# TYPES OF p53/MDM2 INTERACTION INHIBITORS

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**ABSTRACT**

p53 protein maintains the normal apoptosis process in body. Override of p53 function, leads to inhibition of apoptosis and occurrence of cancer. Interaction of p53 protein with MDM2 protein is responsible for inhibition of p53 function. Hence to inhibit this interaction is a good strategy for the cancer therapy. This interaction can be inhibited by antagonist of the MDM2 protein. 3 interacting points (i, i+4, i+7) are there on α-helix of p53 protein. And there are 3 main region or pockets present on the MDM2 protein (Trp 23, Leu 26 and Phe 19). Through which p53 binds with MDM2 protein and interact with each other. For the successful design of the MDM2 inhibitors, it should be shows the 3 pocket binding (Trp 23, Leu 26 and Phe 19 pockets of MDM2). Nutlin was the first MDM2 inhibitor. Nutlin class of compounds and many other compounds have been reported till now. There are three main types of MDM2 inhibitors. Types I inhibitors includes the synthetic peptides, which mimics the conformation of p53 helix. Type II inhibitors includes the non-peptide compounds, which have been further subdivided as Non α-helix mimetics (small molecule inhibitors) and α-helix mimetics. Type III inhibitors covers the natural products like Chlorofusin and its analogues. Compounds belong to Type II (a) have been successfully entered into clinical trials. Much efforts and works has been required for the design and discovery of more MDM2 inhibitors. Optimization of the reported compounds is required, so that apart from type II (a), other types of compounds can also be entered into the clinical trials.

**KEYWORDS:** Cancer, Apoptosis, p53 protein, MDM2 protein, p53/MDM2 interaction inhibitors, types of MDM2 inhibitors, Nutlin.

## INTRODUCTION

p53/MDM2 interaction is responsible for the inhibition of the p53 function. Subsequently, inhibition of the p53 activity will inhibit the natural apoptosis process. And it will leads to cancer. Hence p53/MDM2 interaction is responsible for the occurrence of the cancer.

That's why p53/MDM2 interaction is a potential target for the cancer treatment. There are various approaches to inhibit this interaction. Among them, p53/MDM2 interaction inhibitors or MDM2 inhibitors is prominent approach.

## p53/MDM2 INTERACTION INHIBITORS AND THREE POCKET BINDING

p53 and MDM2, both are protein. This protein-protein interaction is responsible for the cancer genesis. p53 protein has interact with MDM2 protein via α-helical segment. There are 3 main point (i, i+4, i+7), through which p53 protein interact with MDM2 protein. These 3 points of p53 will bind/fits into the 3 different pockets of MDM2 protein. These 3 pockets of MDM2 protein are, Trp 23 pocket, Leu 26 pocket and Phe 19 pocket. So if any compound can mimic the 3 point of α-helical segment of p53, it will bind/fit into the respective 3 pockets of MDM2 protein and will inhibit the p53/MDM2 interaction. 3 pocket binding is necessary for the development of any p53/MDM2 interaction inhibitors. This is a newer approach, so there is not any compound is present in market. Some of the compounds of this class are under clinical trial now. There are mainly 3 type of inhibitors, which are as follow.
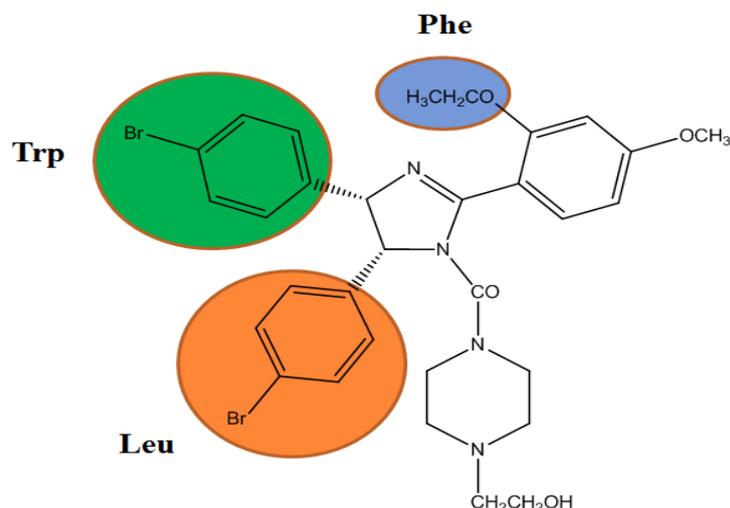
**Figure-1: Three Pocket Binding of Nutlin 2**

**(1) Type I Inhibitors**
These types of inhibitors are synthetic peptides. These peptides are having a structural similarity to the p53 sequence with an acetylated N-terminus to permit entry into the cells. So they can mimic the conformation of the p53 helix and cause non-genotoxic activation p53 function. These types of inhibitors have an advantage of the possibility of high specificity, potency and low toxicity. These synthetic linear peptides can adopt a helical conformation and inhibit the MDM2/p53 interaction.

Use of this linear peptide as drugs has faced some problems, which are as below:
(1) Peptide can adopt random conformations in solution.
(2) Peptide suffers from low cell permeability.
(3) Peptides are proteolytically unstable.

Strategy for overcome this problems is to staple the hydrocarbon with the peptide. Hydrocarbon linker holds the peptide in a helical conformation so it will be always in the helical conformation. and it can be able to permanently bind to MDM2. Hydrocarbon stapling will inhibit the proteolytic degradation and increase the cellular uptake.

Other strategy to overcome the rapid enzymatic degradation is to use D-amino acids for synthetic peptides chain. This type of peptides are far more stable to proteolytic degradation, as enzymes present in the body are only capable of processing L-amino acids due to their stereo-specificity

So, there are some of the approaches to stabilized the peptides. Though many of peptides are reported, which inhibits p53/MDM2 interaction. But there are currently no peptide based inhibitors in clinical trials.

SAH-p53-8 is an example of a stabilized peptide used as an inhibitor of MDM2 protein, showing activity both in vitro with an IC50 of 216 nM in a biochemical assay and in vivo. Its structure is shown in below figure. In the figure curved lines represent the 3 pockets. (Phe, Trp, Leu pockets).
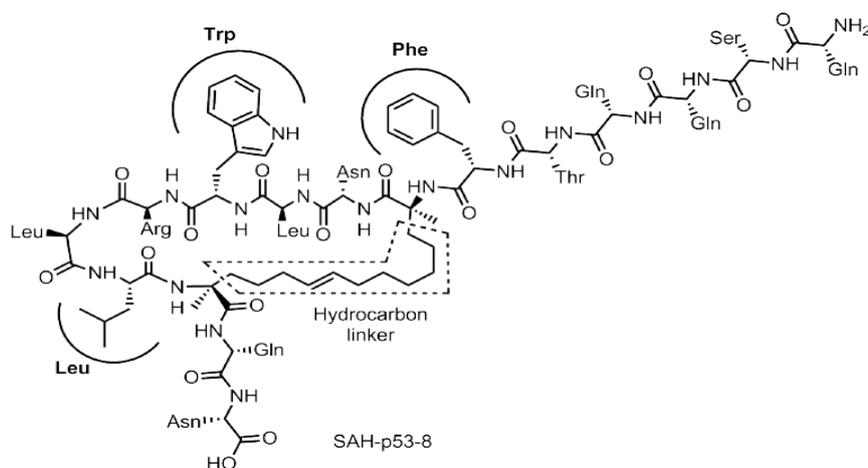


**Figure-2: SAH-p53-8[16]**

## (2) Type II Inhibitors

The compounds, which comes under this types are non-peptide in nature. Further they are subdivided as Non α-helix mimetics and α-helix mimetics.

### (a) Non α-helix mimetics (Functional mimetics)

These types of inhibitors are small non-peptide compounds that place substituents in the same spatial orientation as the p53 helix. They do not mimic the α-helix topography. These types of compounds are the competitive inhibitors of MDM2 protein.

By High Throughput Screening (HTS) campaigns at Hoffmann-La Roche, Vassilev and colleagues were discovered the first compound of this class called as Nutlin. Nutlin contains the cis-imidazoline moiety. Nutlins mimic the three main amino acid residues on p53 (i, i+4, i+7), involved in its interaction with MDM2. Hence, it shows 3 pocket binding. And inhibits the p53/MDM2 interaction. Nutlin group of compounds contains three compounds, Nutlin-1, Nutlin-2 and Nutlin-3 Among them, Nutlin-3 is the most potent compound (IC 50 – 90 nM).

Superimposition of 3D structure of Nutlin on the co-crystal structures of MDM2 protein, shows that one bromophenyl moiety sits deeply in the Trp 23 pocket, the other bromophenyl group occupies the Leu 26 pocket, and the ethyl ether side chain is directed toward the Phe 19 pocket (3 pocket binding).

Assay of Nutlin on various cell lines, shows the dose dependent MDM2 inhibition and activation wild type p53 protein function (not in a cells which contain the mutated or deleted p53). In normal cells, it induced the dose dependent cell cycle arrest but not the cell death. So, small-molecule inhibitors of the p53/MDM2 interaction could provide non-genotoxic therapeutic options for activating p53 function.

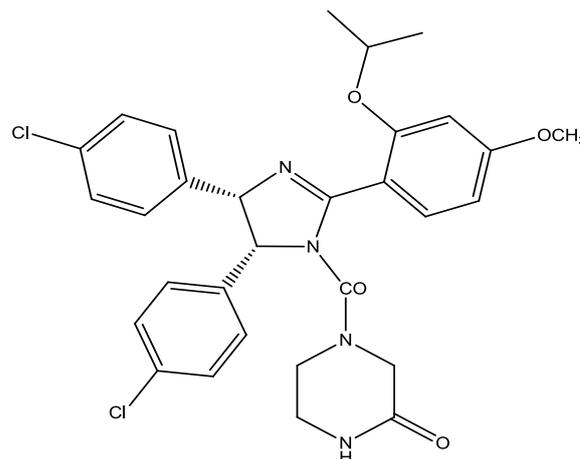Currently, there are total seven compounds of this class, which are under clinical trials.



**Figure-3: Nutlin 3a**

### (b) α-helix mimetics

These types of inhibitors are non-peptide compounds. And mimics the α-helix. They mimic the topography of α-helix and can position the substituents in the same spatial orientation as the 3 different interaction points of p53 helix. With contrast to Functional mimetics, these types of inhibitors are generally more extended.

Hamilton and co-workers discovered the first compound of this class (type-II α-helix mimetics). And it was the terphenyl derivatives. Other reported compounds of this class contains the various chemical derivatives like pyrrolopyrimidine derivatives, spirooligomer derivatives and oligobenzamide derivatives. Oligobenzamide scaffold was reported by wilson and co-workers.

This class of compounds have a good potential of inhibiting the p53/MDM2 interaction, but there are currently no inhibitors from this class present in the clinical trials.
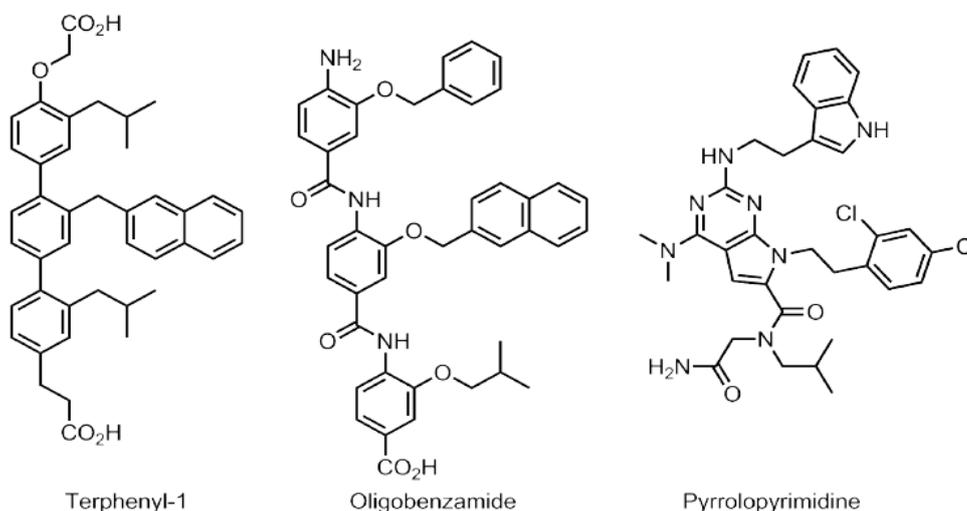


Terphenyl-1          Oligobenzamide          Pyrrolopyrimidine

**Figure-4: Examples of type II (α-helix mimetics) Inhibitors[16]**

**(3) Type III Inhibitors (Chlorofusin)**

It is a natural product and can inhibit the p53/MDM2 interaction. Chlorofusin was first isolated in 2001 by Duncan from the Fusarium sp. Microdocium caespitosum, a type of marine sponge.

Its structure has not been fully confirmed. But the proposed structure of chlorofusin contains the unnatural cyclic peptide and chromophore moieties. In which, azaphilone derived chromophore linked through the terminal amine of ornithine to a cyclic peptide composed of nine amino acid residues. Two of the cyclic peptide amino acids possess a nonstandard or modified side chain, and four possess the D-configuration.

The full peptide and azaphilone structure are required for inhibition of the interaction between MDM2 and p53.

Chlorofusin is p53/MDM2 interaction inhibitors from natural origin. But it has required the little work regarding its detail structure and its potency of binding with MDM2 protein. Currently, there are no chlorofusin based inhibitors present in the clinical trials.
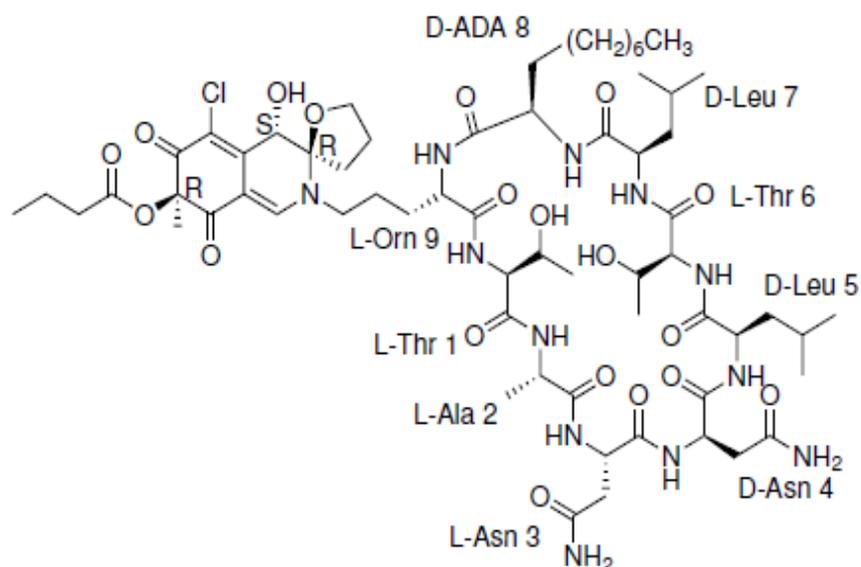


**Figure-5: Chlorofusin**

**DISCUSSION**

From the discovery of Nutlins, many potent MDM2 inhibitors have been discovered. It includes various types of MDM2 inhibitors, like peptidic, non-petidic, α-helix mimetics and non α-helix mimetics. Since this is a newer class so clear classification is not done yet. But generally compounds are divided in the three main types. Though compounds pertaining to three main types can potentially inhibit the MDM2 function, only non-peptide small molecule inhibitors [type II (a)] have been progressed through preclinical and early phase clinical studies. Still there is a need for discovery of the new compounds and optimization of already discovered compound.

**Footnotes**

p53/MDM2 Interaction Inhibitors and MDM2 Inhibitors, both are same.

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