

# Natural Product MDM2 Inhibitors: Anticancer Activity and Mechanisms of Action

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**Abstract:** The *mdm2* oncogene has recently been suggested to be a valuable target for cancer therapy and prevention. Overexpression of *mdm2* is often seen in various human cancers and correlates with high-grade, late-stage, and more treatment-resistant tumors. The MDM2-p53 auto-regulatory loop has been extensively investigated and is an attractive cancer target, which indeed has been the main focus of anti-MDM2 drug discovery. Much effort has been expended in the development of small molecule MDM2 antagonists targeting the MDM2-p53 interaction, and a few of these have advanced into clinical trials. However, MDM2 exerts its oncogenic activity through both p53-dependent and -independent mechanisms. Recently, there is an increasing interest in identifying natural MDM2 inhibitors; some of them have been shown to decrease MDM2 expression and activity *in vitro* and *in vivo*. These identified natural MDM2 inhibitors include a plethora of diverse chemical frameworks, ranging from flavonoids, steroids, and sesquiterpenes to alkaloids. In addition to a brief review of synthetic MDM2 inhibitors, this review focuses on natural product MDM2 inhibitors, summarizing their biological activities *in vitro* and *in vivo* and the underlying molecular mechanisms of action, targeting MDM2 itself, regulators of MDM2, and/or the MDM2-p53 interaction. These MDM2 inhibitors can be used alone or in combination with conventional treatments, improving the prospects for cancer therapy and prevention. Their complex and unique molecular architectures may provide a stimulus for developing synthetic analogs in the future.

**Keywords:** MDM2, MDM2 inhibitors, MDM2-p53 interaction, mechanisms of action, natural product anticancer agents, p53-dependent, p53-independent, p53 wild-type, p53 mutant, small molecule inhibitors of MDM2.

## 1. INTRODUCTION

Carcinogenesis and tumor progression are predominantly caused by alterations in oncogenes, tumor suppressor genes, and other genes regulating cell growth, cell cycle progression, and cell death. Although the loss of a tumor suppressor may or may not lead to oncogenesis, many human tumors possess at least one activated oncogene. The activation of one or more oncogenes, along with exposure to environmental stress or another cellular “hit”, is sufficient to initiate carcinogenesis. The products of oncogenes (*i.e.*, oncoproteins) may be classified into six broad groups: transcription factors (*e.g.*, c-myc), chromatin remodelers (*e.g.*, MLL fusion proteins), growth factors (*e.g.*, EGF), growth factor receptors (*e.g.*, EGFR), signal transducers (*e.g.*, Her2/neu), and apoptosis regulators (*e.g.*, Bcl-2) [1]. Continuous expression of at least one oncogene is often necessary and sufficient for cells to attain unchecked growth and proliferation under conditions normally incompatible with survival. Inhibiting the expression or activity of a dominant oncogene leads to inhibition of tumor growth, demonstrating that overcoming the “oncogene addiction” of the affected cell(s) can stop cancer development and progression [2, 3]. Therefore, targeting oncogene has been suggested as a valid strategy for anticancer drug discovery and development. This review focuses on natural product MDM2 inhibitors, summarizing their biological activities *in vitro* and *in vivo* and the underlying molecular mechanisms of action, along with a discussion on MDM2 biology and development of MDM2 as a viable target for cancer therapy and prevention.

### 1.1. A Brief Historic Perspective of *mdm2* Oncogene

The murine double minute 2 gene (*mdm2*) was originally identified along with two other genes (*mdm1* and *mdm3*) which were overexpressed by more than 50-fold through amplification in a spontaneously transformed mouse BALB/c cell line (3T3-DM) [4, 5]. These genes are located on small, acentromeric extrachromosomal nuclear bodies, called double minutes, and are retained in cells only if they provide a growth advantage. The gene product of *mdm2* was later shown to be responsible for cell

transformation when it was overexpressed [4, 5]. In 1992, Oliner *et al.* cloned the human *mdm2* gene and mapped it to chromosome 12q13-14 [6]. Both the *mdm2* gene and its human counterpart, *hdm2*, consist of 12 exons that can generate many different MDM2 or HDM2 proteins (hereafter referred to as MDM2 for all species) [7]. Alternative splicing of the primary mRNA transcript can result in the generation of different MDM2 isoforms, some of which inhibit the activity of the wild-type protein in a dominant-negative fashion [8, 9]. MDM2 possesses a nuclear localization signal (NLS), a nuclear export signal (NES), a central acidic domain, a C-terminal zinc-finger domain, and a RING finger domain possessing E3 ligase activity [10]. Under normal conditions, MDM2 is expressed in the nucleus, but it translocates to the cytoplasm to mediate the degradation of some of its targets by the proteasome [11-13].

Soon after the identification of the *mdm2* gene, the reason for its transformation potential was discovered. MDM2 was shown to bind the tumor suppressor p53, and inhibit p53-mediated gene transactivation [11]. At the same time, MDM2 gene amplification was observed in over one-third of human sarcomas that retained wild-type p53 [6]. These exciting studies led investigators to hypothesize that the overexpression of MDM2 was another mechanism by which cells could inactivate p53 during the process of transformation. MDM2 affects the p53-mediated transcription of various genes by inhibiting p53-mediated transactivation [8, 11]. MDM2 uses multiple mechanisms to inactivate p53 and inhibit its transcriptional activity [14-16]; it targets p53 for ubiquitination and degradation by the proteasome [15], shuttles p53 out of the nucleus, prevents p53 from interacting with transcriptional coactivators, and recruits known transcriptional corepressors, such as hCtBP2, to p53 [15, 16].

MDM2 has an important role in modulation of several cell cycle proteins. The overexpression of MDM2 has been shown to negatively correlate with the expression of the cyclin-dependent kinase inhibitor, p21 [17, 18]. In breast cancer cells, MDM2 overexpression correlates with a lack of p21 expression [17]. MDM2 can also directly interact with p21 in a p53-independent manner and enhance its degradation [19]. MDM2 also mediates the ubiquitin-independent proteasomal degradation of p21 [20]. In a recent study, Xu *et al.* proved that MDM2-p21 interaction resulted in a conformational change in p21 which depended on the central domain of MDM2; with the nuclear localization of both proteins

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being essential for p21 degradation [21]. MDM2 reverses the growth inhibition at G1 phase imposed by p53 and the retinoblastoma protein (pRb) [22, 23]. Overexpression of MDM2 can overcome the TGF- $\beta$ -related growth inhibition via the pRb-E2F pathway [24]. Transgenic mouse experiments have shown that the expression of a *BLG* ( $\beta$ -lactoglobulin)/*mdm2* transgene (*BLG/mdm2*) in the epithelial cells of the mouse mammary gland causes the mammary epithelial cells to undergo multiple rounds of DNA synthesis without cell division, resulting in polyploidy and tumor formation [25]. This effect of MDM2 on the S phase is independent of the p53 status of the cells [25].

MDM2 also plays an important role in epidermal differentiation [26]. MDM2 overexpression in the granular layer disturbs the differentiation program [27]. In rhabdomyosarcoma, forced expression of MDM2 inhibits MyoD function and consequently inhibits muscle differentiation [28]. MDM2 also ubiquitinates NUMB, a cell fate determinant protein, and influences cell differentiation and survival by inducing the translocation of NUMB to the nucleus and promoting its degradation [29]. The loss of NUMB expression in breast cancer leads to decreased p53 expression and correlates with a poorer prognosis [29].

Additionally, MDM2 is involved in the ribosome biogenesis occurring in both the cell cytoplasm and in the nucleolus of eukaryotic cells. Several proteins contained in the large subunit of the ribosome such as L5 [30], L11 [31], and L23 [32], form a complex with MDM2 in response to ribosomal stress, such as exposure to actinomycin D. These proteins subsequently activate p53 by inhibiting its MDM2-mediated suppression, thereby causing an increase in G1 arrest [30-34]. Our group has discovered another novel MDM2-interacting ribosomal protein, S7, which is present in the small subunit of the ribosome [35]. This protein binds MDM2 *in vitro* and *in vivo*, forming a ternary complex of MDM2-p53-S7, which prevents p53 ubiquitination. The overexpression of S7 inhibits cell proliferation, induces apoptosis, and increases p53 transactivational activity [35]. A later study by Zhu *et al.* demonstrated S7 itself as a substrate for MDM2 E3 ligase in addition to it being a regulator of MDM2 mediated p53 degradation [36].

There are an increasing number of molecules reported to have an interaction with MDM2, which play a role in regulation of MDM2 expression and activity and, in turn, modulate multiple molecular pathways important to the control of cell growth, proliferation and death. We discuss these interactions in the subsequent sections.

## 1.2. MDM2: A Ubiquitin Ligase

E3 ubiquitin ligases are a large family of proteins engaged in regulating the turnover and activity of many proteins [37]. The ubiquitination of proteins occurs through a complex series of steps that involve E1, E2, and E3 proteins. Together with the ubiquitin-activating enzyme, E1, and the ubiquitin-conjugating enzyme, E2, E3 ubiquitin ligases catalyze the ubiquitination of a variety of biologically significant protein substrates, leading to their degradation through the 26S proteasome [37]. MDM2 functions as an E3 ligase that ubiquitinates p53 at several lysine residues [38, 39]. It also has the ability to ubiquitinate itself [40] and various other substrates, such as NUMB [41], pRb [42], and MDMX [43]. The RING motif is common in E3 ligases, and the C-terminal RING finger domain is responsible for the E3 ligase activity of MDM2 [40, 44]. E3 ligases are substrate-specific, and hence, play an important role in the ubiquitin-mediated proteolytic cascade [37]. Low levels of MDM2 activity induce the monoubiquitination and nuclear export of p53, whereas high levels promote the polyubiquitination and nuclear degradation of p53 [45]. MDM2 mediates monomeric p53 ubiquitination on multiple lysine residues [45]. Other proteins that co-operate with MDM2 in the

polyubiquitination and degradation of p53 include p300/CBP [46]. Nevertheless, targeting MDM2 E3 ligase activity is currently one of the major strategies of developing MDM2 inhibitors for cancer therapy.

## 1.3. MDM2-p53 Interaction

The MDM2-p53 interaction has been the hottest focal point in research and discovery of MDM2 inhibitors for cancer therapy. The tumor suppressor p53 is a potent anti-proliferative and pro-apoptotic protein that can be detrimental to normal cells [47]. The cellular level of p53 must be tightly controlled in unstressed cells. It has been well established that MDM2 has a major role in this control. MDM2 and p53 form a sophisticated autoregulatory feedback loop in which the two proteins mutually control each other's cellular level [47]. p53 binds to a promoter of the *mdm2* gene (P1 promoter) and regulates its expression [8, 10]. As the level of MDM2 increases, it binds to and inactivates p53 by directly blocking the p53 transactivational domain and by targeting the p53 protein for ubiquitin-dependent degradation by the proteasome [12, 15]. It was initially reported that MDM2 and p53 bind to each other via their N-terminal domains [48]. Recently, Poyurovsky *et al.* report that MDM2-p53 interaction is decreased upon deletion, mutation or acetylation of the p53 C terminus [49]. Using multiple approaches, they proved that a peptide fragment from the p53 C terminus directly binds the MDM2 N terminus *in vitro*; thus introducing a novel interface for MDM2-p53 interaction [49]. The MDM2 binding site on p53 partially overlaps with its transactivational domain, and thus, MDM2 effectively inhibits p53 transcriptional activity [12, 48]. In addition, as mentioned above, MDM2 serves as an E3 ubiquitin ligase for p53 proteolysis [15]. As a result, both p53 and MDM2 are kept at very low levels in unstressed cells. The crucial role of MDM2 in p53 regulation is strongly supported by the fact that targeted deletion of the *mdm2* gene in mice is embryonic lethal, but *mdm2*<sup>-/-</sup> mice can be successfully rescued by a simultaneous deletion of the *TP53* gene, which encodes p53 [50].

Although it has been demonstrated that MDM2 is the primary regulator of p53 stability and activity, accumulating evidence suggests that MDM2 is not the only protein involved in regulating p53. Recently, three additional proteins, PIRH2 (p53-induced protein with RING-H2 domain that can promote p53 ubiquitination and degradation independent of MDM2) [51], COP1 (constitutively photomorphogenic 1) [52], and ARF-BP1 (ARF-binding protein 1) [53], were discovered to bind p53 and act as p53 ubiquitin ligases. Currently, there is no evidence that any of these p53 ubiquitin ligases can be substitutes for MDM2 in the regulation of p53 stability, which is in agreement with the embryonic lethality of *mdm2* knockout mice.

## 1.4. Interactions with Other Molecules

### 1.4.1. Affectors of MDM2

Many investigations have identified numerous MDM2 interacting molecules that regulate the expression of several key factors involved in cell proliferation, apoptosis, and tumor invasion and metastasis, independent of MDM2's interaction with p53. However, these interactions may have an indirect effect on the degradation of p53. One of the first exemplary proteins discovered to interact with MDM2 was p14<sup>ARF</sup>, an alternate reading frame protein expressed from the *INK4a* locus [54]. The ARF-MDM2 interaction blocks MDM2 shuttling between the nucleus and cytoplasm by sequestering MDM2 in the nucleolus [55]. The transport of p53 from the nucleus by MDM2 is required for the efficient degradation of p53. The sequestration of MDM2 in the nucleolus thus results in the indirect activation of p53 [55]. ARF deregulation results in increased nuclear MDM2 levels, thus decreasing p53, and leading to malignant transformation [56, 57].

Another example of the MDM2-interactive proteins, nucleophosmin (NPM, also known as B23, NO38, or numatrin), stabilizes ARF, increasing its concentration in the nucleolus [58]. NPM also competes for the binding of MDM2 with p53, resulting in decreased p53 degradation.

In addition to p53, other proteins may affect MDM2 transcription and/or function. For example, PTEN decreases both MDM2 stability and transcription [59]. The *mdm2* gene has two promoters, P1 and P2 [60]. PTEN disrupts the activation of the P1 promoter of *mdm2*, thereby decreasing MDM2 expression, independent of p53 [59]. On the other hand, the p72 RNA helicase can co-operate with both P/CAF and p300/CBP to enhance the transcription of MDM2 in both p53-dependent and -independent manners [61]. The Fli-1 Ets transcription factor increases MDM2 transcription; thus decreasing p53 stability and activity [62]. The Ras-Raf-MEK-MAPK pathway also increases MDM2 transcription, and MEK-mediated phosphorylation of MDM2 enhances its export from the nucleus, modulating its function [63].

The phosphorylation of MDM2 by kinases such as ATM kinase impairs the degradation and nuclear export of p53 by MDM2 [64-66]. The phosphorylation of MDM2 by ATM may inhibit HAUSP-mediated MDM2 de-ubiquitination, resulting in a decrease in MDM2 stability and a subsequent increase in p53 stability [67]. Phosphatidylinositol 3-OH-kinase (PI3-kinase) and its downstream target, Akt/PKB serine-threonine kinase, also appear to bind and phosphorylate MDM2 on serines 166 and 186 following mitogen-induced activation, helping to increase the translocation of MDM2 from the cytoplasm into the nucleus, decreasing the transcriptional activity of p53 [68-71]. Other enzymes, such as CK2, PI3K/Akt, and DNA-PK, as well as members of the Ras-Raf-MEK-MAPK pathway, also regulate MDM2 phosphorylation [72]. The phosphatase formed by cyclin G, a regulatory component of the active PP2A holoenzyme, is known to bind and activate MDM2 through dephosphorylation [73]. In fact, cells null for cyclin G expression show increased phosphorylation of MDM2.

Other mechanisms for posttranslational modifications of MDM2 include SUMOylation [74, 75] and acetylation [76]. MDM2 can undergo SUMOylation within its RING finger domain, leading to a decrease in MDM2 auto-ubiquitination, but to an increase in the ubiquitination of p53 [74]. In comparison, the ARF (p14/p19) protein upregulates MDM2 SUMOylation in a p53-independent manner [75]. While the SUMOylation of MDM2 by ARF does not appear to affect the MDM2-p53 loop, it may affect the p53-independent activities of MDM2 [75]. p300/CBP lead to acetylation of MDM2's RING finger domain, decreasing its activity against both p53 and itself [76].

#### 1.4.2. Targets Regulated by MDM2

MDM2 overexpression has been shown to increase the hypoxia inducible factor (HIF)-1 expression levels in colon carcinoma cells, which is followed by a subsequent increase in the expression of vascular endothelial growth factor (VEGF) [77]. In addition, decreased p53 expression (which frequently occurs when MDM2 is overexpressed) results in increased HIF-1 and an increase in VEGF transcription [78]. However, studies have suggested that the effects of the HIF-1-MDM2-p53 interaction may be cell type- and condition-specific [77-81]. In addition, a G-protein-coupled receptor-activated regulator has been implicated in the MDM2-p53 axis [82]. The  $\beta$ -arrestins involved in this signaling pathway bind to MDM2, causing the translocation of MDM2 to the cytoplasm, thus downregulating p53 ubiquitination [82, 83].  $\beta$ -arrestins have also been shown to regulate the ubiquitination of the mitogenic IGF-1R through MDM2 thus serving as an interface for molecular cross-talk between GPCRs and receptor tyrosine kinases [83].

MDM2 has been shown to target pRB and the transcription factor E2F-1 and its binding partner, DP-1, among others [84]. A ternary complex comprising MDM2, pRb, and p53 inhibits the

degradation of p53, and consequently rescues its pro-apoptotic functions [85]. The binding of pRb with MDM2 also regulates the activity of the Sp (specificity protein) transcription factor [86]. It has been reported that the RING domain of MDM2 binds to the androgen receptor (AR) in the nucleus and facilitates its degradation [87]. MDM2 also mediates the NEDDylation of the pro-apoptotic form of p73-TAp73, a process that promotes the translocation of the p73 protein to the cytoplasm and attenuates its p53-transactivational function [88]. The p73 protein, a structural homologue of p53, is also involved in cell cycle regulation and the induction of apoptosis.

Several proteasome-associated proteins other than MDM2 also associate with p53, affecting the MDM2-p53 interaction. For example, gankyrin, a seven-repeat protein commonly overexpressed in early hepatocarcinogenesis and associated with the 19S regulatory complex of the 26S proteasome, facilitates the MDM2-p53 interaction by binding to MDM2 [89]. This results in increased ubiquitination and degradation of p53. Even in the absence of p53, however, the protein enhances the auto-ubiquitination of MDM2 [89]. A de-ubiquitinating protein, HAUSP (herpes virus-associated ubiquitin-specific protease, also known as USP7; ubiquitin specific protease 7), was originally thought to just cleave ubiquitin from p53, thus stabilizing it [90]. However, it was later found to bind to MDM2 as well [91]. The binding of HAUSP to either p53 or MDM2 leads to their de-ubiquitination, and its binding to MDM2 leads to p53 destabilization due to increased MDM2 stability [91]. The proteasome activator PA28 $\gamma$  also regulates the MDM2-p53 interaction (independent of its proteasome-activator function) and serves as a necessary co-factor for p53 degradation [92]. Further, PA28 $\gamma$  binds p21 to regulate its degradation in an ubiquitin-independent manner; it also binds p14/p19ARF and p16 (INK4A) [92]. These observations suggest that the MDM2-interactive proteins, such as PA28 $\gamma$ , p21, and p14<sup>ARF</sup>, may form a complex to enhance the proteasomal degradation of the various proteins.

Recently, it has been shown that the phosphorylated form of Foxo3A is a target for MDM2-dependent ubiquitination and degradation [93, 94]. Foxo3A is responsible for regulating p27, which leads to MDM2-mediated control of cell cycle progression in response to oncogenic growth factor signaling or Ras activation [93, 94]. MDM2 has also been shown to regulate the expression of the anti-apoptotic protein, XIAP, by a novel mechanism [95]. MDM2 binds to XIAP mRNA and enhances its translation, leading to the increased expression of XIAP. This leads to an MDM2-dependent inactivation of caspase-mediated apoptosis [95].

MDM2 has also recently been shown to target E-cadherin for degradation via the 26S proteasome [96]. E-cadherin has a well-characterized role in the epithelial-to-mesenchymal transition, a critical step for the metastatic progression of solid tumors of epithelial origin. MDM2 may also confer TGF- $\beta$  resistance (overcoming G1 arrest) by directly interacting with TGF- $\beta$ 1-activated SMA- and MAD3 (Smad3/4) transcription factors, consequently increasing MDM2 protein expression and destabilization of p53 in human cancer cell lines [24]. Additionally, TGF- $\beta$ 1 expression leads to Smad3 activation and MDM2 expression in murine mammary epithelial cells during the epithelial-to-mesenchymal transition (EMT). These facts suggest that MDM2 may play a critical role in cell migration and metastasis [97].

MDMX, another RING finger ubiquitin ligase, possesses a high degree of homology to MDM2, especially in its N-terminal p53 binding domain [98]. However, although MDMX possesses a RING domain, it is unable to ubiquitinate and degrade p53. MDMX possesses a p53 binding domain at its N-terminus and a RING finger domain at its C-terminus through which it heterodimerizes with MDM2 [98, 99]. Because of its sequence similarity with MDM2 and its ability to inhibit p53-induced transcription when overexpressed, MDMX has been hypothesized to act as a negative



regulator of p53 through physical binding [100]. Despite its inability to facilitate p53 degradation, MDMX seems to be essential for mouse development, because *mdmx*<sup>-/-</sup> mice are embryonic lethal, and similar to the *mdm2*<sup>-/-</sup> mice, can be rescued by the deletion of TP53 [101]. Similar to MDM2, the overexpression of aberrant forms of MDMX has been observed in human tumors and seems to facilitate tumorigenesis [102]. MDMX also has been shown to promote the proteasomal turnover of p21 in cooperation with MDM2 at the G1 phase [103].

We summarize some of the important MDM2 interactive proteins in (Table 1). An exhaustive analysis of all MDM2 interactions is beyond the scope of this review. However, interested readers are directed to several other reviews that provide an excellent discussion on the signaling pathways and interactions of MDM2 with other intracellular proteins and/or receptors [129-135].

### 1.5. MDM2 As a Cancer Target

It has become clear that MDM2 is an important orchestrator of various steps in the oncogenic process. The liberation of p53 from MDM2, which stabilizes the tumor suppressor and activates the p53-mediated pathways, leading to cell cycle arrest and apoptosis, has emerged as an important paradigm for cancer therapy and prevention. Different approaches have been exploited to release p53 from the control of MDM2, including methods to inhibit the MDM2-p53 interaction, MDM2 expression, and MDM2 ubiquitin ligase activity. In addition, inhibiting MDM2's p53-independent activity also becomes one of the major strategies in developing MDM2 inhibitors for cancer therapy and prevention.

#### 1.5.1. Blocking MDM2 Expression (p53-Dependent and -Independent Pathways)

Several studies using antisense oligonucleotides to inhibit MDM2 expression have established the proof-of-concept for this approach in cells and mouse models of human cancer. MDM2 downregulation by antisense oligonucleotides results in p53 stabilization and activation of the p53 pathway in cancer cells *in vitro*, as well as in tumor xenografts in nude mice [136-139]. Interestingly, not only p53 wild-type cells, but also cells that express mutant p53, respond to MDM2 inhibition. Studies have suggested that the p53-independent stabilization of the cyclin-dependent kinase inhibitor, p21, as a result of MDM2 downregulation might contribute to the antitumor activity of MDM2 antisense oligonucleotides and MDM2 inhibitors in these mutant cell lines [140]. Several naturally derived compounds also have been shown to exert their anticancer activities by inhibiting MDM2 interaction with other molecules, independent of p53, such as berberine (disruption of MDM2-DAXX-HAUSP complex) [141]. A comprehensive review of natural products blocking MDM2 expression in a p53-dependent or -independent manner is discussed below.

#### 1.5.2. Modulating MDM2's E3 Ubiquitin Ligase Activity or/and Blocking MDM2-Mediated Ubiquitination of p53

Because the ubiquitination and subsequent degradation of p53 are the major ways in which MDM2 negatively regulates p53, targeting the ubiquitin ligase activity of MDM2 has been pursued as a p53-activating strategy [142, 143]. Recently, small-molecule inhibitors have been identified that specifically target the E3 ligase activity of MDM2 [144]. Several compounds from this class have been shown to inhibit the ubiquitination of p53 *in vitro*, with IC<sub>50</sub> values in the 20–50 μM range. In cancer cells, they activate p53 signaling and induce apoptosis in a p53-dependent manner. However, these compounds have low potency and selectivity. Further optimization of these inhibitors to increase their potency, to increase their E3 ligase specificity, and to eliminate their p53-independent off-target activities is necessary before the potential of this p53-activating strategy can be adequately assessed [144].

#### 1.5.3. Inhibiting MDM2-p53 Binding

MDM2 is unable to downregulate p53 if it is prevented from interacting with the tumor suppressor. Therefore, inhibition of MDM2-p53 binding is a desirable strategy for p53 stabilization and activation. However, targeting protein-protein interactions by small molecules is challenging. Protein-protein interactions usually involve large and flat surfaces that are difficult to interrupt by low molecular weight compounds [145, 146]. However, in the case of the p53-MDM2 interaction, it has been demonstrated that a limited number of amino acid residues are crucial for the binding of the two proteins. In fact, just three amino acids in p53 – Phe19, Trp23 and Leu26 – are essential for the binding between the two proteins, and they are inserted into a deep hydrophobic pocket on the surface of the MDM2 molecule [10, 147]. Thus, one can design small molecules that mimic this interaction.

### 1.6. Synthetic Small Molecule MDM2 Inhibitors

Advances in the understanding of the conformation and structure of MDM2 have sparked the rational development of synthetic small molecule MDM2 inhibitors [148]. Molecular modeling approaches, such as molecular dynamics, pharmacophore-based, and molecular docking, have helped in this structure-based design. The hypothesis that a small-molecule could be developed to bind to MDM2, and thereby inhibit the MDM2-p53 interaction, resulted from the resolution of the crystal structure of MDM2 bound to a peptide from the transactivation domain of p53, which revealed that MDM2 possesses a relatively deep hydrophobic pocket that is filled primarily by three side chains from the helical region of the peptide [147]. Also, the discovery that MDM2-p53 binding is dependent on only three p53 amino acid residues interacting with a discrete MDM2 pocket stimulated efforts to identify potential small molecule inhibitors [10, 147]. 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 p53 responsive genes and activation of the p53 signaling pathway [148]. 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, terphenyls, and many more [142, 148]. There have been concerns whether it is possible to inhibit a protein-protein interaction with a drug-like molecule, and what the effects of unregulated p53 would be on healthy cells. However, experiments with a number of small molecules have proven that it is possible to inhibit this interaction, and a few of these molecules have already progressed to advanced preclinical development or early phase clinical trials [149]. Nearly 20 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, and cis-imidazolines [149]. The spiro-oxindole core structure was discovered using a structure-based *de novo* design strategy to develop entities that could mimic the interaction of Trp 23 in p53 with MDM2 [150].

Despite their different structural motifs, all synthetic inhibitors adhere to the basic hydrophobic pharmacophore model of MDM2. Potent inhibitors belonging to the three major classes of inhibitors have been obtained by the introduction of halide-substituted aromatic groups to improve the pharmacokinetic properties of the molecules [151]. The development of MDM2 E3 ubiquitin ligase inhibitors is less advanced, but it has been pointed out that E3 ligases are, in general, conceptually attractive drug targets. Table 2 provides the structures and describes the major findings of the most potent compounds of the different classes of inhibitors [144, 148-157].

All of the compounds described above target only the MDM2-p53 feedback loop and attempt to reactivate or stabilize p53 in the cell. However, a concern that remains is the fact that p53 activation requires not only stabilization and accumulation of the protein, but also post-translational modifications, such as phosphorylation, acetylation and SUMOylation, in response to genotoxic stress. Although small molecule antagonists of MDM2 prevent p53 from binding to MDM2, they do not technically affect post-translational modifications [151]. Thus, while they are likely to be highly effective against tumors containing wild-type p53, tumors with mutated or non-functional p53 may not be responsive. The possibility of chemoresistance in such tumors and the development of resistance following MDM2 inhibitor treatment need to be investigated. Secondly, it is debatable if the signaling pathways downstream of p53 are intact in tumorous cells. Although defects in the signaling upstream of p53 can be compensated by other molecules, such as HAUSP and COP-1 (which also stabilize p53 and p73), there are few compensatory mechanistic alternatives for defects downstream of p53; small molecular inhibitors such as nutlins that are specifically designed only to target the MDM2-p53 interaction may be rendered ineffective in the latter context. Furthermore, the pharmacokinetic properties and bioavailability of these synthetic compounds still need major improvements before they can be used in the clinical setting.

Among the small molecule MDM2 inhibitors, drugs currently under clinical trials include RG7112 and JNJ-2684165. RG7112 is an imidazoline derivative (belonging to the nutlin family) developed by Hoffman-La Roche and is currently under phase I clinical trial for the treatment of solid tumors [158], hematologic neoplasms and leukaemia [159]. This compound inhibits the MDM2-p53 interaction in a highly selective manner. JNJ-26854165, a tryptamine derivative developed by Johnson & Johnson Company is currently under phase I clinical trial for the treatment of advanced stage or refractory solid tumors in USA [157], non-small cell lung cancer and prostate cancer in Europe [149]. It blocks the degradation of p53 by inhibiting the binding of MDM2-p53 complex to proteasome. Side effects reported with the treatment of JNJ-26854165 include nausea, vomiting, fatigue, anorexia, insomnia, electrolyte imbalance, creatinine elevations, and asymptomatic QTc prolongation, mostly grade 1-2. A patient with higher dose exhibited grade 3 QTcF prolongation, which recovered after stopping the treatment [160]. Since, most of the compounds are in early clinical trials, there are not many reports regarding their adverse effects and efficacy in the clinic. These challenges have led researchers to explore other avenues for the development of MDM2 inhibitors.

## 2. NATURAL PRODUCT MDM2 INHIBITORS

Natural products with their diverse structures and complicated carbon skeletons have always played an invaluable role in development of anti-cancer compounds. Indeed, almost 50% of the antitumor agents approved in the last 50 years of the 20th century are either compounds derived from natural sources or (semi-) synthetic analogs of these products [161-165]. Chemical entities of natural origin may be classified into three major categories, including original natural products, semisynthetic derivatives of natural products, and total synthetics based upon naturally occurring skeletons or with pharmacophores identified from natural products [163-165]. In this review, we also followed the three major categories to summarize the MDM2 inhibitors of natural origin, which could provide both broader and more in-depth insights into the discovery and development of MDM2 inhibitors.

The rationale for developing natural products as MDM2 inhibitors is based on two major factors: that several natural compounds exhibit MDM2 inhibition irrespective of p53 status of cell, and that natural products possess immense chemical diversity

(yielding different kinds of candidate compounds) and often have good "drug-like" properties. The recognized and potential advantages of developing natural products as MDM2 inhibitors over synthetic compounds are discussed below.

In addition to their diversity and abundance, one of the most attractive properties of natural products is their chemical stability. Although a number of synthetic compounds have been designed based on the crystal structure of the MDM2-p53 complex [129, 166, 167], with impressive activity profiles, this design principle has also resulted in some unavoidable drawbacks, such as high molecular weight and chemically unstable compounds. Natural products usually show greater stability than synthetic compounds due to their more stable stereochemical structures. Recently, a homogeneous time-resolved fluorescence (HTRF)-based high throughput screening study helped Allen *et al.* identify chromenotriazolopyrimidine as a potent inhibitor of the MDM2-p53 interaction, with a low micromolar  $IC_{50}$  ( $3.88 \pm 1.48 \mu M$ ) [168]. Unfortunately, this compound was shown to be chemically unstable, and it had poor solubility in DMSO. Even though structural optimization has been carried out based upon cocrystallization studies with MDM2, the poor solubility of the derivatives of the compound can also be predicted owing to the relatively high molecular weight of the compounds.

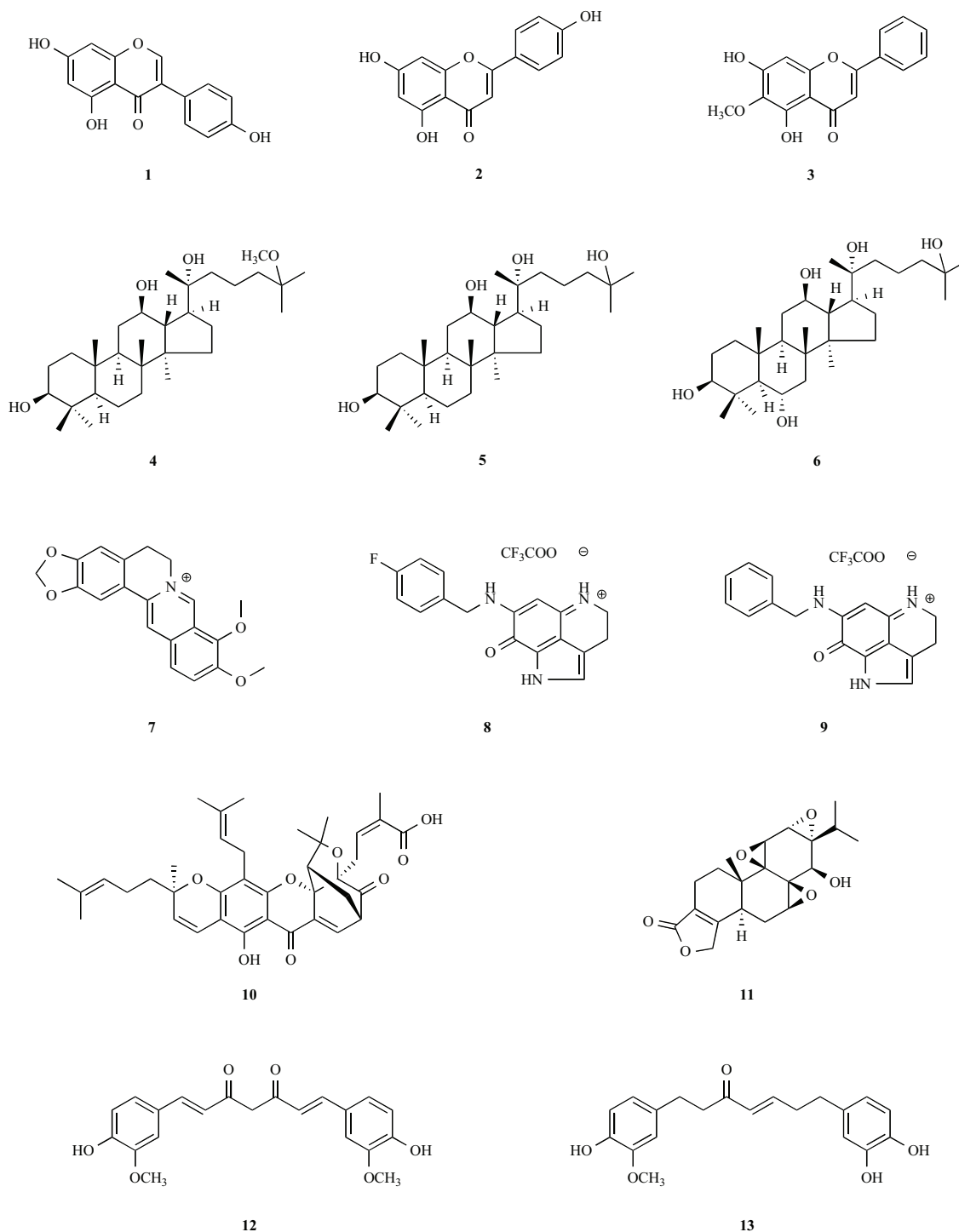
Among the other appealing properties of natural products is the fact that they are often less toxic than synthetic compounds, because many natural products are isolated from dietary vegetables, fruits, or medicinal herbs. As a result they have a long history of use either as food or as folk medicine. A classic example would be curcumin which has been an integral part of the South Asian diet for centuries and has, recently, been investigated for its anti-cancer properties [169]. In turn, none of the synthetics designed as anticancer agents are known to have low toxicity, which has led to a significantly higher rate of failure for such compounds during development. Additionally, natural products often show longer-lasting target effects and fewer side effects compared with synthetic compounds. Recently, a number of reports have demonstrated that several natural products in different classes possess considerable MDM2 binding affinity at micromolar concentrations, which further prompted biologists to screen and develop MDM2 inhibitors from natural resources. Below, we review the natural product MDM2 inhibitors that can be broadly classified into three categories based upon the main strategies that have been used to discover and evaluate them: natural products that inhibit MDM2 expression, those blocking the MDM2-p53 interaction, and those that modulate the E3 ubiquitin ligase activity and/or block the MDM2-mediated ubiquitination of p53.

### 2.1. Natural Products that Inhibit MDM2 Expression

In this section, we review the natural products that inhibit MDM2 expression to emphasize their potential, grouping them according to their carbon frameworks (Fig. (1) and Table 3).

#### 2.1.1. Flavonoids and Isoflavonoids

To the best of our knowledge, genistein (**1**), a soy-derived isoflavone, was one of the first compounds demonstrated to downregulate MDM2 at both the transcriptional and posttranslational levels [170]. Based on the reported chemopreventive activities of genistein in several human cancers, Li *et al.* further demonstrate that the compound directly decreases the MDM2 oncoprotein and *mdm2* oncogene expression levels, independent of the p53 status of the cells. Additional studies have shown that genistein downregulates MDM2 without requiring the known tyrosine kinase inhibitory activity of the compound, and it consequently increases the p21 level. A subsequent *in vivo* investigation confirmed that genistein's antitumor effects are linked to its inhibitory effects on MDM2 expression [170]. Correlatively, synthetic genistein glycosides and 7-O-modified genistein



**Fig. (1).** The chemical structures of natural products that inhibit MDM2 expression. (1) Genistein; (2) apigenin; (3) oroxylin A; (4) 25-OCH<sub>3</sub>-PPD; (5) 25-OH-PPD; (6) 25-OH-PPT; (7) berberine; (8) FBA-TPQ; (9) BA-TPQ; (10) gambogic acid; (11) triptolide; (12) curcumin; (13) 1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)-4E-en-3-heptanone.

derivatives have been shown to have remarkable cytotoxicity in *in vitro* screening studies, and may be promising for the development of new anticancer chemotherapeutics [171-173]. However, it is currently unknown if these genistein derivatives have MDM2 inhibitory effects.

Apigenin (2), a common dietary flavonoid found in many fruits and vegetables, has been reported to induce p53 through the downregulation of MDM2 and to inhibit the phosphorylation of

MDM2 by AKT in ovarian cancer cells [174]. Another flavonoid, oroxylin A (3), a naturally occurring derivative extracted from *Scutellariae radix*, has been shown to induce apoptosis in HepG2 hepatocellular carcinoma cells [175]. During their study, Wu and coworkers observed that oroxylin A stabilized p53 expression at the posttranslational level and consequently induced apoptosis. These changes were brought via downregulating MDM2 expression and interfering with MDM2-modulated proteasome-related p53 degradation [175].

Table 1. MDM2-interactive Proteins and Their Effects

Protein Names	Effect of Interaction with Protein on MDM2	Effect of Interaction with Protein on p53	Effect of MDM2 on Protein	Ref.
14-3-3- $\sigma$	MDM2 stability decreased, translocation to cytoplasm	Increased Stability	No Direct Effect	[104]
TGF- $\beta$	Not Well Understood	No Significant Effect	Not well understood, MDM2 may mediate TGF- $\beta$ resistance	[24, 97]
p14 (ARF)	MDM2 Activity Decreased, MDM2 localized to nucleolus	Increased Stability	MDM2 decreases its transcriptional activity and increases degradation	[55]
Nucleophosmin (NPM, B23)	Inhibits Binding of p53 with MDM2	Increased Stability	Not Well Understood	[58]
Gankyrin (PSM10)	E3 ligase activity of MDM2 increased	Enhanced ubiquitination and degradation	Not Well Understood	[89]
MTBP	Stability Increased	Ubiquitination and degradation of p53 increased	Not Well Understood	[105]
PML	Decreases Ubiquitinating Ability	Protects p53 from Mdm2-mediated inhibition and degradation.	Not Well Understood	[106]
Merlin	Induces MDM2 degradation	Not Well Understood	Not Well Understood	[107]
PCAF	Inhibits binding of MDM2 with p53; stimulates auto-ubiquitination	Decreased Acetylation	MDM2 increases its proteasomal degradation	[108]
Tip60	Localization to PML bodies	Decreased MDM2-mediated NEDDylation; acetylation	MDM2 increases its proteasomal degradation	[109]
JMY	Not Well Understood	Decreased Transcriptional Activity	MDM2 increases proteasomal degradation	[110]
TSG-101	Inhibits Ubiquitination; increased stabilization	Decreased Activity	MDM2 increases its proteolysis	[111]
YY1	Promotes the assembly of the MDM2-p53 complex.	Disrupts the interaction between p53 and the coactivator p300, blocks p300-dependent acetylation and stabilization of p53.	Not Well Understood	[112]
IGFR	Not Well Understood	Increased Stability	MDM2 increases its proteasomal degradation	[113]
Estrogen Receptor $\alpha$	Increased transcription following estrogen ligation by the ER	Increased stability and activity	Interaction with MDM2 decreases its stability; it also increases its activity when p53 is absent	[114]
NF $\kappa$ B	Not Well Understood	Not Well Understood	Pharmacologic inhibition of Mdm2 results in activation-induced apoptosis. MDM2 overexpression leads to p65 subunit induction and chemoresistance	[115,116]
PSD-95	Not Well Understood	Not Well Understood	Promotes ubiquitination and proteasomal degradation	[117]
ENIGMA	Inhibits MDM2 self-ubiquitination	Decreased stability; Increases MDM2 ubiquitin ligase activity toward p53	Not Well Understood	[118]
Siva-1	Not Well Understood	Increases Mdm2-mediated p53 degradation.	Not Well Understood	[119]
elf4e	Increases MDM2 expression	Not Well Understood	Not Well Understood	[120]
VEGF	Not Well Understood	Not Well Understood	MDM2 Positively regulates VEGF mRNA stabilization	[121]
Nucleostemin	Nucleoplasmic mobilization of nucleostemin stabilizes MDM2	Decreases p53 transcriptional activity	Not Well Understood	[122]
Caspase-2	Cleaves MDM2 at asp 367 leading to loss of C-terminal RING domain	Stability Increased	Not Well Understood	[123]
MYCN	MDM2 positively regulated by MYCN	Not Well Understood	Not Well Understood	[124]
S14	Decreased ubiquitination of p53	Increased stability and activity	Not Well Understood	[125]
S100	Not Well Understood	Disrupt extent of MDM2 mediated p53 ubiquitination	Not Well Understood	[126]
HIPK2	Not Well Understood	HIPK2 and p53 colocalize with PML-3 into the nuclear bodies and cooperate in the activation of p53-dependent transcription and induction of apoptosis	MDM2 causes degradation of HIPK2	[127]
HAUSP/USP7	MDM2 stability increased due to de-ubiquitination	p53 stability decreased due to increased MDM2-mediated ubiquitination	Not Well Understood	[67]



(Table 1) contd....

Protein Names	Effect of Interaction with Protein on MDM2	Effect of Interaction with Protein on p53	Effect of MDM2 on Protein	Ref.
MDMX	Stability Increased	Stability increased; NEDDylation	MDM2 increases its ubiquitination	[98]
NUMB	No Known Effect	p53 Activity increased	MDM2 increases its degradation	[29]
p21	Expression Decreased	Decreased Expression of p53	MDM2 induces conformational change in p53 leading to increased proteasomal degradation	[17, 18]
p73	Not Well Understood	Increased stability and transcription; NEDDylation	Interaction with MDM2 decreases its transcriptional activity	[88]
PA28 $\gamma$	Increased Stability	Increased Ubiquitination	Not Well Understood	[92]
pRb	Decreased E3 ligase activity toward p53	Increases p53-mediated apoptosis	MDM2 binding decreases activity	[42]
L5	Decreased Ubiquitination of p53	Increased stability and activity	Not Well Understood	[30]
L11	Decreased Ubiquitination of p53	Increased stability and activity	Not Well Understood	[31]
L23	Decreased Ubiquitination of p53	Increased synthesis and protection from MDM2-mediated ubiquitination	Not Well Understood	[32]
S7	Not Well Understood	Increased stability and activity	Not Well Understood	[36]
S25	Inhibit E3 ligase activity of MDM2	Increased stability and activity, decrease p53 ubiquitination by MDM2	Not Well Understood	[128]
$\beta$ -arrestins	Cytoplasmic localization, decreased E3 ligase activity ( $\beta$ -A2)	Increased protein stability and activity ( $\beta$ -arrestin 2)	Not well understood ( $\beta$ -arrestin 2), increased ubiquitination ( $\beta$ -arrestin 1)	[82, 83]
HIF-1 $\alpha$	Increased activity toward p53	Decreased Stability	MDM2 may increase its stability	[78]
Androgen Receptor	Not Well Understood	Not Well Understood	MDM2 increases its ubiquitination and proteolysis. MDM2 also decreases its transcriptional activity	[87]
E2F1	Not Well Understood	Not Well Understood	p53 represses its activation/function; MDM2 increases its stability and stimulates activity	[42]

### 2.1.2. Ginsenosides

Ginsenosides, a major class of steroid compounds extracted from *Panax* species, are considered to be responsible for the diverse pharmacological activities of *Panax* plants, including their anti-inflammatory, anti-diabetes, and anticancer effects [176]. 20(*S*)-25-methoxyl-dammarane-3 $\beta$ ,12 $\beta$ ,20-triol (25-OCH<sub>3</sub>-PPD, **4**), a novel ginsenoside isolated from the dried leaves of *Panax notoginseng*, has been shown to have considerable cytotoxicity against 12 human cancer cell lines and demonstrated potential as a novel anticancer drug [177]. The anticancer activities of 25-OCH<sub>3</sub>-PPD have been shown in various *in vitro* and *in vivo* models of human prostate [178], pancreatic [179], and lung [180] cancers. The downregulation of the MDM2 protein levels is observed in all of the tested cancer cell lines, which is considered to be at least partially responsible for the anticancer effects of the compound. It is found that 25-OCH<sub>3</sub>-PPD reduces MDM2 transcription in a dose-dependent manner in cancer cells. Additionally, since the greatest effect occurs with the shortest promoter construct (-132 to +33) for MDM2 promoter, it is likely that at least one of the transcription factor binding sites (ETS, AP1, MEF2, or NFAT) within this section is affected by the compound [179].

A total of 11 ginsenosides, including 20(*R*)-dammarane-3 $\beta$ ,12 $\beta$ ,20,25-tetrol (25-OH-PPD, **5**) and 20(*R*)-dammarane-3 $\beta$ ,6 $\alpha$ ,12 $\beta$ ,20,25-pentol (25-OH-PPT, **6**) have recently been isolated and identified from the fruits of *P. ginseng* [181]. One of the compounds, 25-OH-PPD, has remarkable cytotoxicity to cancer cells with low micromolar IC<sub>50</sub> values (10-60  $\mu$ M) and dose-dependent effects on apoptosis, proliferation, and cell cycle progression [181]. The downregulation of the MDM2 protein levels is demonstrated, following exposure to both 25-OH-PPD and 25-OH-PPT [182]. Further investigations are needed to clarify the importance of MDM2 inhibition in their anticancer effects.

### 2.1.3. Natural Isoquinoline Alkaloids

Berberine (**7**), a natural isoquinoline alkaloid from the Chinese medicinal herb *Rhizoma coptidis*, has recently attracted attention due to its diverse pharmacological effects, including anticancer, anti-inflammatory, and antibacterial activities. Zhang *et al.* recently report that berberine could induce apoptosis in acute lymphoblastic leukemia (ALL) cells through downregulation of the MDM2 oncoprotein [141]. Their results suggest that both the high MDM2 expression levels and wild-type p53 status of ALL cell lines are necessary for the proapoptotic effects of berberine, and that the compound persistently downregulates the MDM2 level to subsequently activate p53 in a steady-state manner, but does not inhibit MDM2 mRNA expression. It is further discovered that berberine also decreases DAXX expression at the transcriptional level, and subsequently prevents the formation of the MDM2-DAXX-HAUSP complex, resulting in the persistent self-ubiquitination of MDM2 in ALL cells [141]. In addition, numerous analogs and derivatives of berberine, such as palmatine, coralyne, and sanguinarine, have been reported to possess remarkable cytotoxicity to cancer cells, and are also expected to be natural product MDM2 inhibitors [183].

### 2.1.4. Makaluvamine Analogs

The makaluvamines, a class of marine pyrroloiminoquinone alkaloids isolated from sponges of the genus *Zyzya*, have been demonstrated to have considerable *in vitro* and *in vivo* anticancer activities, which are initially thought to result from the inhibition of topoisomerase II [184]. Further studies indicate that the cytotoxicity of the makaluvamines could not be attributed solely to the inhibition of topoisomerase II, but also resulted from the direct DNA damage that occurs under the reductive activation conditions caused by the makaluvamines [185].

In order to further explore the novel mechanisms of action of makaluvamines, more than 40 makaluvamine analogs have been



synthesized and evaluated for their anticancer activities in our laboratories [186, 187]. Their notable activities prompted the further consideration of four novel makaluvamine analogs, including 7-(4-fluorobenzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1H)-one (FBA-TPQ, **8**), 7-(phenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1H)-one (PEA-TPQ), 7-(3,4-methylenedioxyphenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1H)-one (MPA-TPQ), and 7-(3,4-dimethoxyphenethylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1H)-one (DPA-TPQ). As expected, the compounds have broad-spectrum anticancer activities, with nanomolar IC<sub>50</sub> values against thirteen human cancer cell lines [188]. Further studies have shown an increase in p53/p-p53 and a decrease in the MDM2 expression level in both *in vitro* and *in vivo* models of breast cancer [188]. A follow-up study using ovarian cancer models have also confirmed that the activation of the MDM2-p53 feedback loop is largely responsible for the anticancer activities of FBA-TPQ [189].

Another synthetic makaluvamine analog, 7-(benzylamino)-1,3,4,8-tetrahydropyrrolo [4,3,2-de]quinolin-8(1H)-one (BA-TPQ, **9**) has been shown to dose-dependently inhibit cell growth, induce apoptosis and cell cycle arrest, and decrease the growth of xenograft tumors, all of which have been partially attributed to the action of BA-TPQ on the p53/MDM2 interaction [190]. We have also found the downregulation of the MDM2, cyclin D1, Cdk2, Cdk4, Cdk6, and E2F1 protein levels and the upregulation of the p53 protein level in MCF-7 cells. Interestingly, similar effects on these cell cycle/proliferation-related proteins also are observed in MCF-7 p53 KD cells and MDA-MB-468 (p53 mutant) cells, suggesting that BA-TPQ has a p53-independent mechanism of action [190]. MDM2 appears to be a major target of BA-TPQ, and p53-independent oncogenic effects of MDM2 may be inhibited in these cells. Reports about natural makaluvamines, makaluvamine derivatives, and synthetic makaluvamine analogs are still being published [191], and the data suggest that this class of compounds may yield a number of potential therapeutic agents for cancer, some of which may exert their effects by inhibiting MDM2.

### 2.1.5. Xanthonoids

Gambogic acid (GA, **10**), a polyprenylated xanthone, is the major active ingredient of gamboges, which is derived from *Garcinia hanburyi*, which grows in Southeast Asia [192]. It has a long history of use as folk medicine due to its broad spectrum of activities. Recent pharmacological studies have shown that GA has potent anti-tumor activity, and that the compound exerts its effects by acting as an apoptosis inducer in cancer cell lines. Kasibhatla *et al.* report that GA targets the transferrin receptor (TfR or CD71) [192]. However, Gu *et al.* demonstrate that GA exerts its potent antitumor activity by modulating the MDM2-p53 pathway [193]. GA up-regulates p53 protein expression, but has no influence on p53 mRNA level. A subsequent study suggests that the downregulation of MDM2 at both the transcriptional and posttranscriptional levels be responsible for the p53 activation, subsequently triggering cancer cell apoptosis. In addition, a xenograft study also validates that GA inhibits the growth of p53 wild-type tumors, but not p53-null tumors [193]. Further research by Rong *et al.* confirms the downregulation of MDM2 at both the transcriptional and posttranscriptional levels by GA [194]. GA reduces the MDM2 expression in a concentration- and time-dependent manner, regardless of the p53 status of the cells. In addition, both the P1 and P2 promoters of *mdm2* are responsive to GA, resulting in a decrease in the *mdm2* mRNA expression level. GA also increases the protein level of p21, regardless of the p53 status of the cells, probably due to the alleviation of the binding of MDM2 to p21 and the stabilization of p21. A further *in vivo* investigation shows that GA inhibits tumor growth in a mouse xenograft model, possibly by downregulating MDM2 [194]. Furthermore, several GA derivatives, such as gambogic acid [195], *N*-(2-ethoxyethyl)gambogamide [196], and *N*-(carboxy-

methyl)gambogamide [197], have been reported to have antitumor activities *in vitro* and *in vivo*. However, it remains to be determined if their anticancer activities can be attributed to MDM2 inhibition.

### 2.1.6. Diterpenes

Several reports have highlighted the anticancer efficacy of natural diterpenes, of which taxol is the best-known, as mitotic inhibitors that can be used for cancer chemotherapy [198]. In recent years, several natural diterpenes have been reported to exert their anticancer activities by modulating the p53/MDM2 pathway. Kiviharju *et al.* have demonstrated that low concentrations of triptolide (**11**), a diterpene triepoxide from *Tripterygium wilfordii* Hook. f., inhibit cell proliferation and induce cell senescence, and at high concentrations induce apoptosis, which is partly attributed to the nuclear accumulation of p53 [199]. Further studies show that triptolide exposure decreases the levels of both the MDM2 and p21 proteins [199]. Yang *et al.* report that triptolide inhibits MDM2 phosphorylation and increases p53 expression in a dose-dependent manner [200]. In addition, Carter *et al.* also report that triptolide is more effective for stabilizing p53 and activating p53 signaling than nutlin3a, an MDM2 inhibitor, and that it decreases the levels of MDM2 and XIAP [201]. Li *et al.* report a novel synthetic triptolide analog, (14*S*)-14,21-epoxytriptolide, has significant selective *in vivo* anticancer activity, especially against human prostate (PC-3) and ovarian (SK-OV-3) cancers, and has high efficacy against multidrug resistant cancer cell models [202]. (14*S*)-14,21-epoxytriptolide could be a promising candidate for further development of novel MDM2 inhibitors because of its chemical relationship with triptolide [202].

### 2.1.7. Diarylheptanoids

Curcumin (diferuloylmethane, **12**), a dietary polyphenol, is the major active ingredient of *Curcuma longa* (known as the spice turmeric). Increasing evidence has suggested that curcumin can affect numerous molecular targets and influence diverse biochemical and molecular cascades [169]. We have demonstrated that curcumin exerts anticancer activity by downregulating MDM2 transcription through the PI3K/mTOR/ETS2 pathway [203]. The p53-independent downregulation of MDM2 expression is seen in both human normal and cancer cell lines after treatment with curcumin, resulting in p21 upregulation. Further investigations in PC3 cells, including control (parental) PC3 cells, and PC3 cells with stable knockdown or overexpression of MDM2, have demonstrated that MDM2 expression is at least partially responsible for the p53-independent anticancer activity of curcumin, and that curcumin exposure also contributes to the sensitization of human cancer cells to chemotherapy and radiation through MDM2, regardless of the p53 status of the cells [203]. Moreover, the biological significance of curcumin has also prompted medicinal chemists to synthesize curcumin derivatives, which has led to several potent new candidate chemotherapeutic agents [204, 205]. A more thorough elucidation of the molecular mechanisms for these derivatives is still needed, considering that MDM2 expression may also be a target for these compounds. Much attention has been paid to various analogs of curcumin, such as diarylheptanoids [206]. The most potent diaryheptanoid, 1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)-4*E*-en-3-heptanone (**13**), induces S phase arrest and apoptosis by upregulating ATF3 and stabilizing p53 and downregulation of MDM2 [206].

## 2.2. Natural Products Blocking the MDM2-p53 Interaction

As a result of the elucidation of the crystal structure of the MDM2-p53 complex, especially the identification of the three crucial amino acids in p53 (Phe19, Trp23, and Leu26) required for the binding of these two proteins, hundreds of peptides, synthetic compounds, and natural products have been designed, synthesized, or screened for their ability to target the MDM2-p53 interaction, preventing MDM2 binding to p53, stabilizing p53 protein and

activating p53 functions. This strategy has resulted in several classes of MDM2 inhibitors under development. The examples of natural products that are currently known to block the MDM2-p53 interaction are listed in Fig. (2) and Table 3.

### 2.2.1. Chalcone Derivatives

Chalcones and their derivatives have exhibited broad spectrum and potent anticancer activities. Recently, this class of natural compounds has been revealed to inhibit the MDM2-p53 interaction by a two-site ELISA and multidimensional NMR spectrometry [207]. The ELISA data show that compounds **14** and **15** have the best inhibitory activities, with  $IC_{50}$  values of 49 and 117  $\mu\text{M}$ , respectively, and that compounds **16** and **17** have modest activities, with  $IC_{50}$  values of 206 and 250  $\mu\text{M}$ , respectively. Accordingly, NMR titration experiments also have demonstrated that these chalcone derivatives are bound in or near the tryptophan (Trp23) binding pocket of MDM2, and that compounds **14** ( $K_D = 90 \mu\text{M}$ ) and **15** ( $K_D = 150 \mu\text{M}$ ) have better activities than compounds **16** ( $K_D = 220 \mu\text{M}$ ) and **17** ( $K_D = 244 \mu\text{M}$ ). Their activities are attributed to the presence of the chlorophenyl group and augmented by increasing the number of chloro-substitutions [207]. In addition, among several newly synthesized chalcone derivatives tested for their anticancer activities is a boronic-chalcone derivative, 3,5-bis-(4-boronic acid-benzylidene)-1-methyl-piperidin-4-one (**AM114**, **18**), that has the most potent inhibitory activity [208]. AM114 treatment leads to p53 and p21 accumulation and apoptosis, but does not significantly disrupt the MDM2-p53 interaction [209].

### 2.2.2. Hexylitaconic Acid and its Derivatives

Hexylitaconic acid (**19**) is an itaconic acid derivative isolated from a culture of the marine-derived *Arthrinium* sp fungi, which was initially separated from a marine sponge from Toyama Bay in the Sea of Japan [210, 211]. The compound has been identified as a new inhibitor of the MDM2-p53 interaction by an ELISA assay, blocking MDM2-p53 interaction in a dose-dependent manner, with an  $IC_{50}$  value of 50  $\mu\text{g/mL}$  [210]. However, a SAR study measuring the inhibitory activities of three derivatives and two analogs of hexylitaconic acid, including a monomethyl ester (**20**), a dihydro derivative (**21**), a dihydro derivative of monomethyl ester (**22**), itaconic acid, and succinic acid, reveals that no significant inhibitory activities at the concentration of 50  $\mu\text{g/mL}$  [210].

### 2.2.3. Chlorofusin

Chlorofusin (**23**), isolated from the extract of the fungal strain *Microdochium caespitosum*, is considered to be among the first MDM2-p53 interaction inhibitor derived from a natural source [212]. Duncan *et al.* first reported its inhibitory effects ( $IC_{50} = 4.6 \mu\text{M}$ ) on the MDM2-p53 interaction by using the DELFIA-modified ELISA assay [212]. The results of that study prompted Duncan *et al.* to continue their investigation of the interaction between chlorofusin and the N-terminal domain of MDM2 by surface plasmon resonance. They found that it had an affinity constant ( $K_D = 2.11 \times 10^5 \text{ M}^{-1}$  or 4.7  $\mu\text{M}$ ) that was consistent with the  $IC_{50}$  obtained in their previous study [213]. As a result of the increasing interest in this exciting lead compound, total synthetic studies of this natural product have been carried out, leading to the reassignment of the chromophore-relative stereochemistry, assignment of the absolute stereochemistry, and a series of analogs and structural moieties have been constructed [214-220]. Clark *et al.* have evaluated the inhibition of MDM2-p53 binding by chlorofusin, its seven chromophore diastereomers, and several key analogs and partial moieties by the Searcey assay, showing that all of the tested chromophore diastereomers are effective inhibitors of the MDM2-p53 interaction, and three of them, (4*R*,8*S*,9*S*)-chlorofusin (**24**), (4*S*,8*R*,9*S*)-chlorofusin (**25**), and (4*S*,8*S*,9*R*)-chlorofusin (**26**), exhibit equipotent effects ( $IC_{50} = 8 \mu\text{M}$ ) with chlorofusin [220]. Two of these have unnatural 4*S* configurations and one of them is the enantiomer of the natural product. However, the cyclic peptide moiety and chromophore moiety of chlorofusin

and the simple derivatives synthesized using these features do not show significant inhibitory activities on the protein-protein binding. Based on these findings, it is concluded that neither the chromophore nor the absolute stereochemistry is essential for inhibiting the MDM2-p53 interaction.

### 2.2.4. Hoiamide and its Derivatives

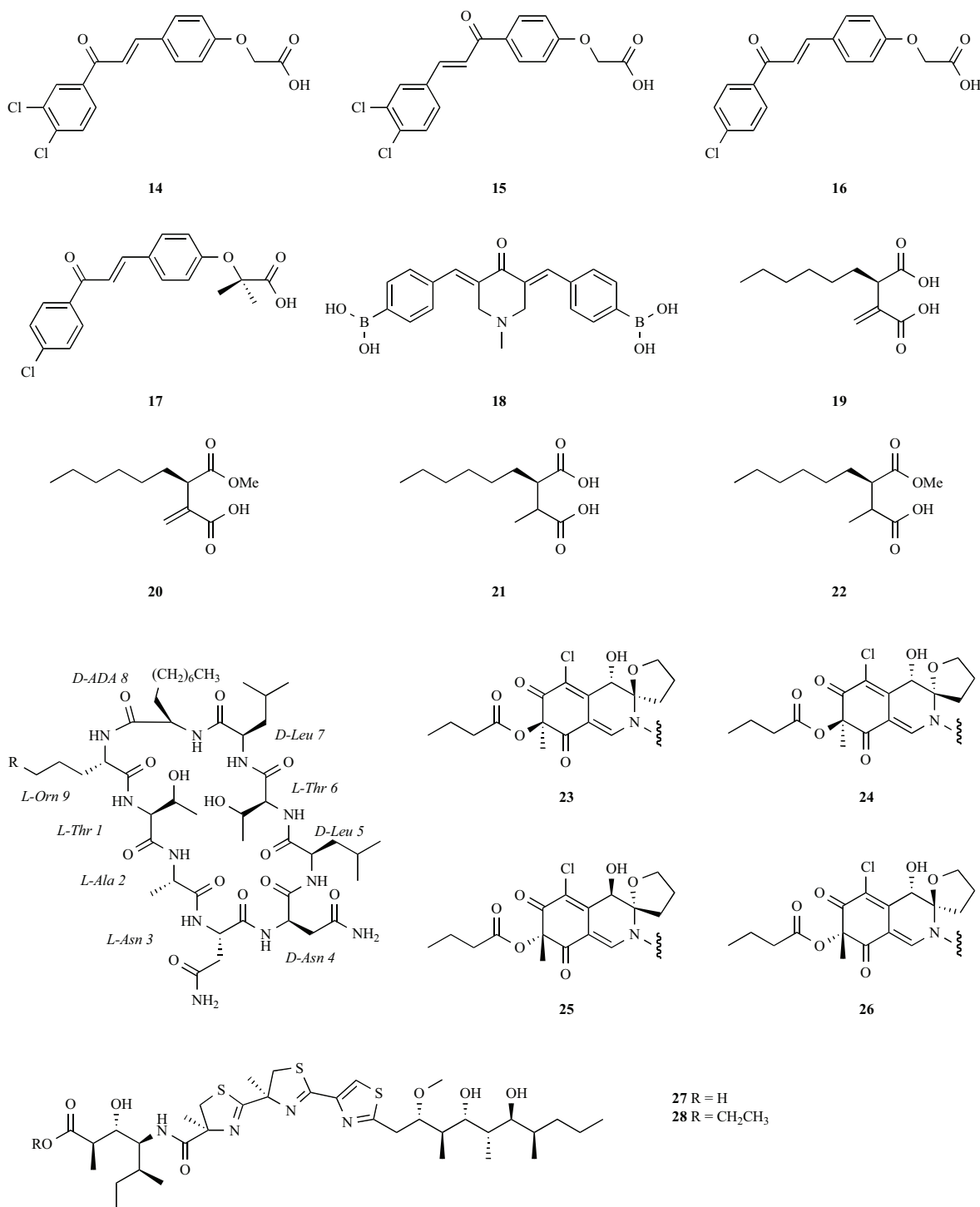
A bioassay-guided isolation of two cyanobacterial extracts from Papua New Guinea has led to the purification of hoiamide D (**27**) and C (**28**), two polyketide synthase/non-ribosomal peptide synthetase-derived natural products [221]. Hoiamide D is obtained in both its carboxylic acid and conjugate base forms, and hoiamide C is considered to be an artifact that formed during the extraction process. The inhibitory activity of hoiamide D in the carboxylic acid form on the MDM2-p53 interaction is examined by a HTR-FRET-based competition assay. The carboxylate anion exhibits significant inhibitory effects on MDM2-p53 binding, with an  $EC_{50}$  value of 4.5  $\mu\text{M}$ . However, this compound has minimal cytotoxicity to mammalian H460 cells at a concentration of 40  $\mu\text{M}$ . Even though there is no direct proof that hoiamide C inhibits MDM2-p53 binding, this compound may also be a potential inhibitor due to the structural relationships between the two hoiamides [221].

## 2.3. Natural Products that Modulate the E3 Ubiquitin Ligase Activity and/or Block the MDM2-Mediated Ubiquitination of p53

As the major negative regulator of p53, MDM2 controls p53 ubiquitination and subsequent degradation by its ubiquitin ligase (E3) activity, for which the C-terminal RING finger domain is required. An increasing number of studies have demonstrated that many small molecule inhibitors can specifically inhibit the E3 ligase activity of MDM2, preventing the degradation of p53, thereby stabilizing it. In this section, we review the natural product inhibitors falling into this category, and classify them based on their chemical structures (Fig. (3) and Table 3).

### 2.3.1. Sesquiterpene Lactone

Sesquiterpene lactones have been considered to be a promising natural resource for developing anticancer drugs, such as artemisinin and its derivatives, which mainly exert their anticancer activities by inducing apoptosis [222]. Several sesquiterpene lactones are reported to exert their anticancer activities by modulating the MDM2-p53 feedback loop. Gopal *et al.* report that parthenolide (**29**), 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 suppressors [223]. Their results demonstrate that parthenolide treatment induces an increase in p53 and MDM2 modification in a time- and dose-dependent manner and that the change in MDM2 coincides with the parthenolide-dependent HDAC1 depletion. Parthenolide induces the dissociation of p53-bound HDAC1 and the ubiquitination of p53-bound MDM2, resulting in p53 activation. In addition, the protein levels of PUMA and p21 are increased after parthenolide exposure, further confirming the activation of p53 [223]. In order to improve the water solubility of parthenolide (**29**), Nasim and Crooks have designed and synthesized a series of aminoparthenolide analogs through diastereoselective conjugate addition, by which several primary and secondary amines are added to the  $\alpha$ -methylene- $\gamma$ -butyrolactone moiety of parthenolide [224]. The anticancer activities of these 17 derivatives are evaluated in NCI 60 cancer cell lines. A tyramine derivative shows cytostatic effects and cytotoxicity against lymphoblastic leukemia cells, and a (*R*)-(1,2,3,4-tetrahydro-1-naphthyl)amino derivative exhibits cytostatic effects in human anaplastic large T-cell lymphoma cells at nanomolar concentrations [224]. Further studies on this class of natural product derivatives are needed, especially to determine their mechanism of action, and to elucidate whether the MDM2-p53



**Fig. (2).** The chemical structures of natural products that block the MDM2-p53 interaction. (14–17) Chalcone derivatives (No names reported); (18) AM14; (19) hexylitaconic acid; (20–22) hexylitaconic acid derivatives (No names reported); (23) chlorofusin; (24) (4*R*,8*S*,9*S*)-chlorofusin; (25) (4*S*,8*R*,9*S*)-chlorofusin; (26) (4*S*,8*S*,9*R*)-chlorofusin; (27) hoiamide D; (28) hoiamide C.

pathway is one of the major targets, since these derivatives are closely related to parthenolide.

### 2.3.2. Sempervirine (An Indole Alkaloid)

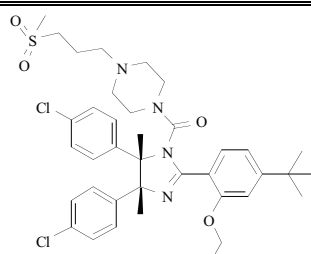
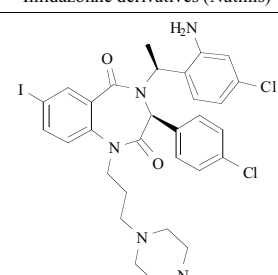
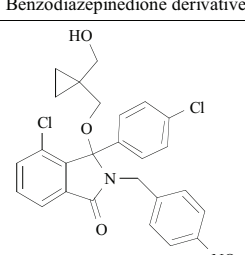
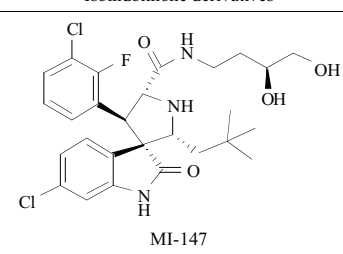
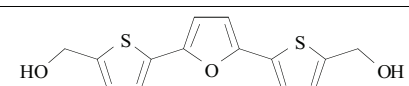
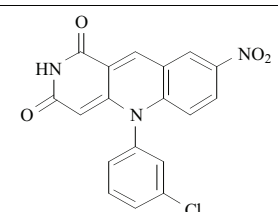
Sempervirine (30), a natural indole alkaloid, has been identified as an inhibitor of MDM2's E3 ligase activity from more than 144,000 natural product extracts evaluated by a high-throughput electrochemiluminescent screen [225]. Sempervirine inhibits the MDM2-mediated ubiquitination and degradation of p53, resulting

in the accumulation of p53. This compound also preferentially induces apoptosis in transformed cells and cancer cells expressing wild-type p53, warranting further studies of its anticancer activity [225].

### 2.3.3. Pyridoacridine Alkaloids

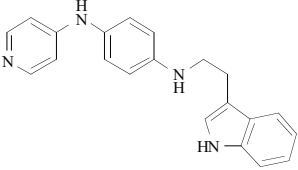
Using the same high throughput screening method described above, an extract of the ascidian *Lissoclinum cf. badium* from the coast of Papua New Guinea has been identified as an inhibitor of

**Table 2. Synthetic MDM2 Inhibitors and their Potential Targets**

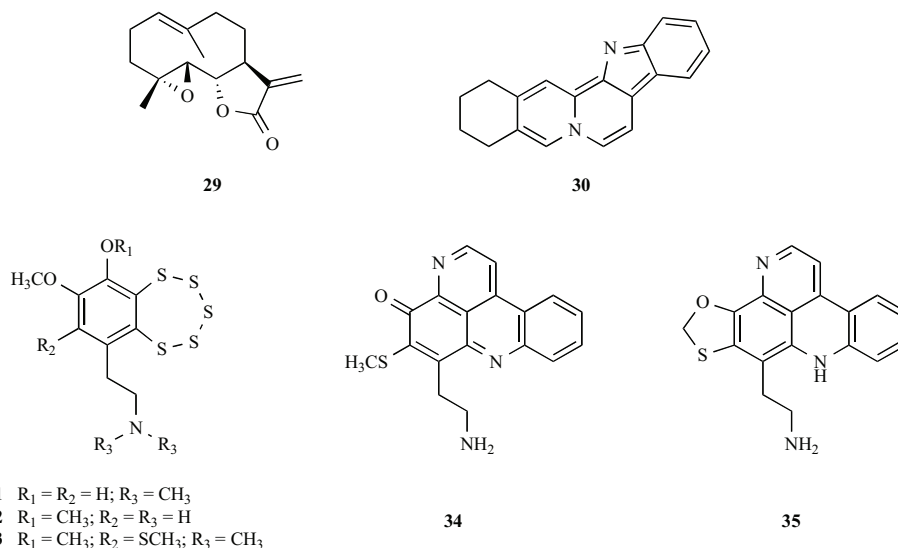
Name and Structure	Mechanism of Action	<i>In vitro</i> Evidence	<i>In vivo</i> Evidence	Current Status	Ref.
 <p>RG7112 Imidazoline derivatives (Nuttlins)</p>	Inhibits the MDM2-p53 interaction	IC <sub>50</sub> = 17 nM for the most potent compound, but not RG7112 (data not disclosed yet)	Not reported for RG7112	Phase I clinical trial for the treatment of solid tumors and hematological neoplasms, and treatment of leukemia	[141, 142, 144]
 <p>TDP665759 Benzodiazepinedione derivatives</p>	Inhibits the MDM2-p53 interaction	IC <sub>50</sub> = 0.7 μM	Inhibited tumor growth in A375 melanoma xenografts in female nude mice (synergism with Doxorubicin)	Preclinical development	[145]
 <p>Isoindolinone derivatives</p>	Inhibits the MDM2-p53 interaction	IC <sub>50</sub> = 0.17±0.2 μM	Not reported	Unknown/not reported	[146]
 <p>MI-147 Spiro-oxindole derivatives</p>	Inhibits the MDM2-p53 interaction	K <sub>i</sub> = 0.6 nM	Inhibited tumor growth in SJSA-1 and LNCaP tumor xenografts in nude mice.	Preclinical development	[143, 147]
 <p>RITA</p>	Inhibits the MDM2-p53 interaction by binding to p53	K <sub>D</sub> = 1.4 nM	Inhibited tumor growth in HCT116 and HCT116 p53 <sup>-/-</sup> xenografts in SCID mice	Unknown/not reported	[148]
 <p>HL198C 5-Deazaflavin derivatives</p>	Inhibits the E3 ligase	IC <sub>50</sub> = 20 μM	Not reported	Unknown/not reported	[137]



(Table 2) contd...

Name and Structure	Mechanism of Action	In vitro Evidence	In vivo Evidence	Current Status	Ref.
 JNJ-26854165 Tryptamine derivatives	Inhibits the E3 ligase	Not disclosed	Inhibited tumor growth in NCI-H1373 non-small cell lung cancer xenografts, PC-3M orthotopic prostate and p53 mutant HT-29 colon xenografts, and U87 glioblastoma xenografts	Phase I clinical trials for the treatment of advanced stage or refractory solid tumors in the USA and non-small cell lung cancer and prostate cancer in Europe.	[137, 149, 150]

IC<sub>50</sub>, Inhibition of MDM2 binding to p53, or inhibition of E3 ubiquitin ligase activity; K<sub>i</sub> or K<sub>D</sub>, Affinity constants.



**Fig. (3).** The chemical structures of natural products that modulate MDM2's E3 ubiquitin ligase activity and/or block the MDM2-mediated ubiquitination of p53. (29) Parthenolide; (30) sempervirine; (31) isolissoclinotoxin B; (32) varacin; (33) *N,N*-dimethyl-5-methylvaracin; (34) diplamine B; (35) lissoclinidine B.

MDM2's E3 ligase activity [226]. A subsequent study has led to the purification and identification of five novel pyridoacridine alkaloids, including isolissoclinotoxin B (31), varacin (32), *N,N*-dimethyl-5-methylvaracin (33), diplamine B (34), and lissoclinidine B (35). The MDM2-inhibitory activity of these five compounds are evaluated in a MDM2 electrochemiluminescence assay, demonstrating that isolissoclinotoxin B (31) has the best activity, with an IC<sub>50</sub> value of 58.6 μM, and lissoclinidine B (35) displays weaker activity, with an IC<sub>50</sub> value of 98.1 μM. However, a further study suggested that lissoclinidine B (35) inhibits the ubiquitination and degradation of p53, selectively kills transformed cells expressing wild-type p53, and could be a promising lead candidate for the development of new anti-MDM2 chemotherapeutic agents [226].

In summary, we see a variety of natural compounds obtained from diverse sources, which possessing highly dissimilar carbon skeletons have the capability of inhibiting the MDM2 oncoprotein. However, in most cases, we do not know if MDM2 inhibition is indeed a major anticancer mechanism for those compounds. This sort of information would be highly valuable while designing combination therapy and for devising methods to counter drug resistance. Studies involving elucidating the effect of a particular compound in presence or absence of MDM2 (*i.e.* involving MDM2 overexpressing stably transfected or inducible cell lines, MDM2 knockout systems) should be performed to understand the actual importance of MDM2 inhibition in mediating the anticancer effects of the drug.

### 3. FUTURE DIRECTIONS AND CONCLUSIONS

As discussed in the previous sections, MDM2 is one of the most important factors controlling the oncogenic process. In addition to being a negative regulator of p53, it interacts with several other proteins involved in cell cycle progression and growth. The disruption of the MDM2-p53 interaction with small molecule inhibitors has been validated as a potentially viable and attractive cancer therapeutic strategy. Current drug discovery efforts in this area are focused on the binding of p53 to the N-terminal domain of MDM2, which has been extensively characterized at the molecular level [148]. Less work has been done on the development of molecules to target other regions of MDM2, such as its RING finger domain. As detailed in this review [148] and other reviews [117, 129, 130, 227-230], there are numerous proteins in addition to p53 that interact with MDM2. Many of these interactions enhance MDM2's ability to downregulate p53, and their interaction with MDM2 should be explored as the basis for novel cancer therapeutic strategies. Targeting these interactions may provide alternative or complementary approaches to targeting the MDM2-p53 interaction. It is also necessary to focus on MDM2 itself as a drug target and not limit studies to its role in the MDM2-p53 feedback loop. The p53-independent oncogenic activities of MDM2 that can be investigated include, but are not limited to (i) its binding to, and destabilization of, p21, (ii) its binding to the p53 homologue, p73, (iii) its destabilization of NUMB, (iv) its positive modulation of the HIF-1 transcription factor, (v) its modulation of ribosomal proteins, (vi) role of MDM2 in modulation of apoptotic proteins,

**Table 3. Natural Product MDM2 Inhibitors and their Potential Targets**

No.	Compounds	Origin	Chemical Class	<i>In vitro</i> Effects	<i>In vivo</i> Effects	Target(s)	Ref.
1	Genistein	N	Isoflavonoid	Reduces MDM2 protein and mRNA levels in human cell lines of breast, colon, and prostate cancer; primary fibroblasts; and breast epithelial cells	Inhibits MDM2 expression and the growth of PC3 xenograft tumors	MDM2 Expression	[159]
2	Apigenin	N	Flavonoid	Downregulates MDM2 and inhibits the phosphorylation of MDM2 in ovarian cancer cells	Not Reported	MDM2 Expression	[163]
3	Oroxlylin A	N	Flavonoid	Downregulates MDM2 expression and interferes with the MDM2-modulated proteasome-related p53 degradation	Not Reported	MDM2 Expression	[164]
4	25-OCH <sub>3</sub> -PPD	N	Ginsenoside	Decreases MDM2 protein levels in cell lines of prostate, pancreatic, and lung cancers; Inhibits MDM2 transcription in pancreatic cancer cell lines	Inhibits the growth of PC3, Panc-1, and A549 xenograft tumors	MDM2 Expression	[166-169]
5	25-OH-PPD	N	Ginsenoside	Inhibits MDM2 expression in pancreatic and prostate cancer cell lines.	Inhibits the growth of Panc-1 and PC3 xenograft tumors	MDM2 Expression	[170, 171]
6	25-OH-PPT	N	Ginsenoside	Inhibits MDM2 expression in prostate cancer cell lines	Not Reported	MDM2 Expression	[170]
7	Berberine	N	Isoquinoline Alkaloid	Downregulates the MDM2 protein level and subsequently activates p53, but does not inhibit MDM2 mRNA expression	Not Reported	MDM2 Expression	[134]
8	FBA-TPQ	SN	Makaluvamine	Inhibits MDM2 expression in breast, colon, and ovarian cancer cell lines	Inhibits MDM2 expression and growth in MCF7 and OVCAR-3 xenograft tumors	MDM2 Expression	[177, 178]
9	BA-TPQ	SN	Makaluvamine	Inhibits MDM2 expression in breast cancer cell lines	Inhibits MDM2 expression and growth in MCF7 and MDA-MB-468 xenograft tumors	MDM2 Expression	[179]
10	Gambogic Acid	N	Xanthonoid	Downregulates MDM2 at both the mRNA and protein levels	Inhibits the growth of HepG2 and H1299 xenograft tumors	MDM2 Expression	[181, 182]
11	Triptolide	N	Diterpenoid	Decreases the levels of MDM2 and p21 proteins; Inhibits MDM2 phosphorylation, and increases p53 expression in a dose-dependent manner; Stabilizes p53 and activates p53 signaling	Not Reported	MDM2 Expression	[188-190]
12	Curcumin	N	Diarylheptanoid	Reduces MDM2 protein and mRNA and enhances p21 <sup>Waf1/CIP1</sup> protein expression	Inhibits the growth of PC3 xenograft tumors	MDM2 Expression	[192]
13	1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)-4E-en-3-heptanone	N	Diarylheptanoid	Inhibits MDM2 expression	Not Reported	MDM2 Expression	[195]
14	No Name Reported	ND	Chalcone Derivative	IC <sub>50</sub> = 49 μM; K <sub>D</sub> = 90 μM	Not Reported	MDM2-p53 Interaction	[196]
15	No Name Reported	ND	Chalcone Derivative	IC <sub>50</sub> = 117 μM; K <sub>D</sub> = 150 μM	Not Reported	MDM2-p53 Interaction	[196]
16	No Name Reported	ND	Chalcone Derivative	IC <sub>50</sub> = 206 μM; K <sub>D</sub> = 220 μM	Not Reported	MDM2-p53 Interaction	[196]
17	No Name Reported	ND	Chalcone Derivative	IC <sub>50</sub> = 250 μM; K <sub>D</sub> = 244 μM	Not Reported	MDM2-p53 Interaction	[196]
18	AM114		Chalcone Derivative	No significant inhibition of the MDM2-p53 interaction	Not Reported	MDM2-p53 Interaction	[197, 198]
19	Hexylitaconic Acid	N	Hexylitaconic Acid	IC <sub>50</sub> = 250 μg/mL	Not Reported	MDM2-p53 Interaction	[199]

(Table 3) contd....

No.	Compounds	Origin	Chemical Class	<i>In vitro</i> Effects	<i>In vivo</i> Effects	Target(s)	Ref.
20	No Name Reported	ND	Hexylitaconic Acid Derivative	No significant inhibition of the MDM2-p53 interaction	Not Reported	MDM2-p53 Interaction	[199]
21	No Name Reported	ND	Hexylitaconic Acid Derivative	No significant inhibition of the MDM2-p53 interaction	Not Reported	MDM2-p53 Interaction	[199]
22	No Name Reported	ND	Hexylitaconic Acid Derivative	No significant inhibition of the MDM2-p53 interaction	Not Reported	MDM2-p53 Interaction	[199]
23	Chlorofusin	N	Cyclic Peptide	IC <sub>50</sub> = 4.6 μM; K <sub>D</sub> = 4.7 μM	Not reported	MDM2-p53 Interaction	[201, 202]
24	(4 <i>R</i> ,8 <i>S</i> ,9 <i>S</i> )-Chlorofusin	ND	Cyclic Peptide	IC <sub>50</sub> = 8 μM	Not Reported	MDM2-p53 Interaction	[209]
25	(4 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> )-Chlorofusin	ND	Cyclic Peptide	IC <sub>50</sub> = 8 μM	Not Reported	MDM2-p53 Interaction	[209]
26	(4 <i>S</i> ,8 <i>S</i> ,9 <i>R</i> )-Chlorofusin	ND	Cyclic Peptide	IC <sub>50</sub> = 8 μM	Not Reported	MDM2-p53 Interaction	[209]
27	Hoiamide D	N	Hoiamide	EC <sub>50</sub> = 4.5 μM	Not Reported	MDM2-p53 Interaction	[210]
28	Hoiamide C	ND	Hoiamide	None reported	Not Reported	MDM2-p53 Interaction	[210]
29	Parthenolide	N	Sesquiterpene Lactone	Induces the dissociation of p53-bound HDAC1, ubiquitination of p53-bound MDM2, and p53 activation.	Not Reported	E3 Ubiquitin Ligase	[212]
30	Sempervirine	N	Indole Alkaloid	Inhibits the ubiquitination and degradation of p53 by MDM2, and leads to accumulation of p53	Not Reported	E3 Ubiquitin Ligase	[214]
31	Isolissoclinotoxin B	N	Pyridoacridine Alkaloid	IC <sub>50</sub> <sup>b</sup> = 58.6 ± 4 μM	Not Reported	E3 Ubiquitin Ligase	[215]
32	Varacin	N	Pyridoacridine Alkaloid	IC <sub>50</sub> <sup>b</sup> > 295 μM	Not Reported	E3 Ubiquitin Ligase	[215]
33	<i>N,N</i> -dimethyl-5-methylvaracin	N	Pyridoacridine Alkaloid	IC <sub>50</sub> <sup>b</sup> = 120.8 ± 9 μM	Not Reported	E3 Ubiquitin Ligase	[215]
34	Diplamine B	N	Pyridoacridine Alkaloid	IC <sub>50</sub> <sup>b</sup> = 101.3 ± 4 μM	Not Reported	E3 Ubiquitin Ligase	[215]
35	Lissoclinidine B	N	Pyridoacridine Alkaloid	IC <sub>50</sub> <sup>b</sup> = 98.1 ± 6 μM	Not Reported	E3 Ubiquitin Ligase	[215]

N, Natural product; ND, Natural product derivate; SN, Synthetic compound with a pharmacophore from a natural product; IC<sub>50</sub>, Inhibition of MDM2 binding to p53 measured by ELISA, DELFIA-modified ELISA assay, or Searcy assay; K<sub>D</sub>, Affinity constants between compounds and the N-terminal domain of MDM2 measured by NMR titration experiments or surface plasmon resonance; EC<sub>50</sub>, Inhibition of the MDM2-p53 interaction measured by HTR-FRET-based competition assay; IC<sub>50</sub><sup>b</sup>, Inhibition of the ubiquitin ligase activity measured by an electrochemiluminescence assay.

and (vii) interaction with androgen receptor (may be important in prostate cancer management).

Another area that needs a close attention is the role of MDM2 in cell migration and metastasis. Until now, most research on MDM2 has focused on its role in the degradation of p53 or its effects on the cell cycle and apoptosis. Studies by Yang *et al.* and Araki *et al.* proved that MDM2's influence spreads much farther and wider than was previously thought [96, 97]. Given that research into the epithelial-mesenchymal transition is still in its early stages, it is possible that MDM2 may be identified as a molecule that can be targeted to regulate this process as well.

Although MDM2 overexpression is oncogenic and is associated with a poorer prognosis in most situations, in certain scenarios overexpression of MDM2 may be associated with a good prognosis. For example, MDM2 overexpression correlates with a favorable prognosis in estrogen receptor alpha positive breast cancer [231]. Also, in malignant melanoma IGF-1R, an oncogenic receptor tyrosine kinase, can be downregulated by MDM2-mediated ubiquitination, targeting it for proteasomal degradation [113]. Thus, while there may be some stipulations to using MDM2 inhibitors for cancer therapy, in most scenarios, MDM2 inhibition appears to have potential as a novel and effective anticancer approach.

Further, MDM2 also inactivates the tumor suppressor pRb, and stimulates the E2F1/DP1 transcription factors, resulting in the promotion of the G1 to S phase transition [42, 85]. It has also been shown to confer a growth advantage to cells, even in the absence of p53 and pRb, causing them to overcome the G1 cell cycle blockade induced by p107 [232]. These examples are given to emphasize the crucial role that this molecule plays in the cell cycle machinery, and the numerous opportunities still remaining that could be investigated for anticancer drug discovery targeting MDM2.

In addition, MDM2 has several domains that can be additional targets for therapy, including its NLS, NES, and its C-terminal RING finger domain [10]. To date, most drugs have been investigated for their ability to inhibit the N-terminal domain-mediated interaction with p53 [142, 151]. Altering the posttranslational modifications, including phosphorylation and SUMOylation, may also represent novel approaches for inhibiting MDM2 [72]. The p53-binding domain has been extensively targeted, and a few agents have focused on the E3 ligase activity of the RING finger domain [143, 144, 148]. However, the other regions of MDM2, including the binding domains for other proteins, as well as the other functional domains, have not been considered. Also, recently, other domains in p53 that bind to

MDM2 (such as the C-terminus of p53) have been identified [49]. This information may also be potentially used to develop a new generation of small molecule compounds targeting these new sites. Further, the numerous MDM2-interactive (p53-dependent and -independent) proteins can also be targeted to improve the therapeutic response to cancers [129].

A recent study shows that the proteasome inhibitor velcade (also known as bortezomib), when combined with nutlin, exhibited synergistic activity against myeloma [233]. These results and other studies employing similar combinations, provide the framework for further development of MDM2 inhibitors that can be used either alone or in combination with conventional and novel therapies to increase the anticancer efficacy and improve the patient outcome.

Natural compounds present an attractive area for the development of MDM2 inhibitors. Unlike synthetic drug development, which can cost billions of dollars to develop from the bench to the bedside, many natural compounds have known safety and pharmacokinetic profiles; having been used as part of traditional medicines or in supplements. Their chemical diversity and complex structures can also serve as elegant progenitors for novel synthetic compounds. The natural product inhibitors of MDM2 have been shown to target both the p53-dependent and -independent activities of MDM2. Importantly, many of them have shown impressive activity in p53 mutant or non-functional tumor environments, thus eliminating the need for p53 dependent mechanisms to exert their effects. On the other hand, synthetic small molecule inhibitors (SMIs) of MDM2 are known to restore the apoptotic and cell cycle regulatory functions of p53 by disrupting the MDM2-p53 interaction. Therefore, in principle, these SMIs are ineffective against p53 mutant tumors, which is a hallmark of most cancers. Although their mechanisms need to be more clearly defined before definitive conclusions can be made, many of the naturally derived compounds appear to decrease MDM2 expression and/or activity in animal models, suggesting that they could be effective cancer therapeutic or preventive agents. However, what is lacking is a systematic study of chemically-related natural MDM2 inhibitors in established, well characterized cancer models to ascertain the relationships between the structures and inhibitory activities of these compounds. Such data could be used to develop novel semi-synthetic derivatives with improved efficacy, pharmacokinetic, and bioavailability profiles.

Targeting individual interactive molecules or the interaction of MDM2 with specific co-factors or regulators (dependent or independent of p53) can provide an effective therapeutic strategy. However, more research is needed to elucidate the role(s) of each of these interactions, and to define the circumstances under which the interaction(s) can be successfully targeted. The use of modern combinatorial libraries and high throughput screening techniques, coupled with an increasingly in-depth understanding of the biochemistry and molecular biology of MDM2 will help us to develop novel MDM2 inhibitors.

## CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

## ACKNOWLEDGEMENTS

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