellular resistance to topoisomerase II inhibitors [191].

3. STRATEGIES TO TARGET MDM2 FOR CANCER THERAPY

As aforementioned, in most situations, MDM2 overexpression is oncogenic and is associated with late stage disease, resistance to chemotherapy and radiotherapy, and poor prognosis, making it a valid and valuable target for developing cancer therapeutics. We and others first tested the value of MDM2 as a cancer target in preclinical models using antisense

oligonucleotides (ASOs) and RNA interference (siRNA) to inhibit MDM2 expression. The results have validated that MDM2 inhibition in cells and mouse models of human cancer is indeed a viable approach to suppress tumor cell growth *in vitro* and *in vivo* [33, 192–197]. To date, investigations on the MDM2-p53 interaction has provided a basis for the design of novel small molecule therapeutics aiming at inhibiting MDM2 activity and eventually reactivating the wild-type p53 function [198–202]. *In vivo* studies have shown restoration of wild-type p53 function can lead to the suppression of soft tissue sarcoma, lymphomas, and liver cancers [202].

Studies on the crystal structure of the N-terminal domain of MDM2 reveal a deep hydrophobic cleft on which the p53 peptide binds as an amphipathic alpha helix, a structure also present in other pro-apoptotic proteins such as Bax [203]. In fact, three amino acids in p53 (Phe19, Trp23 and Leu26) are essential for the binding between p53 and MDM2, and they are inserted into the deep hydrophobic depression on the surface of the MDM2 molecule [203]. Various approaches followed in the discovery of novel small molecule inhibitors of MDM2-p53interaction have been recently developed. The major strategies that can be used to target MDM2 includes: (i) blocking MDM2 expression; (ii) inhibiting the E3 ubiquitin ligase activity of MDM2; (iii) inhibiting the MDM2-p53 interaction; and (iv) targeting the protein-protein complex in relation to MDM2 interactive proteins.

Cutting-edge technologies in structure biology, bioinformatics, computer-aided drug design, and high throughput screening, have led to the identification of numerous molecules belonging to different classes of chemical structures that have significant MDM2 inhibitory effects. The list of natural compounds and synthetic small molecules capable of selectively inhibiting MDM2 and/or the MDM2-p53 interaction keeps increasing rapidly. In the following sections, we will focus primarily on specific and selected molecules that have (or might have) a strong association at the clinical level.

3.1. Inhibiting MDM2 Expression

Several early studies by our group [32, 192–194, 197] and others [204, 205] using antisense oligonucleotides to inhibit MDM2 expression have established the proof of principle for this approach in cell and mouse models of human cancer. MDM2 down-regulation in various cancer cells results in p53 stabilization and activation of the p53 pathway in cancer cells *in vitro*, as well as in tumor xenografts in nude mice. Interestingly, not only p53 wild-type cells, but also cells that express mutant p53, respond to MDM2 inhibition [32, 197]. It has been suggested that the p53-independent stabilization of the cyclin-dependent kinase inhibitor, p21, due to MDM2 down-regulation might contribute to the antitumor activity of MDM2 antisense oligonucleotides and MDM2 inhibitors in these mutant cell lines [200]. MDM2 inhibition also results in chemosensitization and radiosensitization of cancer cells in various *in vivo* and *in vitro* models [33, 206].

Other gene targeting strategies include the use of ribozymes and RNA interference techniques. MDM2 ribozymes induce cell growth arrest and increase apoptosis *in vitro* [207]. RNAi-mediated MDM2 knockdown leads to similar effects on proliferation, survival, apoptosis, and cell cycle progression [208]. Also, aptamers targeting MDM2 provide another

line of attack [209]. More recently, several groups have used novel approaches to deliver MDM2-siRNA for cancer therapy [210, 211].

In the past few years, we and others have investigated the value of MDM2 as a molecular target for natural product chemotherapeutic and chemopreventive agents [212–214]. Several well-known chemopreventive agents such as curcumin, genistein, and ginsenosides have been demonstrated to down-regulate MDM2 oncoprotein expression. These compounds were able to influence MDM2 levels in tumors with wild-type p53 as well as those with non-functional or mutant p53. For a comprehensive discussion on natural product MDM2 inhibitors, readers are directed towards a recent review [215].

3.2. Inhibiting MDM2-p53 Binding

MDM2 will be unable to down-regulate p53 if it is prevented from interacting with p53 protein. Therefore, inhibition of MDM2-p53 binding appears to be a desirable strategy for p53 stabilization and activation. However, targeting protein-protein interactions by small molecules is challenging. Generally, protein-protein interactions are difficult to interrupt by low molecular weight chemical entities as they involve large, flat interfaces with buried surfaces [216]. However, in the case of the MDM2-p53 interaction, it has been demonstrated that a limited number of amino acid residues (namely, Phe19, Trp23 and Leu26 in the N-terminal domain of p53) are crucial for the binding of the two proteins [203]. There exists a narrow, continuous, hydrophobic pocket on the MDM2 protein for p53 binding with the aforementioned amino acids critically regulating the interaction. Therefore, it is reasonable to expect that a synthetic molecule with three hydrophobic groups projecting into this pocket would essentially mimic the key amino acid residues of p53, thus competitively inhibiting the MDM2-p53 interaction. The nutlin class of MDM2 inhibitors developed by Roche Company from the screening of a large combinatorial library is based on this principle [198–200]. These inhibitors possess the capability to displace p53 from MDM2 *in vitro* with nanomolar potency (IC_{50} = 90 nM for nutlin-3a). Crystallographic studies demonstrate that nutlins bind to the p53-interacting domain of MDM2 in a way that closely resembles the molecular interactions of the crucial amino acid residues from p53 [198].

3.3 Modulating E3 Ubiquitin Ligase Activity of MDM2

The ubiquitin ligase activity of MDM2 is crucial for its negative regulation on p53 protein stability, indicating that this may be a valid drug target. Recently, small molecule inhibitors have been identified that specifically target the E3 ligase activity of MDM2. These compounds inhibit the ubiquitination of p53 *in vitro*, with IC_{50} values in the micromolar range [217–221]. They are able to activate p53 signaling, inducing apoptosis in a p53-dependent manner. The first report on MDM2 E3 ligase inhibitors dates back to 2002 with the arylsulfonamide, bisarylurea, and acylimidazolone compounds being discovered in an MDM2-mediated p53 ubiquitination chemical library screen [218]. They do not affect the physical interaction between MDM2 and p53, probably inhibiting MDM2 in an allosteric fashion by blocking a structural rearrangement of MDM2 necessary for p53 ubiquitination [218]. Selective inhibition of p53 ubiquitination is therapeutically desirable as opposed to total loss of MDM2 ubiquitin ligase activity (which will also inhibit MDM2 autoubiquitination, leading to enhanced MDM2 levels). In addition, several natural product

MDM2 inhibitors have been shown to induce MDM2 autoubiquitination and degradation, although the exact mechanism of action remains to be revealed [215].

4. SMALL MOLECULE MDM2 INHIBITORS

4.1. SMIs Disrupting MDM2-p53 Interaction

Advances in the understanding of the conformation and structure of MDM2 have sparked the rational design of small molecule MDM2 inhibitors. Selective binding of another molecule to the surface of MDM2 can prevent the MDM2-p53 interaction, leading to the accumulation of p53 in the nucleus, and the subsequent induction of pro-apoptotic and anti-proliferative p53 functions. Most new chemical entities developed as MDM2 inhibitors are based on this principle [222]. High throughput screening methods, combined with combinatorial library synthesis, have led to the development of a number of small molecule MDM2 inhibitors with diverse chemical structures. These include chalcones, piperazine-4-phenyl derivatives, nutlins (cis-imidazolines), spiro-oxindoles, sulfonamides, benzodiazepinediones, isoindolinones, and terphenyls among others [221, 223–225]. Their corresponding binding domains on MDM2 and/or p53 proteins are shown in (Fig. 3).

4.1.1. SMIs Binding to MDM2—The imidazoline derivatives (better known as Nutlins) are one of the first small molecules for selective inhibition of MDM2-p53 binding [198]. They act by mimicking the three critical amino acid residues (Phe19, Trp23, Leu26) within the hydrophobic pocket of MDM2. Crystallographic studies demonstrate that the chlorophenyl moieties perfectly fit deeply into the Leu26 and Trp23 pockets while the isopropylphenyloxy moiety mimics Phe19 with the phenyl groups serving as a connector to place the phenyloxy group into the Phe19 pocket. Nutlins do not induce p53 phosphorylation on Ser15, suggesting that they are non-genotoxic, selective inhibitors. Studies also suggest that the 4-methoxy functionality in nutlin3 mimics p53 Leu22. Nutlin-3, the most potent analog of the series, induces p53 levels and enhances p53 transcriptional activity. It has been shown to be effective in various cancer models with wild-type p53, such as neuroblastoma, colon, mantle cell lymphoma, breast and osteosarcoma [202, 226–228]. Nutlin-3 also activates p53 and induces apoptosis and cellular senescence in myeloid and lymphoid leukemic cells [229, 230]. An interesting observation has been that androgen deprivation followed by two weeks of Nutlin-3 administration in LNCaP bearing nude mice leads to a greater tumor regression and dramatically increases survival, indicating cross talk between p53, MDM2, and the androgen receptor [231].

This class of compounds have been shown to have effects on other proteins as well. In the absence of functional p53, Nutlin-3 disrupts p73-MDM2 interaction and increases p73 transcriptional activity, leading to greater apoptosis and growth inhibition in hepatocellular carcinomas [232]. Interestingly, Nutlin-3a, a compound that was designed to inhibit MDM2-p53 binding, has been shown to interfere with the MDM2-E2F-1 interaction [233]. Simultaneous treatment of a p53 mutant peripheral nerve sheath tumor cell line with Nutlin-3a and cisplatin leads to an increase in E2F-1 levels and E2F-1-mediated apoptosis [234]. Furthermore, E2F-1 knockdown inhibits Nutlin-3-mediated apoptosis [234]. Nutlin-3 also inhibits VEGF via inhibition of HIF-MDM2 interaction [235]. Nutlin-3 has also been

shown to inhibit the protein expression of NFκB target genes ICAM-1 and MCP-1 in a p53-dependent manner [236]. ICAM-1 and MCP-1 are involved in cell migration and metastasis, suggesting a role for Nutlin-3 in the treatment of advanced diseases. The nutlin series of compounds have been shown to cause cell death via interaction with proteins such as TRAIL, PUMA, and others [229]. Nutlin therapy has also been shown to sensitize different cell lines such as laryngeal, lung, and prostate cancer cells to ionizing radiation [237]. Nutlin-resistant cell lines have wild-type MDM2 but mutations in the p53 DNA binding and dimerization domains. This chemoresistance can be overcome by the p53 targeting agent RITA [238]. Interestingly, the nutlins do not bind the MDM2 homolog MDMX. Furthermore, despite the similarity between MDM2 and MDMX, MDM2 inhibitors such as Nutlin-3 are far less effective against MDM4. However, MDMX inhibitors not only can activate p53 and induce apoptosis in breast cancer cells, but also synergize with MDM2 inhibitors for p53 activation and induction of apoptosis [239]. Nutlins-3a and −3b (inactive enantiomer) have been demonstrated to be substrates of multi-drug resistance protein-1, and act to reverse multi-drug resistance by saturating the efflux capabilities of these transporters [240]. However, several nutlins developed in the early phases failed to proceed to the trials due to poor pharmacokinetic properties [241].

Derivatives structurally related to nutlins include pyrrolidine-2-carboxamide derivatives, and imidazothiazole derivatives (with a proline moiety) [223]. A series of compounds with 3-imidazolyl-indole scaffolds as dual inhibitors of MDM2/MDMX-p53 interaction have been identified [242]. Spiroxindole derivatives represent another class of MDM2-p53 inhibitors designed to mimic the Trp23 residue that is the most critical for binding of p53 to MDM2. MI-63, MI-219, MI-319, and MI-147 belong to this class. Oxindoles were synthesized using a *de novo* approach to mimic the indole ring and also the side chain of Trp23. Shangary and others then used a substructure search technique to identify natural products with an oxindole substructure [202], such as spirotryprostatin A and alstonisine. MI-219, the clinical grade compound, is shown to alter the functional activity of MDM2 through enhancing its auto ubiquitination and degradation [243]. MI-219 causes p53 activation by blocking the MDM2-p53 interaction in wild-type p53 cells but exhibits much lower activity in cells with mutant p53, which is consistent with its mechanism of action as a specific inhibitor of the MDM2-p53 protein-protein interaction [202]. However, in the studies, MI-219 failed to achieve tumor regression in various xenograft models of human cancer though it was able to completely inhibit tumor growth [244]. Recently, the group has reported the synthesis of a series of highly potent diastereomeric spiro-oxindoles, in which stereochemistry affects binding affinity to MDM2 greatly [245]. Optimization of the pharmacokinetic properties of one of the analogs reported therein has yielded MI-888 (K_i = 0.44 nM) with highly selective activity in inhibiting the growth of wild-type p53 cancer cell lines [296, 297]. MI-888 exhibits very good oral bioavailability in rats along with complete tumor regression in mice bearing osteosarcoma (SJSA-1) and acute leukemia (RS4;11) xenografts [297]. An analogue of MI-888 has been advanced into Phase I clinical development [246].

Other MDM2 binding SMIs include chalcone and the NAcylpolyamine derivatives. The latter class represents cyclic peptides in β-sheet confirmation which inhibit the association of both MDM2 and MDMX with p53 [247]. The chal-cone derivatives have been shown to have anticancer activities in a number of cancer cells. Several derivatives prepared from

Licochalcone-A, obtained from licorice root, inhibit MDM2-p53 interaction by binding near the Trp23 pocket of MDM2 [248]. A second generation of chalcone derivatives bearing a boronic fragment has also been synthesized and it is believed that boronic acid analogs may form a stronger salt bridge with Lys51 than corresponding chalcone analogs of caxboxylic acid moiety [248–250]. Surprisingly, the DNA damaging agent 5-fluorouracil has been demonstrated to stabilize and activate p53 by blocking MDM2 feedback inhibition through ribosomal proteins [251]. This finding warrants further investigation into the mechanism of current chemotherapeutic agents to unravel if they activate p53 by inhibiting MDM2.

Recently, Amgen has developed a series of piperidinone based compounds [252]. These compounds also interact with the Trp23, Leu26, and Phe19 residues in MDM2 apart from ordering the N-terminal residues of MDM2, thus resulting in very high affinity for MDM2 binding (MDM2 IC_{50}= 1.1 nM) [252]. In a mouse SJSA-1 tumor xenograft model, oral administration of AM-8553 at 200 mg/kg once daily resulted in partial tumor regression, demonstrating its excellent antitumor activity and clinical translational potential [252].

4.1.2. SMIs Binding to p53—The molecule RITA (Reactivation of p53 and Induction of Tumor cell Apoptosis) acts by binding to the N-terminal of p53 and reactivating p53 function [249]. It is shown that RITA acts not only on wild-type p53, but also on mutant p53. RITA may bind directly with mutant p53 or by disrupting the inhibitory complex that mutant p53 forms with p63 and smad2, or the complex with p73. However, some investigators have demonstrated that RITA does not block MDM2-p53 binding [253, 254]. Therefore, a better understanding of the binding mode of this class of compounds is required for further development of them as effective anti-cancer agents.

4.2. SMIs InhibitingMDM2 E3 Ubiquitin Ligase Activity

The 5-deazaflavin compounds inhibit the E3 ligase activity of MDM2 by targeting the RING finger domain of the oncoprotein. These compounds are also known as HDM2 ligase inhibitor (HLI series). Treatment of these compounds results in p53 activation, despite causing stabilization of both p53 and MDM2. This activation is speculated to result from the direct binding and inhibition of the RING finger domain, the functional domain necessary for MDM2's E3 ligase activity. These compounds also affect the E2 ligase activity. However, at high concentrations, HLIs also cause cell death in p53 mutant cells [255].

Selective inhibitors of E3 ligase include three chemically distinct classes of compounds (namely, benzsulfonamides, ureas, and imidazolones). These are selective inhibitors of MDM2 E3 ligase, with little or no effect on auto ubiquitination of MDM2. They comprise of simple reversible inhibitors of MDM2 that bind to it in a manner noncompetitive with respect to the substrates, Ub-Ubc4 (E2) and p53. The lead candidate of tryptamine derivatives, JNJ26854165, blocks the degradation of p53 by inhibiting the binding of MDM2-p53 complex to proteasome. Treatment with this compound induced p53-mediated apoptosis in wild-type p53 cells, while in mutant p53 cells, JNJ26854165 induced S-phase cell cycle arrest and E2F1-mediated apoptosis. Notably, the compound is active against Nutlin-3a resistant samples, indicating involvement of a pathway other than MDM2-p53 inhibition [109, 220, 256].

Sempervirine, a natural indole alkaloid identified by a high throughput electrochemiluminescent screen that screened more than 100,000 natural compounds, has also been identified as an inhibitor of MDM2's E3 ligase activity [257]. Sempervirine inhibits the MDM2-mediated ubiquiti-nation and degradation of p53, resulting in the accumulation of p53. This compound also preferentially induces apoptosis in wild-type p53 expressing cancer cells, meriting further investigation of its anticancer activity [257].

4.3. SMIs Inducing MDM2 Post-Translational Modifications

Based on the reported chemopreventive activities in several human cancers, we have demonstrated that the soy-derived isoflavone genistein directly down-regulates MDM2 at both the transcriptional and post-translational levels, independent of the p53 status [212]. Our studies have also shown that genistein down-regulates MDM2 and increases p21 levels, independent of tyrosine kinase inhibitory activity of the compound. Another common flavonoid, apigenin, has been shown to induce p53 via MDM2 attenuation and to inhibit the phosphorylation of MDM2 by Akt (which, in turn, increases MDM2 stability and p53 degradation) in ovarian cancer cells [258]. Similarly, the sesquiterpene lactone parthenolide, isolated from the European feverfew herb, *Tanacetum parthenium*, induces MDM2 ubiquitination and proteasomal degradation in an ATM-dependent manner, subsequently activating p53 and other MDM2-regulated tumor suppressor [259, 260]. Berberine, a natural isoquinoline alkaloid obtained from the herb *Rhizoma coptidis* and used in Traditional Chinese Medicine, has been widely investigated due to its myriad pharmacological effects, including anticancer, anti-inflammatory, and antibacterial activities. Berberine induces apoptosis in acute lymphoblastic leukemia (ALL) cells through downregulation of the MDM2 oncoprotein in wild-type p53 ALL cell lines [261]. Berberine also decreases DAXX transcription, and subsequently prevents the formation of the MDM2-DAXX-HAUSP complex, resulting in the persistent self-ubiquitination of MDM2 in ALL cells [261].

4.4. SMIs Inhibiting MDM2 Expression

Several natural products have shown inhibitory effects on MDM2 gene expression [215]. Ginsenoside saponins has been known to regulate multiple steps in cell proliferation, modulate the expression of tumor suppressors, oncogenes, growth factors, cell death mediators, pro-inflammatory molecules, and protein kinases [262]. These anticancer activities follow a well-defined structure-activity relationship. Over the past few years, our group has identified two new ginsenoside products, 20(*S*)-25-hydroxy-dammarane-3β,12β, 20-triol and its methoxyl derivative (25-OCH-PPD and 25-OCH_3-PPD) which exhibit excellent anticancer activity against various human cancers, including prostate, pancreatic, lung, and breast cancers by decreasing MDM2 protein levels both *in vitro* and *in vivo*. 25-OCH_3-PPD also inhibits the transcriptional activity of MDM2 in cancer cells [263–267].

Curcumin, a dietary polyphenol, also down-regulates MDM2 transcription through the PI3K/mTOR/ETS2 pathway and curcumin exposure sensitizes human cancer cells to chemotherapy and radiation *via* MDM2 [213]. Semisynthetic derivatives of the marine alkaloids such as the makaluvamines have been shown to decrease the levels of MDM2 oncoprotein, to cause apoptosis by the caspase-mediated pathway, and to inhibit the PI3k-Akt pathway [268–274].

5. CLINICAL DEVELOPMENT OF SMALL MOLECULE MDM2 INHIBITORS

5.1. Nutlins (*Cis*-Imidazolines)

A cis-imidazoline derivative belonging to the Nutlin family entered clinical trials in late 2007/early 2008. This compound codenamed RG-7112 molecule has just completed phase I clinical trials in patients with solid tumors, and hematologic neoplasia (ClinicalTrials.gov; identifiers: and). This multicenter, open trial was conducted in the United States and France and designed to determine the maximum tolerated dose and the optimal dosing schedule of RG-7112, administered as monotherapy in patients with advanced solid tumors. A first cohort of patients received the starting dose of 20mg/m^2/day, once daily for 10 days in each 28-day cycle. RG7112 treatment was seen to stabilize the p53 protein and induce p53 target genes such as CDKN1A, NOXA, PUMA, FAS and BAX. The compound exhibited good dose tolerance and positive effects in the treatment of acute or chronic leukemia and patients responded well to an escalation in dosage. It is reported that a single patient with AML who has been leukemia-free for 9 months subsequent to RG7112 treatment [275]. Reductions in lymph node and spleen size, as well as in circulating leukemia cells, were noticed in chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) conditions [276–278].

RG-7112 has also been tested in patients with well-differentiated liposarcoma prior to debulking surgery (ClinicalTrials.gov; identifiers:) [277]. The study conducted in France sought to study biomarker evidence of MDM2 and p53 pathway alterations in a clinical setup. The results of these initial proof of mechanism studies are preliminary since a very small number of patients (20 patients) were enrolled. Although, the data indicate that RG7112 does reach its target in a solid tumor and correlates with increased p53 and p21 levels, increased MIC-1 levels (induced by p53), and decreased proliferation, the data did not reach statistical significance. Also, as the tumors were not microdissected, it may be possible that the heterogeneous tumor tissue contained only a small fraction of cells responsive to RG7112 treatment. The most frequent adverse event seen was hematological toxicities. Thus, RG7112 may be a part of neoadjuvant therapy in combination with existing clinically approved non-genotoxic chemotherapeutics. This will help prevent aggravating the hematological toxicities that are a hallmark of standard DNA damaging drugs. Further information on the molecule’s pharmacokinetic profile and adverse effects are awaited. Currently, an ongoing clinical trial is recruiting participants for a multicenter, open-label, Phase Ib study for RO5045337 (the oral formulation of RG7112) that will evaluate the safety, pharmacokinetics and efficacy of this drug in combination with doxorubicin in patients with soft tissue sarcoma. Another small molecule MDM2 inhibitor, RO5503781, presumably with a similar structure, has been launched into clinical trials, presently recruiting participants for a multicenter, open label, dose-escalating study to evaluate the safety, pharmacokinetics, pharmacodynamics and efficacy in patients with advanced malignancies except leukemia. In the clinical trials, this drug is being tested as a single agent or in combination with cytarabine (ClinicalTrials.gov; identifiers: and).

5.2. Spiroxindoles

SAR405838 (MI-888), an analogue of the MI series (MI-219: clinical prototype), discovered by Shangary *et al.* at the University of Michigan, was launched into phase I clinical trials by Sanofi S.A in 2012 (ClinicalTrials.gov; identifier:). This study, as of date, is still recruiting participants and no results are available. The study aims to assess the drug's efficacy in patients with dedifferentiated liposarcoma and determine safety and the maximum tolerated doses (MTD).

5.3. Tryptamine Derivative (JNJ-26854165/Serdemetan)

Another MDM2 inhibitor in clinical trials is the E3 ubiquitin ligase inhibitor JNJ-26854165 (ClinicalTrials.gov; identifier:) [220, 279, 280]. The first observations from the clinical trials were presented in 2009. This orally bioavailable drug has just completed phase I clinical trials for the treatment of advanced stage or refractory solid tumors in USA [276]. The starting dose of JNJ-26854165 in the trial was 4 mg/day as a single oral dose. Side effects reported with the treatment of JNJ-26854165 included nausea, vomiting, fatigue, anorexia, insomnia, electrolyte imbalance, creatinine elevations, and asymptomatic QTc prolongation. No hematological or cardiovascular toxicities were observed. A single patient with higher dose exhibited a grade 3 QTcF prolongation which was reversed after discontinuation of the treatment. Linear pharmacokinetics was seen in 20 to 400 mg dose range, with the preclinically determined therapeutic concentration being achieved at doses above 300 mg [276]. The levels of p53 were upregulated in skin while MDM2 levels were enhanced in tumors (probably due to loss of auto ubiquitination function) in a dose dependent manner. Similarly, the levels of MIC-1, a plasma macrophage inhibitory cytokine, a member of the TGF-β super family and induced by p53, were also increased in dose-dependent manner. A dose of 350 mg was used on expanded cohort of patients to confirm maximum tolerated dose, and a separate trial was started with alternate dosing schedule (150 mg twice a day) to minimize QTc prolongation.

5.4. Other Compounds in Clinical Trials

A novel chemical moiety thioureidobutyronitrile (Kevetrin) is also being launched into Phase I dose escalation and safety clinical trials this year. In preclinical studies, Kevetr in activates p53, induces p21 and PUMA (p53 up-regulated modulator of apoptosis), a p53 activated proapoptotic protein (ClinicalTrials.gov; identifier: NCT 01664000). Recently, Novartis has launched an orally active MDM2-p53 interaction inhibitor, codenamed CGM097, into clinical trials for treatment of p53 wild-type tumors (ClinicalTrials.gov; identifier:).

In addition to these MDM2 small molecule inhibitors, other compounds in clinical development include MK-8242 (or SCH 900242), which is being tested in two phase I clinical trials (ClinicalTrials.gov; identifier: and). This compound is being tested as a single agent in patients with advanced solid tumors and in combination with cytarabine in acute myelogenous leukemia (AML) subjects. Lately, Daiichi Sankyo Inc. has commenced phase I clinical trial of DS-3032b (ClinicalTrials.gov; identifier:) in patients with advanced solid tumors or lymphomas.

Of note, several of the naturally occurring compounds such as ginseng saponins and curcumin have been extensively studied for efficacy and safety in clinical setups, although they were not initially investigated as MDM2 inhibitors. They present a veritable treasure house of MDM2 inhibitors for future development. In (Fig. 4), we present the structures of several prototype MDM2 inhibitors both synthetic and natural origin, while in (Table 3) we summarize the mechanisms of action of these prototype synthetic and natural SMIs along with their clinical development statuses.

6. DISCUSSION AND FUTURE DIRECTIONS

Disruption of the MDM2-p53 interaction with small molecule inhibitors is an attractive cancer therapeutic strategy. However, drug discovery efforts in this area have been primarily focused on strategies to inhibit the binding of p53 to the N-terminal domain of MDM2 and several novel scaffolds (both from rational drug synthesis and natural sources) have been developed [286–293]. Some of these compounds such as the dihydroimidazothiazole derivatives synthesized by scientists at Daiichi employ structural modifications of the nutlins followed by subsequent optimization of potency and pharmacokinetic behavior [289]. We now know that other domains in MDM2 are also involved in the MDM2-p53 interaction and mutations in these domains are associated with cancer [294]. In fact, it has been reported that the ligands binding to the MDM2 acid domain cause p53-mediated inhibition of cell growth and induce apoptosis [295]. This highlights the importance of the acidic domain in addition to the N terminus as a potential target for small molecular MDM2 inhibitors. Directly inhibiting the MDM2-p53 interaction, as a means of restoring p53 wild-type functions, is potentially useful in the treatment of cancers which harbor wild-type p53, but there still exist concerns as to how viable this concept would be clinically: how efficient will a chemical moiety in inhibiting a highly specific protein–protein interaction? What will be the adverse effects of unleashing a potent pro-apoptotic molecule like p53 on healthy cells? And since p53 transcriptionally activates MDM2, will increased p53 lead to increased MDM2 levels due to the MDM2-p53 feedback loop? Present evidence is in favor of these inhibitors; with a number of them entering clinical trials, the ultimate proof of concept may be just around the corner. More than twenty different chemical classes have been claimed to inhibit the MDM2-p53 interaction, but the majority of studies have been directed toward three classes: benzodiazepinediones, spiro-oxindoles (the MI series of compounds), and cis-imidazolines (Nutlins). Further preclinical and clinical studies on other compounds may provide more information on the value of targeting MDM2 and MDM2-p53 interaction.

In order to critically evaluate the mechanism of action and therapeutic potential of a MDM2 inhibitor, it should have the following desirable “drug-like” properties: (i) high binding affinity and specificity towards MDM2, (ii) high cytotoxicity in cancer cells with wild-type p53, and (iii) a highly desirable pharmacokinetic (PK) profile [202]. The p53-binding site of MDM2 is highly hydrophobic, and therefore all the non-peptide drugs that have been developed are, necessarily, lipophilic, and, thus lack aqueous solubility. It is true that several potent MDM2 inhibitors have been tested in animal models of human cancer for their anticancer activity. However, some of these compounds such as Nutlin 3A and MI-219 were not able to achieve complete tumor regression and also showed variable activity in different cancer types (Nutlin-3 and MI-219 inhibit tumor growth completely in SJSA-1 derived

xenografts but show minimal activity against HCT-116 colon cancer xenografts) [244]. These results were consistent with data obtained from *in vitro* cell experiments [244, 296]. It is noteworthy that the antitumor activity of these compounds (nutlins as well as the MI series) was achieved at doses that caused no visible toxicity to the animals, as evidenced by body weight and gross organ morphology at necropsy [198–200, 244, 246, 275, 276, 296, 297]. Since derivatives of nutlins (such as RG7112) with optimized pharmacological parameters (with respect to bioavailability) are able to achieve tumor regression (either partial or complete), it is evident that potent and highly optimized MDM2 inhibitors can achieve impressive anticancer activity in animal models of human cancers [275]. Indeed, researchers at University of Michigan have carried out structural modifications in their MI-series based on the stereo-chemical properties of these compounds alongwith optimization of the pharmacokinetic parameters (by adding more “biological friendly” side chains that increase bioavailability) [244–246]. These efforts have yielded MI-888 which exhibits excellent oral bioavailability alongwith complete tumor regression in two animal models of human cancer [246, 296, 297]. An analogue of MI-888 has also advanced into phase-I clinical trials [297]. An interesting study by Azmi *et al.* details the use of the essential trace element zinc along with the MDM2 antagonist, MI-219 [298]. Zinc is an important part of the p53 biochemistry, with p53 binding to DNA through a structurally complex domain stabilized by zinc atom [298]. The MDM2 protein also carries a C-terminal RING domain that coordinates two zinc atoms, which are responsible for p53 nuclear export and proteasomal degradation [298]. Zinc chloride supplemented MI-219 regimen suppresses the p53 feedback MDM2 activation, thus increasing its efficacy [298].

However, till date, two MDM2 inhibitors (compounds inhibiting the MDM2-p53 interaction), Nutlin-3 and MI-219, appear to meet enough “drug-like” criteria. In addition, the benzodiazepinedione compound TDP665759, with an IC_{50} value of 704 nM appears to be another suitable compound. On the contrary, other small molecule inhibitors that target the MDM2-p53 interaction have either exhibited modest cytotoxic activities in cells or do not possess high binding affinity and/or specificity towards MDM2. Moreover, information on their cellular mechanisms of action, *in vivo* anti-cancer activity in preclinical set-up, cellular specificity for cancerous cells versus normal cells has not yet been reported.

Inhibitors of the MDM2-p53 interaction are unique in the aspect that, unlike, several traditional chemotherapeutics, these compounds induce p53 accumulation and activation in a non-genotoxic manner (without inducing DNA damage or requiring ATM/ATR-dependent p53 phosphorylation). Thus, these compounds do not technically affect p53 post-translational modification pathways such as phosphorylation, acetylation and sumoylation that cause p53 activation in response to genotoxic stress.

Although these inhibitors are highly effective against tumors containing wild-type p53, tumors with mutated or nonfunctional p53 may not be responsive. In fact, mutant p53 is known to stabilize MDM2. Considering that MDM2 has multiple p53-independent oncogenic activities, compounds such as nutlins that have been developed to specifically inhibit MDM2-p53 interaction would display poor effects on p53-inactivated tumors. The development of resistance following MDM2 inhibitor treatment still needs to be further investigated. Moreover, the pharmacokinetic properties and bioavailability of synthetic

MDM2 inhibitors need major improvements before clinical use can be permitted. For instance, several initial analogs in the nutlin series have inadequate bioavailability *in vivo* [202].

As aforementioned, MDM2 is one of the most important oncogenes in the entire process of cancer. In addition to being a negative regulator of p53, it interacts with numerous other proteins involved in diverse cellular functions ranging from cell proliferation, apoptosis, to metastasis and angiogenesis, indicating that these signaling pathways have a potential to be explored as therapeutic targets. Targeting these interactions may provide alternative or complementary approaches to targeting the MDM2-p53 interaction, especially for the treatment of cancers with mutant p53 or loss of p53 function. Given the p53-independent activities of MDM2 in cancer, it becomes necessary to focus on MDM2 itself as a drug target. The oncogenic activities of MDM2 that have potential to be translated into possible molecular targets for cancer therapy include: (i) binding and destabilization of p21, (ii) binding and inhibition of the p53 homologues, p63 and p73, (iii) positive modulation of HIF-1α transcription factor/VEGF [299], (iv) interaction with ribosomal proteins, (vi) inhibition of MDM2 by the tumor suppressor ARF, (vii) inhibition of pRb, (viii) its interaction with hormone receptors such as androgen receptor and estrogen receptor, and (iv) its role in epithelial-mesenchymal transition and metastasis. However, more studies are required to elucidate the role(s) of these interactions, and to define the circumstances under which these interaction(s) can be successfully targeted. The use of system biology and modern high throughput drug screening techniques, combined with an increasingly in-depth understanding of the biochemistry and molecular biology of MDM2 will help us to develop novel MDM2 inhibitors.

MDM2 expression is regulated at multiple levels through various cellular mechanisms. Therefore, it is imperative to understand these mechanisms to better develop therapeutic strategies for MDM2 inhibition. In addition to p53, the transcription of MDM2 is regulated by other transcription factors such as NF-κB [300], Fli-1 [301], ETS [213], AP1 [302], and NFAT1 [303]. Inhibition of these transcription factors may provide another strategy to overcome MDM2 deregulation in cancer cells. Indeed, we have seen that curcumin suppresses MDM2 though the inhibition of ETS transcription factor. Thus, we believe a therapeutic regimen combining an MDM2 transcriptional inhibitor may benefit those harboring high level of MDM2 and/or inactivated p53.

Another mechanism by which MDM2 can be prevented from destabilizing p53 is by inhibiting its E3 ubiquitin ligase activity. However, not much progress (in terms of number of new chemical entities generated) has been made on MDM2 E3 ubiquitin ligase inhibitors on account of the biological complexity of the ubiquitination process. It is hoped that a better understanding at the molecular level of how exactly MDM2 functions as an E3 ubiquitin ligase will facilitate structure-based drug design and discovery. Conceptually, E3 ligases are very attractive drug targets as they mediate a majority of protein destruction mechanisms. Moreover, targeting MDM2 E3 ubiquitin ligase may have a broader spectrum of activity in cancers exhibiting mutant and non-functional p53. In fact, a study employing a combinatorial regimen of bortezomib (a proteasome inhibitor) along with nutlin shows excellent activity against myeloma [304, 305].

At present, most of the available MDM2 inhibitors lack activity against MDMX. Although Nutlin 3a and MI-219 activate p53 in cancer cells with overexpressed MDM2, they do not have similar effects in cells with overexpressed MDMX. MDM2 inhibitors are not able to bind to MDMX although MDMX has similarities in the N-terminal p53-binding domains to that of MDM2. Subtle structural differences in the MDMX-p53 interaction pocket drastically reduce the binding affinity of both Nutlin 3a and MI-219 for MDMX. This observation is supported by *in vitro* binding studies which demonstrate that Nutlin 3a is 500-fold less potent against MDMX compared with MDM2 [306]. MDM2 and MDMX possess non-redundant roles in modulating p53 activity and are the major antagonists of p53 *in vivo*. Therefore, dual antagonists for both MDM2 and MDMX may effectively reactivate p53 in cancer. RO-5963 has been identified as a dual inhibitor of MDM2-p53 and MDMX-p53 interactions. Structural analyses suggest that the compound induces p53 activity via formation of MDM2-MDMX homodimers and heterodimers [306].

Finally, natural compounds present an attractive area for the development of MDM2 inhibitors as they have been shown to target both the p53-dependent and -independent activities of MDM2, exhibiting impressive activity even in p53 mutant or non-functional conditions, thus eliminating the need for p53-dependent mechanisms to exert their effects. On the other hand, most synthetic small molecule inhibitors of MDM2 are known to restore the pro-apoptotic and anti-proliferative functions of p53 by disrupting the MDM2-p53 interaction and, therefore, are less effective in p53 mutant tumors. Further research on the reasons for the effectiveness of naturally occurring compounds in a p53 mutant scenario may provide clues about the structural requirements of such p53-independent MDM2 inhibitory activity. The unique frameworks of the natural compounds can inspire to develop novel synthetic and semi-synthetic derivatives with improved efficacy, pharmacokinetic, and bioavail-ability profiles, thus opening new avenues in MDM2-p53 research.

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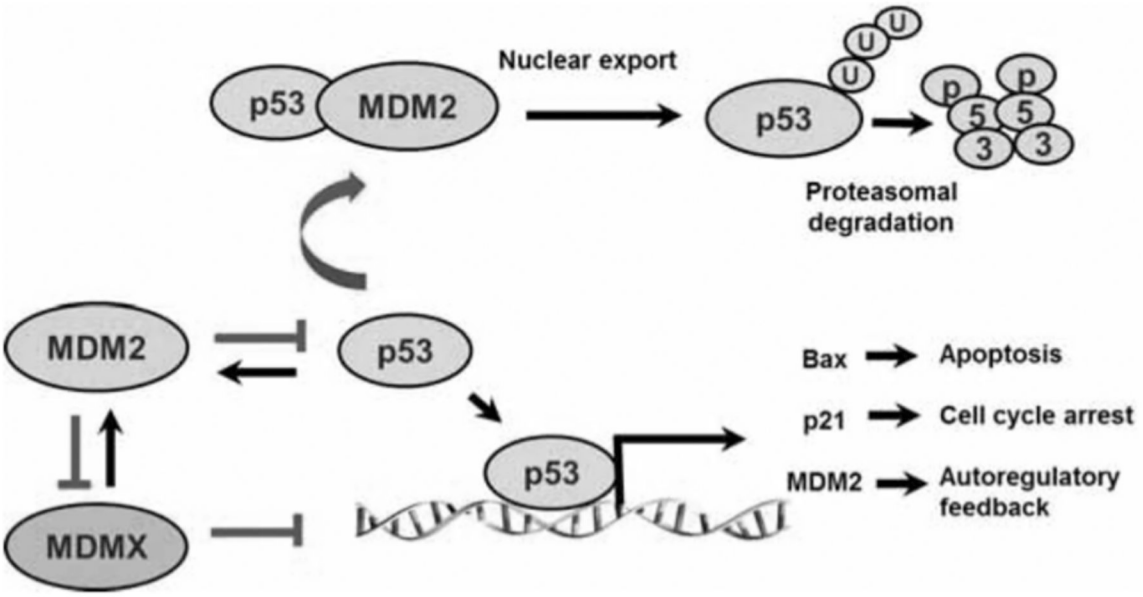

Fig. (1). The MDM2-p53 Interaction.

MDM2 binds to p53 and mediates its ubiquitination, leading to the nuclear export of p53 and proteasomal degradation. The MDM2-p53 interaction also masks the transactivation domain of p53, inhibiting its target gene expression. In turn, p53 induces the transcription of MDM2, forming a negative feedback loop that tightly maintain p53 protein at low level in normal cells.

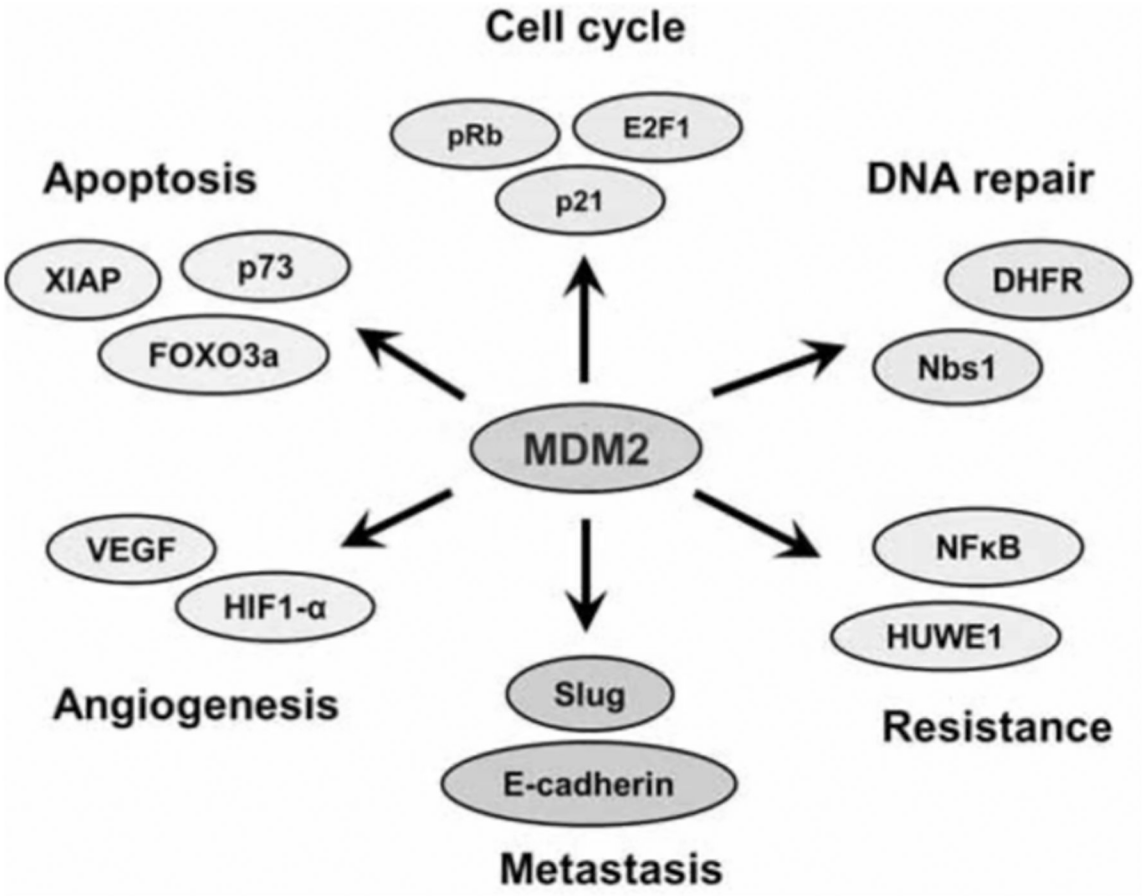

Fig. (2). MDM2: p53-independent activities.

MDM2 regulates a variety of cellular proteins and contributes to multiple processes of carcinogenesis and cancer progression in a p53-independent manner (see text for more information).

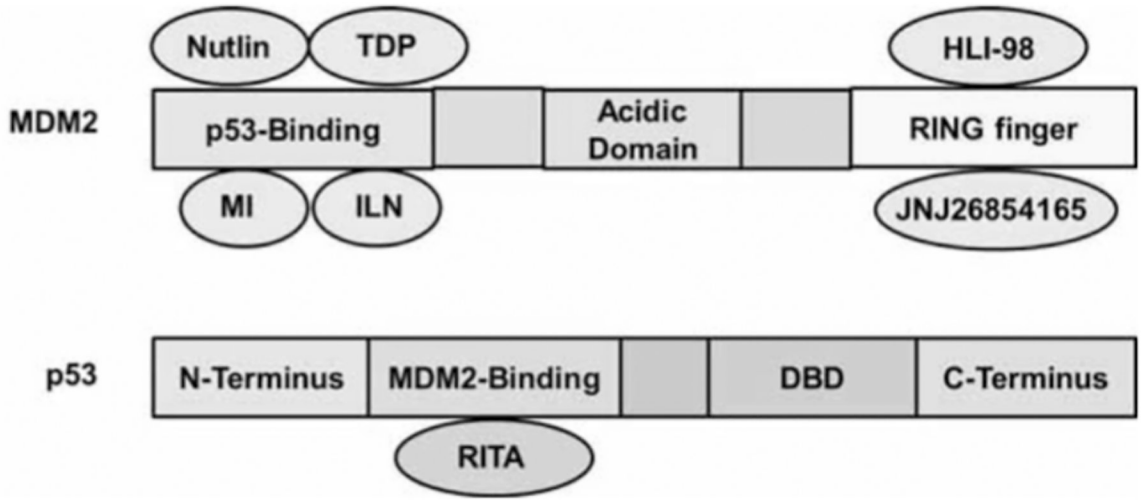

Fig. (3). Schematic representation of the MDM2 and p53 proteins, and the binding areas for small-molecule inhibitors.

Nutlin, cis-imidazoline; TDP, benzodiazepinedione; MI, spiro-oxindoles; ILN, isoindolinone; HLI-98, 5-deazaflavin; JNJ-26854165, tryptamine; RITA, thiophene; RING finger: E3 ubiquitin ligase domain; DBD: DNA binding domain. Binding of either HLI98 or JNJ-26854165 to MDM2 RING finger domain inhibits its E3 ubiquitin ligase activity.

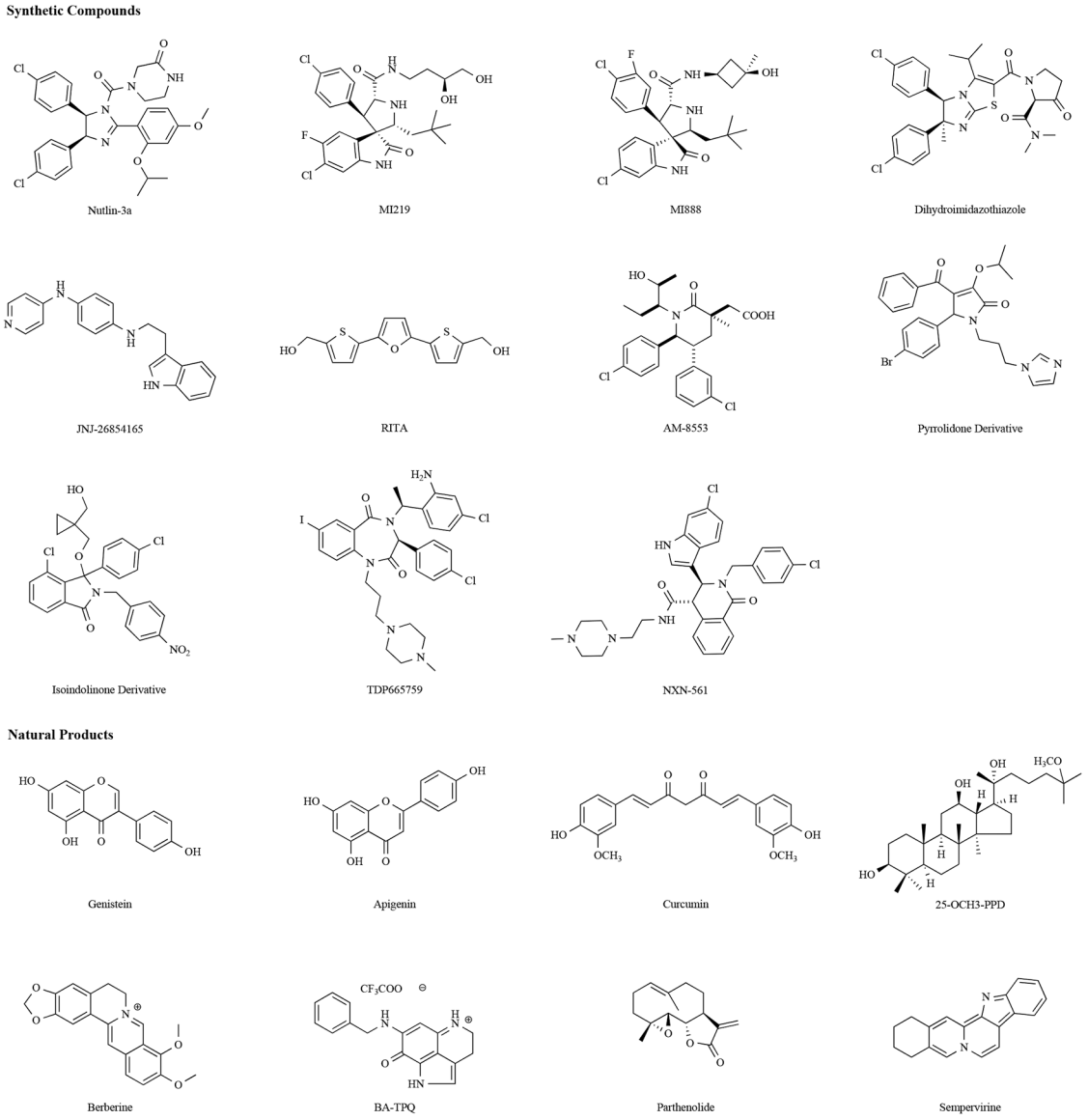

Fig. (4).

The chemical structures of representative synthetic small molecule and natural product MDM2 inhibitors.

Table 1.

Summary of Proteins Modulating the MDM2-p53 Loop

MDM2-p53 Loop Modulator	Outcome	Mechanism	References
ARF	Stabilizes and/or activates p53	Disrupts MDM2-p53 interaction, sequesters MDM2 to nucleolus, and promotes MDM2 degradation	[68, 69, 86]
NPM	Stabilizes and/or activates p53	Competes with p53 for MDM2 binding	[87]
HIPK2	Stabilizes and/or activates p53	Prevents MDM2-mediated p53 nuclear export and ubiquitination	[88]
PML	Stabilizes and/or activates p53	Protects p53 from MDM2-mediated inhibition and degradation by sequestering MDM2 into nucleus	[89–92]
14–3-3-σ	Stabilizes and/or activates p53	Disrupts MDM2-p53 interaction by translocating MDM2 into cytoplasm	[74]
PCAF	Stabilizes and/or activates p53	Disrupts MDM2-p53 interaction and stimulates MDM2 auto-ubiquitination; Acetylates p53 in response to DNA damage	[93, 94]
SENP3	Stabilizes and/or activates p53	Binds to MDM2 and inhibits p53 ubiquitination	[95]
Tip60	Stabilizes and/or activates p53	Disrupts MDM2-p53 interaction by localizing p53 to PML bodies; Inhibits NEDDylation of p53 by MDM2 and promotes p53 acetylation	[96–98]
Caspase-2	Stabilizes and/or activates p53	Cleavage of MDM2 at Asp-367, leading to loss of C-terminal RING domain	[99]
pRb	Stabilizes and/or activates p53	Forms trimeric complex with p53 and MDM2 to protect p53 pro-apoptotic activity	[100, 101]
RYBP	Stabilizes and/or activates p53	Interacts with MDM2 to decrease MDM2-mediated p53 ubiquitination	[67]
TRIAD1	Stabilizes and/or activates p53	Binds to p53 C-terminus and facilitate its dissociation from MDM2	[102, 103]
Aurora-A	Stabilizes and/or activates p53	Prevents MDM2-p53 interaction by phosphorylating p53 at Ser-106	[104]
Ribosomal proteins (L5/L11/L23/L26/S7/S14 /S25/S27/S27L/S27a)	Stabilizes and/or activates p53	Bind to the central acidic domain of MDM2 and inhibits its E3 ubiquitin ligase activity towards p53	[50–63]
PAK1IP1	Stabilizes and/or activates p53	Interacts with MDM2 and inhibits p53 ubiquitination	[105]
DNAJC7	Stabilizes and/or activates p53	Dissociates MDM2 from p53	[106]
Otubain-1	Stabilizes and/or activates p53	Binds to MDM2 cognate E2, UbcH5 and inhibits MDM2-mediated p53 ubiquitination	[107]
MDMX	Destabilizes and/or inactivates p53	Hetero-oligomerization of MDM2 and MDMX via their RING domains enhances p53 ubiquitination	[75, 84, 108–110]
SENP2	Destabilizes and/or inactivates p53	Desumolyates MDM2 and permits its binding and ubiquitination of p53	[111]
Enigma	Destabilizes and/or inactivates p53	Binds to MDM2 and forms a ternary complex with p53; inhibits MDM2 self-ubiquitination and E3 ligase activity towards p53	[112]
YY1	Destabilizes and/or inactivates p53	Promotes assembly of the MDM2-p53 complex, and blocks p300-dependent p53 acetylation and stabilization.	[113, 114]
RNF2	Destabilizes and/or inactivates p53	Binds with both p53 and MDM2 and promotes MDM2-mediated p53 ubiquitination	[65, 66]
PA28γ	Destabilizes and/or inactivates p53	Binds with both p53 and MDM2 and increases MDM2-mediated p53 ubiquitination	[64]

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MDM2-p53 Loop Modulator	Outcome	Mechanism	References
HAUSP	Destabilizes and/or inactivates p53	Deubiquitinates MDM2 leading to increased p53 degradation	[115]
SKI	Destabilizes and/or inactivates p53	Increases MDM2 stability by stimulating sumoylation of MDM2	[116]
Siva-1	Destabilizes and/or inactivates p53	Binds to both p53 and MDM2 and enhances MDM2-mediated p53 ubiquitination and degradation	[117]

Table 2.

MDM2 Gene and/or Protein Alterations in Human Cancers

Cancer Type	MDM2 Expression	Clinical Outcome	References
Breast	*mdm2* gene amplification	Poor prognosis; reduced survival; advanced metastasis	[148, 149]
Prostate	MDM2 protein overexpression	Increased tumor growth and metastasis	[150–152]
Lung	*mdm2* gene amplification MDM2 protein overexpression	Poor prognosis; decreased overall survival	[153–155]
Colorectal	MDM2 protein overexpression	Predictor of metastasis; correlation with advanced stage of the disease	[156–159]
Esophagus	*mdm2* gene amplification	Shorter survival Increased chemo/radioresistance	[24, 26, 160, 161]
Osteosarcoma	*mdm2* gene amplification	Advanced disease and metastasis	[24, 26, 162]
Glioblastoma	*mdm2* gene amplification	Poor prognosis	[24, 26]
Liposarcoma	*mdm2* gene amplification	Poor prognosis	[163]
Endometrial	SNP309 polymorphism *mdm2* gene amplification	Increased risk	[24, 164]
Gastric	MDM2 protein overexpression	Poor prognosis; increased risk	[165]
Pancreatic	MDM2 protein overexpression	Increased risk	[166]
Ovarian	SNP309 polymorphism MDM2 protein overexpression	Poor prognosis; increased risk	[167, 168]
Bladder	SNP309 polymorphism MDM2 protein overexpression	Increased risk	[169, 170]
Leukemia	SNP309 polymorphism MDM2 protein overexpression	Poor prognosis	[171–173]
Melanoma	MDM2 protein overexpression *mdm2* gene amplification SNP309 polymorphism	Poor prognosis; increased risk	[174–178]

Table 3.

Representative Synthetic and Natural-origin Small Molecule Inhibitors of MDM2

Prototype Compound	Molecular Target	Mechanism of Action	Clinical Status	Ref.
1. Synthetic Small Molecule Inhibitors				
Nutlin 3A (Cis-imidazolines)	MDM2 N-terminal p53-binding pocket	Disrupts MDM2-p53 interaction	Phase I clinical trial completed; Combination studies ongoing	[109, 198–200, 275, 276]
JNJ-26854165 (Tryptamine derivative)	MDM2 RING finger domain	Inhibits MDM2 E3 ubiquitin ligase activity; p53-independent activity	Phase I clinical trials	[109, 202, 276]
TDP665759 (Benzodiazepinedione derivatives)	MDM2 N-terminal p53-binding pocket	Disrupts MDM2-p53 interaction	No clinical translation as of yet	[281, 282]
Isoindolinone derivative	MDM2 N-terminal p53-binding pocket	Disrupts MDM2-p53 interaction	No in-vivo activity reported	[283]
MI-219/MI-888 (Spiro-oxindole derivatives)	MDM2 N-terminal p53-binding pocket	Disrupts MDM2-p53 interaction; Induces MDM2 autoubiquitination	Analog-MI 773 (SAR SAR405838) is in Phase I clinical trials	[202, 241, 243]
NXN-561 (Isoquinoline derivative)	MDM2 N-terminal p53-binding pocket	Disrupts MDM2-p53 interaction	No *in vivo* studies reported	[284]
Pyrrolidone derivative	MDM2 N-terminal p53-binding pocket	Disrupts MDM2-p53 interaction	No clinical translation as of yet	[285]
HLI-198C (5-Deazaflavin derivatives)	MDM2	Inhibits MDM2 E3 ubiquitin ligase activity; p53-independent activity	Unknown/not reported	[255, 282]
AM-8553 (Piperidinone derivatives)	MDM2 N-terminal p53-binding pocket	Disrupts MDM2-p53 interaction	Preclinical studies in mouse SJSA-1 tumor xenograft; orally active	[252]
2. Natural Origin Small Molecule Inhibitors				
Parthenolide	MDM2 RING finger	Induces MDM2 ubiquitination in ATM dependent mannerresulting in p53 activation	Not clinically tested in cancer	[259]
Sempervirine	MDM2 RING finger	Inhibits MDM2 E3 ubiquitin ligase activity, preventing both MDM2 autoubiquitination as well as MDM2 mediated p53 ubiquitination.	No clinical translation as of yet	[257]
Genistein	MDM2	Inhibits MDM2 protein expression; p53-independent activity	Clinically tested	[212]
Apigenin	MDM2	Inhibits MDM2 protein expression; Inhibits MDM2 phosphorylation by Akt	Clinically tested	[258]
25-OCH3-PPD	MDM2	Inhibits MDM2 protein expression; p53-independent activity	No clinical translation as of yet	[214, 263, 265–267]
Curcumin	MDM2	Inhibits MDM2 transcription through PI3K/mTOR/ETS2 pathway; p53-independent activity	Clinically tested	[213]

Prototype Compound	Molecular Target	Mechanism of Action	Clinical Status	Ref.
1. Synthetic Small Molecule Inhibitors				
Berberine	MDM2	Inhibits MDM2 protein expression; Induces MDM2 autoubiquitination	Not clinically tested in cancer	[261]