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Review Article

REVIEW OF DESIGN APPROACHES AND CLINICAL PROGRESS OF MDM2 INHIBITORS

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ABSTRACT

Activation of the oncogenes and inhibition of the apoptotic function of the p53 protein is a gateway for the cancer genesis. Interaction of the MDM2 protein with p53 protein is responsible for the inhibition of the p53 function. Inhibiting the p53-MDM2 interaction by drug will lead to the p53 release in the cancer cells. And can restart the apoptosis in the cancer cell. Computational methods successfully used for the design and development of the new, potent MDM2 inhibitors. Researchers and pharma companies used rational approach like target-based drug design or ligand-based drug design to develop the novel MDM2 inhibitors. The number of MDM2 inhibitors, has been designed by the computer-aided drug design and in-silico studies. In clinical studies, MDM2 inhibitors are led by RG7112. RG7112 completed its phase-1 trials in 2016, and recently it is under phase-2 trials. Along with RG7112, the number of potent MDM2 inhibitors entered the clinical trials successfully. It indicates the successful development of this class (MDM2 inhibitors). MDM2 inhibitors were found very effective in various studies for the treatment of various kinds of cancers. They have good selectivity for the tumor cells over the normal cells. It induced the dose dependent cell cycle arrest only; in the normal cells. In studies, MDM2 inhibitors successfully detached the p53 protein from the MDM2 protein. And restart the cell-killing function of the p53 protein in the cancer cells. Hence, MDM2 inhibitors can selectively kill the cancer cells over the normal cells.

Keywords*:* cancer, p53-MDM2 interaction, MDM2 inhibitors, p53 activators, CADD, in silico study

INTRODUCTION

p53-MDM2 PPI is responsible for the inhibition of p53 and apoptosis in the tumor cells. It is very rational that; we can reactivate the apoptotic function of the p53 protein in the tumor cells by inhibiting p53-MDM2 interaction. So, reactivation of p53 will selectively kill the cancer cells[1].

p53 reactivation can be achieved using: (a) Inhibition of p53-MDM2 interaction (2) Direct activation of the mutant p53 protein function.
To successfully activate the p53 function in the cancer cells, we must have required a clear insight into the p53-MDM2 PPI[2]. To inhibit this important PPI in cancer cells, we need to explore the MDM2 protein and its structure.

With the advancement of the computation methods, we can easily study the MDM2 protein structure and p53-MDM2 complex. These structures and complexes can be evaluated by using chemo-informatics. Therefore, In-silico study and chemo informatics can be efficiently used to design the novel ligand (MDM2 inhibitors) by this data[3].

In silico means the study which is performed on the computer or via computer simulation. In addition to In vivo and In vitro, In silico study is related to biology and biological experiments. Though in silico study is a newer approach, it is most widely used to study and predict drug interaction with the body and pathogens[4].

Computer-Aided Drug Design (CADD) is mainly applied for drug design and drug discovery. It speeds up the process of drug discovery[5]. And by using this, we can bypass the expensive and tedious lab work and clinical trials. With the help of computer simulations, we can produce and screen drug candidates more effectively.

One of the best techniques of the in-silico study is the docking study. For the virtual screening of the hit compound or by reverse engineering to identify the target, the Docking method is most widely used. So, the best CADD method for drug design is molecular docking simulation. Through molecular docking, we can predict the binding of the ligand with the receptor (MDM2 protein) in 3D mode; and can score that binding with different orientations of the ligand[6]. By scoring function, we can predict the affinity (free energy of binding). When experimental structural information of the ligand and protein/target is not available, in that case; docking is helpful for the structural analysis of the ligand and protein interaction.

Following the discovery of the Nutlins (Figure 1), many MDM2 inhibitors/p53 activators have been reported. CADD method was found very helpful in the development of this class (MDM2 inhibitors). Various reported MDM2 inhibitors have been

designed and developed based upon the available details of the target protein (MDM2) binding site and existing reported ligands.

Researchers utilize this rational approach; they used the Ligand-based drug design or Target-based drug design to design newer ligands (MDM2 inhibitors).

DESIGN APPROACHES

Target-based drug design

MDM2 protein consists of a deep hydrophobic pocket in its structure. And this hydrophobic pocket has been packed/filled by the three side chains of the p53 protein (during the interaction)[7].

There are three main amino acids residues in the p53 side chain, which play a role in the binding with MDM2 protein; these three amino acids (Phe19, Trp23; and Leu26) sits/fit into the MDM2 pocket (3 pocket binding) during the PPI. Trp23 is important for the p53-MDM interaction because it forms the hydrogen bond with the MDM2 pocket; also having hydrophobic interaction with that[8].

The crystal structure of the p53-MDM2 complex, provides this binding site details of a target (MDM2 protein). From studying this complex, it is clear that there are three crucial binding sites on the MDM2 protein, that is Phen19 binding site/pocket, Trp23 binding pocket; and Leu26 binding site. For any MDM2 inhibitors, it has required to bind within these hydrophobic pockets (three-pocket binding)[9].

These data provide the basis for the designing of the newer ligand (MDM2 inhibitors). CADD method and HTS screening have been used to identify the ligands which can bind in these three hydrophobic pockets.

Using the CADD technique, we can design the compounds that are identical with the three residues and topology of the side chains of the p53 protein. Hence, it will easily mimic the p53 protein by three pocket binding in MDM2 protein. That is how we can spare the binding of the p53 protein with the MDM2 protein, and p53-MDM2 PPI can be inhibited[10].

Researchers at various pharmaceutical companies have to identified the various structural moiety, which can bind/fit into these three MDM2 protein pockets at the active site. The special orientation of these moieties; has been defined by the docking simulation. Then joining these structural moiety/motifs together with the backbone provided the newer compounds of this class which can bind with MDM2 protein pockets (Figure 1).

After designing the ligand, the next step is to quantify its interaction with the active site of the MDM2 protein. The ligand interaction with receptor protein can be characterized by binding affinity (binding free energy). Molecular docking is the most popular CADD technique, to estimate the binding affinity between ligand and receptor (MDM2 protein).

High binding affinity represents the tight binding with target and strong inhibitors of the MDM2 protein. Hence, good binding affinity inhibitors can easily reactivate the p53 function and trigger the p53 dependent apoptosis in the cancer cells[11].

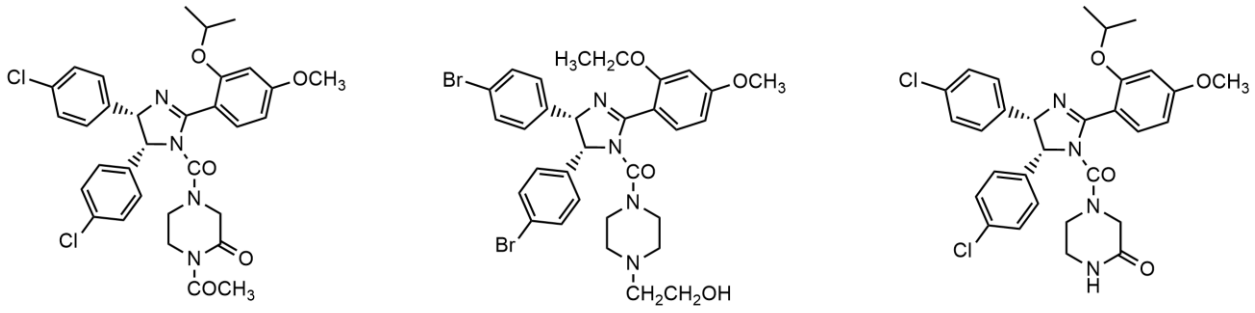
Many reported MDM2 inhibitors had been developed based upon the data of the MDM2 active site/binding site (Figure 1).

Ligand-based drug design

In 2004, Vassilev and his colleagues at Roche Pharmaceutical reported the first potent MDM2 inhibitor, "Nutlin", which can reactivate the p53 function by MDM2 inhibition. Nutlin is a cis-imidazoline analogue and having the IC50 value of 100 to 300 nM[12].

Nutlin is a group of compounds (Figure 1), which contains the nutlin 1, nutlin 2 & nutlin 3. They all had different MDM2 inhibition potency. As of nutlin 1 and 2, nutlin 3 is also the stereo-active compound. In nutlin 3, one of its enantiomer nutlin 3a is 150 times more active than its enantiomer 3b. Nutlins class of compounds can successfully mimic the p53 side chain's residue; and it is having three-pocket binding[13].

Superimposition of 3D structure of Nutlin 2 on the co-crystal structure of MDM2 protein shows that (Figure 3), one bromo-phenyl ring of the nutlin 2 fits into the Trp23 pocket of MDM2 protein. The other bromo-phenyl ring sits into the Leu26 pocket and the ethyl ether side chain directed towards the Phen19 pocket[14] (Figure 3). So, it is showing the three-pocket binding (Figure 2).



Nutlin 1 **Nutlin 2** **Nutlin 3a**

Figure 1: Structure of Nutlins

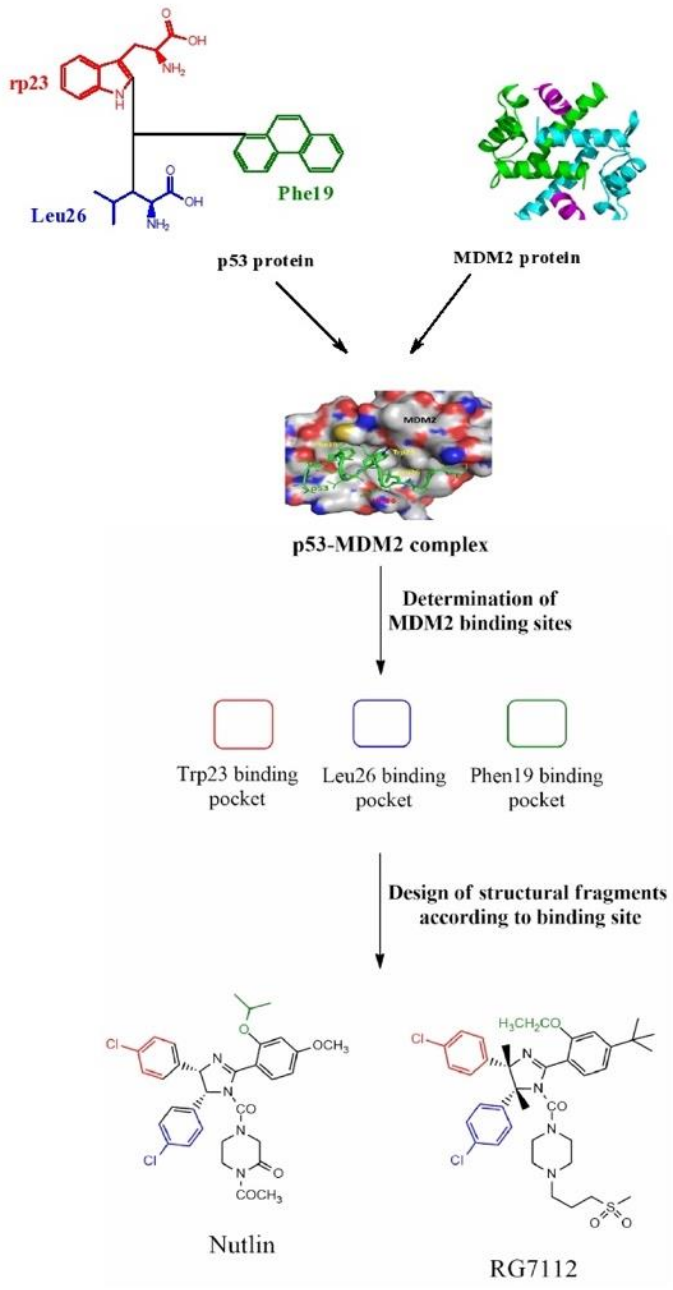


Figure 2: Target Based Drug Design Approach

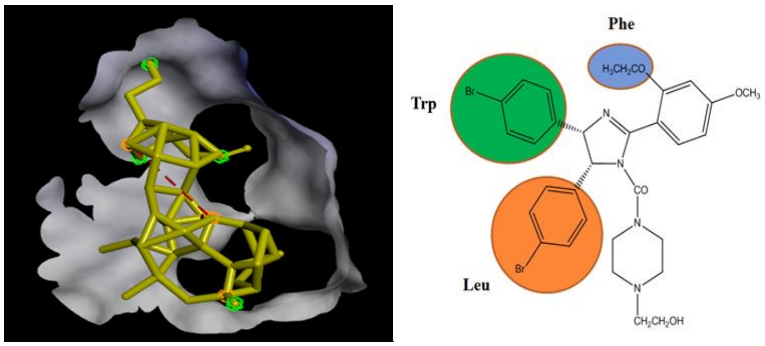


Figure 3: Crystal Structure of the Nutlin-2 bound MDM2 Protein[15]

This study provides the basis for the ligand-based drug design of the novel MDM2 inhibitors. From the Nutlin 2 binding data, it is clear that; two halo-phenyl rings and one alkyl ether containing phenyl ring are necessary for the three pockets binding into the MDM2 protein. And all reported non-peptidic MDM2 inhibitors consist of the central scaffold. The central scaffold provides the backbone to the compound[16]. The central scaffold may contain the heterocyclic ring, annulated ring, and acyclic linear moieties (in some compounds).

A number of MDM2 inhibitors, were designed based upon the Nutlin template (Figure 4). Using the ligand-based drug design approach, we can design the MDM2 inhibitors, which have the structural features the same as the nutlins[17].

With the help of CADD techniques, the researcher replaces the crucial structural motifs (two halo-phenyl rings and one alkyl ether containing phenyl ring) of the nutlin with other equivalent motifs. Isosteric replacement of the nutlin's crucial motifs gives the newer structural fragments/moieties[18]. Putting all the moieties required for the three-pocket binding, then all these moieties will be joined with the central scaffold (heterocyclic ring).

So, ultimately newer pharmacophore has been developed. After evaluating the pka (logP) value of the new pharmacophore, a polar tail may be added to it. The design molecule must have required the polar side chain for the good logP value and bioavailability[19]. These results in newer MDM2 inhibitors (Figure 4). Like nulins, most of the inhibitors have multiple chiral centres.

Recently, we are working on the development of this class of compounds.

CLINICAL STUDY OF MDM2 INHIBITORS

After discovering the Nutlins, numbers of p53-MDM2 interaction inhibitors have been reported by various researchers and companies. The reported MDM2 inhibitors either contain natural moieties or synthetic moieties[20].

RG7112 (R7112, RO5045337) was the first compound to enter the phase-1 clinical trials in 2012. RG7112 contains the cis-imidazoline derivative, so chemically; it is similar to the nutlins. Hoffmann-La Roche pharmaceuticals were obtained by the lead optimization of the nutlins[21].

RG7112 is an orally administered agent. It inhibits p53/MDM2 interaction and proteasomal degradation of p53. So p53 getting stabilized and reactivate the p53 function in the cancer cells[22].

The substantial research results into the number of potent compounds of this class. And many of the p53 activators have been successfully entered into the clinical trials. Structures and IC50 values of the various p53 activators (via MDM2 inhibition) which are under clinical trials are documented in Table 2 and Figure 5.

The IC50 values of the MDM2 inhibitors which are under clinical trials are documented in Table 2[10].

Table 1: MDM2 Inhibitors under Clinical trials[22-26]

Types	Compound	Phase of Clinical Trial	Status	Originator
Non-peptidic, organic small molecules	RG7112 (RO5045337)	2	Active	Hoffmann-La Roche
	RG7388 (RO5503781) (Idasanutlin)	1	Active	Hoffmann-La Roche
	MI-77301 (SAR405838)	1	Active	University of Michigan, Advanced into clinical trials by Sanofi in 2012
	MK-8242 (SCH 900242)	1	Active	Merck
	AMG232	1	Active	Amgen Biopharma
	DS-3032b	1	Active	Daichi Sankyo
	HDM201	1	Active	Novartis
	CGM097	1	Active	Novartis
Stapled Peptides	ALRN-6924	1	Active	Aileron Therapeutics and Roche

Table 2: IC50 values of the MDM2 inhibitors

Compound	IC 50 value (nM)
RG7112 (RO5045337)	18
RG7388 (RO5503781) (Idasanutlin)	06
MI-77301 (SAR405838)	100-200 (Cell lines: SJSA-1, RS411, LNCaP, HCT116)
MK-8242 (SCH 900242)	20
AMG232	0.6
DS-3032b	5.57
HDM201	0.21
CGM097	1.7

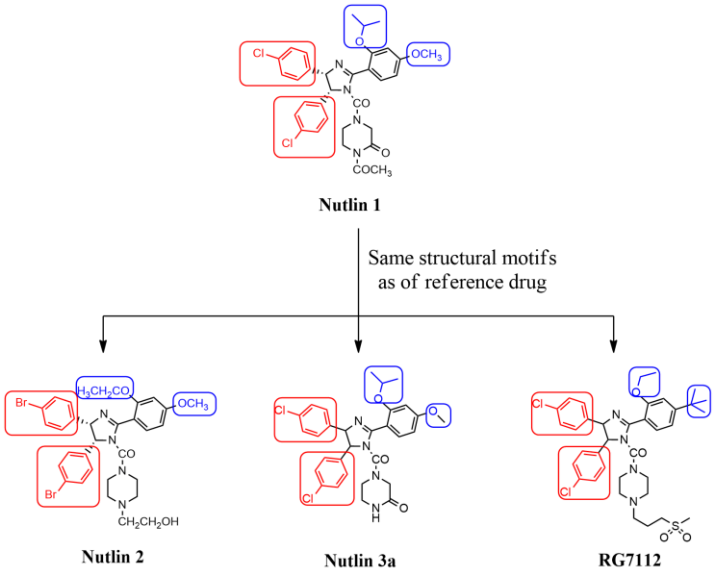


Figure 4: Ligand Based Drug Design Approach

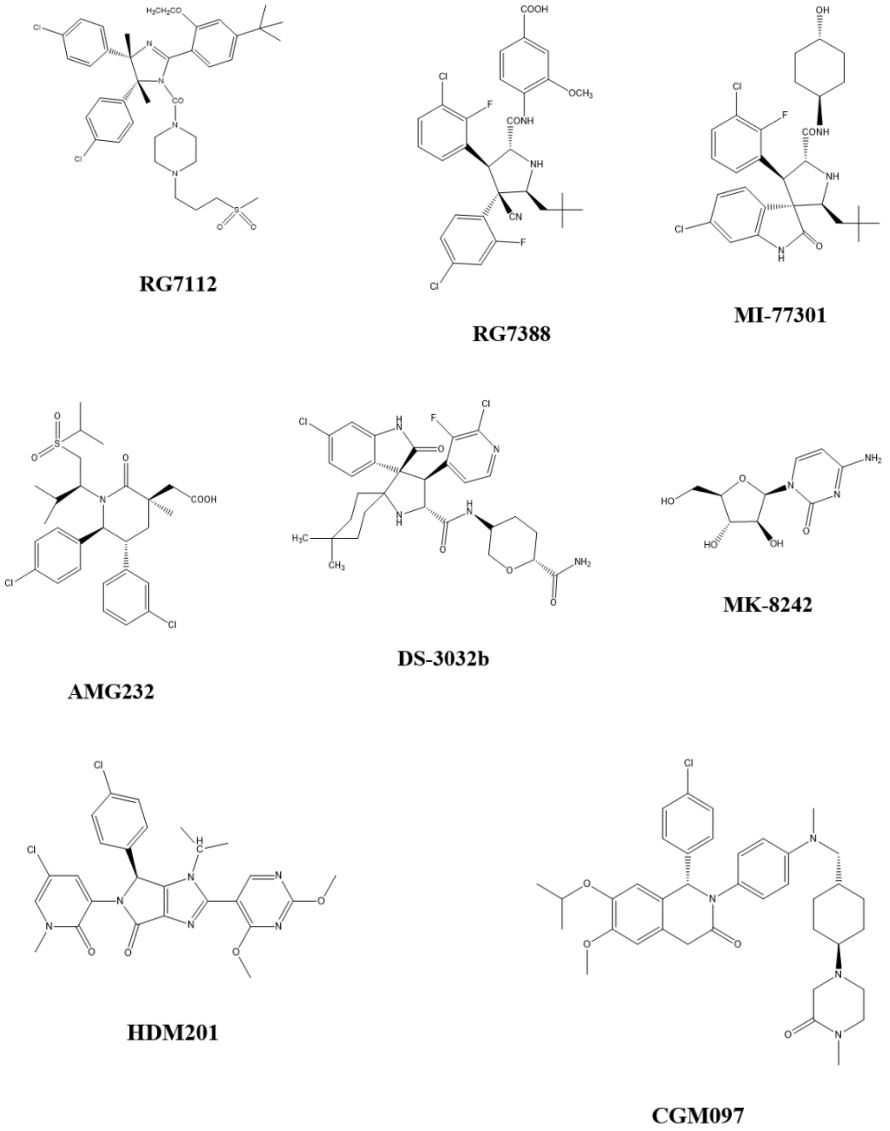


Figure 5: Structures of the MDM2 Inhibitors under Clinical trials[10, 23-26]

The first eight compounds in Table 1 are small molecular MDM2 inhibitors. They are non-peptidic small organic molecules. Many small molecule MDM2 inhibitors are at the upper limits of a molecular weight cut-off (Lipinski's rule of five)[27].

The last compound in Table 1 is a staple peptide type MDM2 inhibitor.

Alrn-6924

It is synthetic peptides (stapled) in nature. Synthetic peptides have been designed based on the template of p53 protein. So, synthetic peptides have a structural similarity to the p53 sequence with an acetylated N-terminus to permit entry into the cells. That's why; they can mimic the conformation of the p53 helix and cause non-genotoxic activation p53 function. These types of inhibitors have the advantage of high specificity, potency; and low toxicity. These synthetic linear peptides can adopt a helical conformation and inhibit the MDM2/p53 interaction[18].

The use of this linear peptide as drug (MDM2 inhibitor) has faced some problems, which are as follows[18] : (1) Peptide can adopt random conformations in solution. (2) Peptide suffers from low cell permeability. (3) Peptides are proteolytically unstable.

Stapling of hydrocarbon with the peptide can overcome these limitations. The hydrocarbon linker holds the peptide in a helical conformation, so it will always be in the helical conformation; and it can be able to bind to MDM2 permanently. Hydrocarbon stapling will inhibit proteolytic degradation and increase cellular uptake[27].

BIOLOGICAL EFFICACY OF THE MDM2 INHIBITORS

Most of the anti-neoplastic drugs trigger the indirect activation of the p53 protein function. The effect of every anti-neoplastic drug is only because of indirect activation of the p53 function. As p53 is a tumor suppressor and apoptotic in nature, so its level rises in the cells, it kills the cancer cells via apoptosis[1]. At the same time, MDM2 inhibitors induced the direct activation of the p53 protein and apoptosis in cancer cells.

MDM2 inhibitors should have the followings effect in the cells: (a) Degradation and nuclear export of the p53 protein must be prevented by the compound. (b) The compound can induce cell-cycle arrest in the G1and G2 phases and/or apoptosis[28].

Clinical data evidence that; p53 activators can liberate the p53 protein from the MDM2 protein. So p53 getting accumulated in the nuclei of cancer cells. And this will reactivate the p53 function as well as its pathways. And ultimately, proliferating cancer cells have been effectively blocked in G1 and G2 phases (G2/M phase fraction and nearly complete depletion of the S-phase)[12]. These effects trigger cell cycle arrest and cell death/apoptosis in cancer cells.

The short-coming in this approach is that p53 function and apoptosis have only occurred in the cancer cells which bear the wild-type p53 protein, not in the cancer cells which contain the mutated/deleted p563 protein. Therefore, low cytotoxicity occurs in the cancer cells, which contain the mutant p53 protein.

CONCLUSION

Inhibition of the p53-MDM2 PPI is the most promising approach for cancer therapy. By using this approach, we can selectively target the cancer cells. And we can protect the normal cell. p53 activation can be achieved by many strategies. After the discovery of the nutlins in 2004, a number of compounds have been reported from the natural origin as well as from the synthesis. Design and subsequent development of the reported MDM2 inhibitors up to the lead compound by the CADD techniques is proved as efficient. In-silico methods like docking have been implemented by the researchers for the development of novel MDM2 inhibitors. Their logP profile, bioavailability, and cell permeability to the target cells are important parameters for the clinical developments of the MDM2 inhibitors. The clinical progress of MDM2 inhibitors is good. Total nine MDM2 inhibitors are recently under clinical trials. Clinical data shows good potency of the MDM2 inhibitors to kill the cancer cells. MDM2 inhibitors selectively blocked the tumor cells in G1 and G2 phases and ultimately killed them. They show a low toxicity profile to the normal cells. So, its arise hope for the development of a new and potent class of anticancer agents. On the other hand, even though many MDM2 inhibitors does not follow the Lipinski rule for the molecular weight, they have shown the promising result in the clinical trials. But all the results and data of clinical trials are preliminary. So, it requires complete clinical trials and confirmatory studies to evaluate its role in cancer therapy.

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