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Insilico designing and development of potent drug inhibitor to MDM2 protein in cancer through molecular docking studies

[*]Pravallika vadlamudi[1], Manas Ranjan Barik[2].

[1]Department of pharmaceutical Chemistry, Nimra college of pharmacy, Vijayawada, A.P., India
[2]DNA LABS INDIA, Hyderabad, A.P., India.

ABSTRACT

Cancer is a class of diseases characterized by out-of-control cell growth. Cancer is a leading cause of death worldwide.The p53 tumor suppressor is one of the principal mediators of cell-cycle arrest and the activation of apoptosis in response to cellular injuries . In normal unstressed cells, p53 is regulated by a feedback loop with the negative regulator protein MDM2 (murine double -minute clone 2, referred to as human double –minute clone 2, HDM2 , in humans) . A well -known mechanism for the loss of wild –type p53 activity in cancer cells is the overexpression of MDM2.The murine double minute 2 (MDM2) protein facilitates G1 to S phase transition by activation of E2F-1 and can enhance cell survival by suppressing wild-type p53 function.Murine DM2 (MDM2) protein is overexpressed in a variety of neoplasms, including acute leukemias, myelodysplastic syndrome, chronic lymphocytic leukemia and lymphomas , multiple myelomas etc. Blocking the MDM2-p53 interaction to reactivate the p53 function is a promising cancer therapeutic strategy. Activation of the p53 protein protects the organism against the propagation of cells that carry damaged DNA with potentially oncogenic mutations. This can be attained by designing a molecule which can bind to P53 transactivation site of Mdm2 and further this Mdm2 protein cannot bind with P53 .The aim of present study is designing a small molecule(antagonist) having capability to bind with the over expressed Mdm2 protein and blocking its path to bind with p53 tumour suppressor protein that is having sufficient absorption and free of hepatotoxicity and carcinogenicity.

Keywords: cancer, MDM 2protein, , ADME Docking.

INTRODUCTION

Nearly 40 000 articles published in the past 26 years have established the tumor suppressor p53 as one of the most important molecules in human cancer [1,2]. The main function of p53 is to organize cell defence against cancerous transformation. In this complex role, p53 coordinates a signal transduction network, the p53 pathway, that evolved to minimize the consequences of oncogenic stress[3,4]. p53 is a potent transcription factor that is activated in response to diverse stresses, leading to induction of cellcycle arrest, apoptosis or senescence . In addition, transcription-independent activities of p53 [5] can further enhance and/or differentiate cellular responses to stress, which are precisely controlled by p53 to assure that individual cells choose the irreversible path of self destruction only as a last resort [6].Although the regulation of the p53 pathway is not fully understood at the molecular level, it has been well established that activated p53 is detrimental to cancer progression .This is why cancer cells have developed multiple mechanisms for disabling p53 function. In fact, p53 is one of the most –frequently altered proteins in human cancer. The TP53 gene is deleted or mutated and , thus, inactive as a transcription factor in _ 50% of all human tumors [7]. Restoring p53 function to cancer cells with mutant p53 has been shown to induce tumor cell death, but the identification of pharmacologically relevant agents that can do this in vivo is still lagging [8,9].

Activation of p53 that have retained its wild –type conformation has also been considered an attractive therapeutic s trategy [10]. Although 50% of all human tumors express wild-type p53, many are thought to have inadequate p53 function due to abnormalities in p53 regulation or defective signalling in the p53 pathway [1]. One mechanism for suppressing p53 uses its negative regulator murine double minute 2 gene product (MDM2) [11]. MDM2 is overproduced in many human tumors due to an amplification of a chromosome segment including the MDM2gene, or overexpression of the protein without gene amplification [12–14]. As a result, p53 function is effectively suppressed without the need for mutation . Indeed, tumors with MDM2 gene amplification almost exclusively express wild -type p53 [14]. Therefore, by inhibiting MDM2 one might re-activate p53 in cancer cells, leading to their demise. However, the therapeutic utility of p53 activation by MDM2 antagonists will depend on several critical factors: (i) MDM2 is not the only known negative regulator of p53 and, therefore , MDM2 –free p53 might not be fully activated; (ii) defective p53 signaling in cancer cells with wild-type p53 might attenuate or disable the response to MDM2 antagonist; and (iii) possible growth suppressive and/or apoptotic activity of p53 in normal tissues might narrow or eliminate the therapeutic window of p53 activators. Here, the most –recent developments in this novel therapeutic strategy are discussed with an emphasis on small –molecule approaches for MDM2 inhibition. The tumor suppressor p53 is a potent anti –proliferative and pro-apoptotic protein that can harm normal cells. This is why the cellular level of p53 is accurately controlled in unstressed cells. It has been well established that MDM2 has a major role in this regulation. p53 and MDM2 form an autoregulatory feedback loop by which the two proteins mutually control their cellular levels . p53 binds to the promoter and regulates the expression of the Mdm2 gene, one of its transcription targets . As the level of MDM2 rises, it binds and inactivates p53 by directly blocking p53 transactivation domain and by targeting p53 protein for ubiquitin-dependent degradation in proteasome [13,15]. MDM2 and p53 bind to each other via their Nterminal domains . The MDM2 binding site of p53 partially overlaps with its transactivation domain and this is why MDM2 effectively inhibits p53 transcriptional activity [16]. In addition, MDM2 serves as an E3 ubiquitin ligase for p53 and its binding facilitates p53 proteolysis [17–19]. 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 mice can be successfully rescued by a concomitant deletion of the TP53 gene [20,21].A large body of evidence has established MDM2 as a crucial negative regulator of p53 and the major suppressor of p53 function in tumors with aberrant MDM2 expression [22,23]. Thus, by liberating p53 from MDM2 one might stabilize the tumor suppressor and activate the p53 pathway, leading to not only wild-type p53 cells but also cells that express mutant p53 have responded equally wel l to MDM2 inhibition.

MATERIALS AND METHODS

Target Identification:

The methods of Target identification extract useful knowledge from the raw data and help to focus on the relevant items of data. The most sophisticated aspect is the generation of new insights through the combination of information from different sources. Knowledge on the three- dimensional structure (fold) of a protein provides clues on its function and aids in the search for inhibitors and other drugs. To retrive and validate the Mdm2 protein sequence using computational tools such as **NCBI, UniProtKB, GeneCards,** etc. The X-ray structure of unliganded human MDM2 with the p53 transactivation domain was used in the present study (PDB code: 1ZIM).For docking purpose the structure was minimized by 500 steps using the conjugate gradient protocol and employing the CHARMM force field implemented in **Discovery Studio** software .

Chemical Library:

A chemical library or compound library is a collection of stored chemicals usually used ultimately in high throughput screening. The chemical library can consist in simple terms of a series of stored chemicals. Each chemical has associated information and its physiochemical properties with information such as the chemical structure, molecular formula, weight, logP, hydrogen bond donor, hydrogen bond acceptor, etc. characteristics of the compound. For this library of screening **Accelyrs Discovery Studio, ChemSpider, PubChem, ChemBank**, etc. databases were used. There are millions of compounds available in these databases. Through the help of these tools we can find a new compound against a Mdm2 protein and tested for their ability to modify / inhibit the target protein. In compound screening the major part to test that compound is having druglkeness or must passed ADME properties.We have used **Accelyrs Discovery Studio** for the present work**.**

Lead Optimization:

There are many tools available for designing of lead/drug such as **Discovery Studio, HyperChem, ChemDraw, ChemSketch**, etc. When a drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents. Activity is generally dosage-dependent and it is not uncommon to have effects ranging from beneficial to adverse for one substance when going from low to high doses. Activity depends critically on fulfilment of the ADME criteria. To be an effective drug, a compound not only must be active against a target, but also possess the appropriate ADME (Absorption, Distribution, Metabolism, and Excretion) properties necessary to make it suitable for use as a drug. The drug must possess the TOPKAT parameter for its novel properties. TOPKAT is nothing but the properties prediction of that drug. The properties such as molecule's bioavailability, it is carcinogenic o r not , lethal dose (LD50) , value of developmental toxicity prediction etc. The all values are calculated by protocols of **Discovery studio.**

Molecular Simulation and Docking :

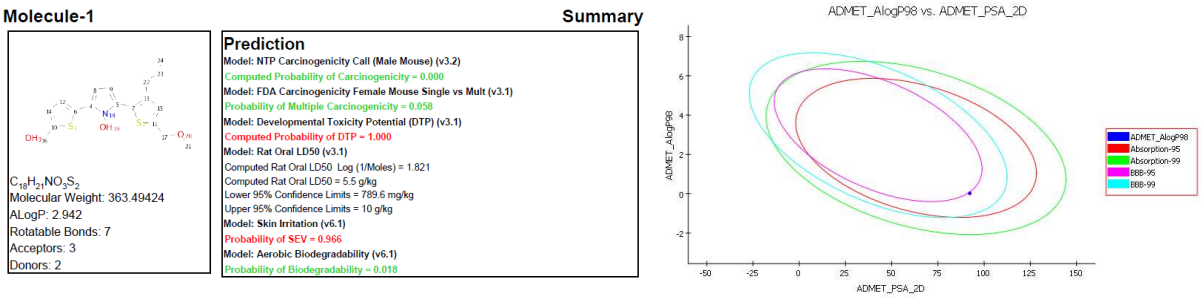
High-throughput screening (HTS) of compound libraries is used to discover novel leads for drug development. When a structure is available for the target, computer -based screening using molecular docking may also be considered. Molecular docking is a compute simulation procedure to predict the conformation of a receptor- ligand complex, where the receptor is usually a target protein and the ligand is either a small designed molecule. It can also be defined as a simulation process where a ligand position is estimated in a predicted or pre- defined binding site. Molecular docking simulations may be used for reproducing experimental data through docking validation algorithms, where protein-ligand conformations are obtained *in silico* and compared to structures obtained from Xray

crystallography or nuclear magnetic resonance. Furthermore, docking is one of main tools for virtual screening procedures, where a library of several compounds is "docked" against one drug target and return s the best hit. Before docking study, we need to minimize the energy of both molecule (ligand) and receptor (target molecule).. These all study carried out through Discovery studio. With the help of this tool we can see the prope r intermolecular

bonds between ligand-receptor complexes. There were three intermolecular hydrogen bonds seen in the complex of receptor and screened molecule.

RESULTS

From the designed library of molecule very few candidates screened out from the ADME and TOPKAT parameter. The best candidate molecule has been selected for further analysis. By using molecular simulation and docking technique the best drug candidate were identified which is satisfied the all rules and possess the inhibitor property. The inhibitor shows the highest binding affinity towards the receptor cavity is chosen for the best drug candidate molecule among synthesized library . The drug pentacosahydrogen (3S)-3-[(2R)-1-amino-3- methoxypropan-2-yl 6,7-dimethoxy-4-methyl-dihydroquinoxaline-1-thiol passing all ADME and TOPKAT parameter as shown in Graph 1 below**.**



Graph 1 Graphical representation of ADMET absorption.

Figure1:Toxicity prediction like NTP carcinogenicity, biodegradability, Rat oral LD50 and LC50 properties of designed drug molecule.

DOCKING RESULT:

The selected drug candidate undergo docking simulation with the protein , pdb id 1ZIM and resulted in dock score 11.79. The resul t has been proposed that a group of amino acid residues located on the binding cavity such as Asp-859, Glu-892 in target protein of Mdm2. This interaction / affinity plays an important role in ligand binding.

Table 1: A set of designed compound displaying and their molecular properties and ADMET properties.

Name	Mol wt.	LogP	HDonor	HAcceptor	Admet BBB	ADMET Absorption Level	ADMET Solubility	ADMET Hepatoxicity	ADMET Hepatoxicity probability	ADMET CYP2D6 PROB.
2-[5-(hydroxymethyl)thiophen-2-yl]-5-[5-(methoxymethyl)-3-propylthiophen-2-yl]-1*H*-pyrrol-1-ol	363.494	3.45	2	3	0.518	0	-0.55	1	0.58	0
5-({[(2*R*,3*R*)-3-hydroxy-5-(hydroxymethyl)-2,3-dihydrothiophen-2-yl]methoxy}methyl)-2-(hydroxymethyl)thiophene-3-ol	304.382	0.116	4	6		0	-0.47	1	0.7	0

Figure 2: Binding orientation of designed compound 5-({[(2*R*,3*R*)-3-hydroxy-5-(hydroxymethyl)-2,3-dihydrothiophen-2-yl]methoxy}methyl)-2-(hydroxymethyl)thiophene-3-ol with the target associated protein 1zim.

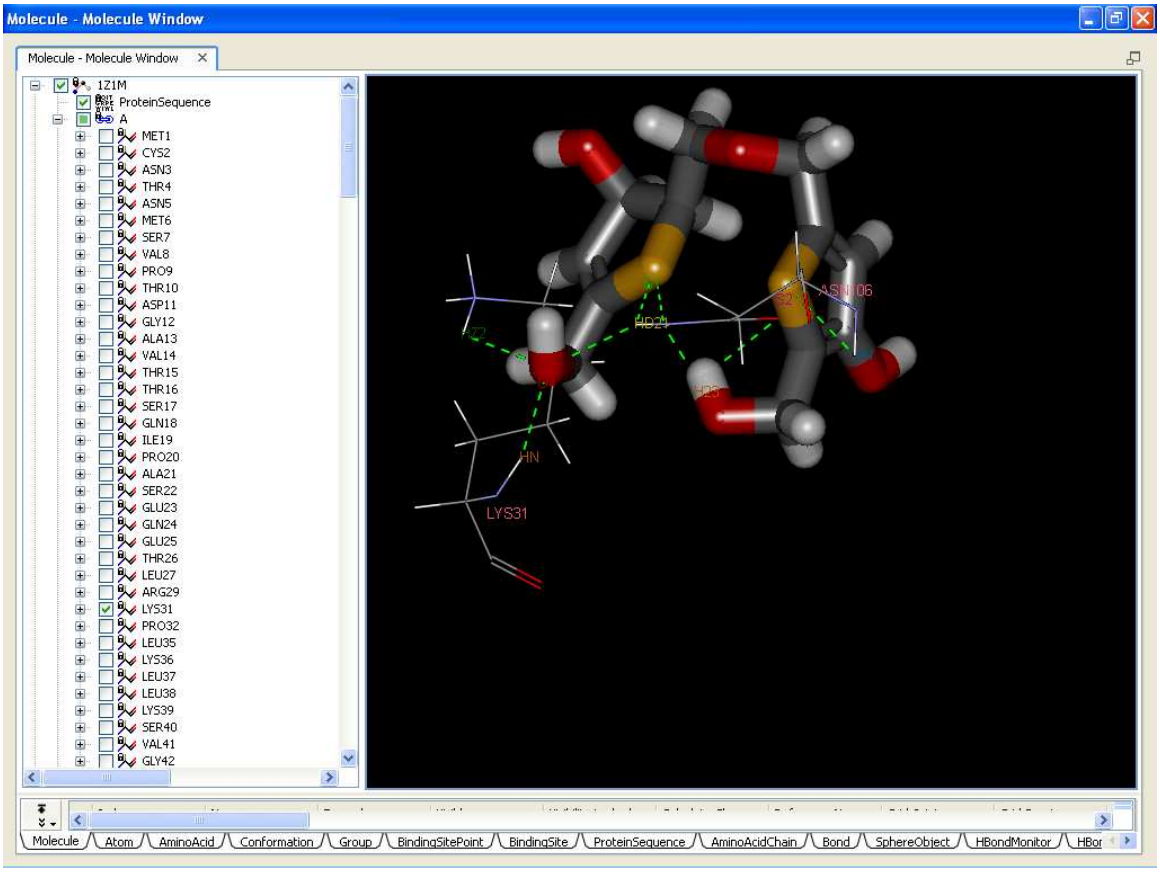


Table-2: The list of inhibitors with their C-Docker interaction energy to active site of target receptor

Name	C-DOCKER energy	C-DOCKER Interaction energy	CHARAMm energy	Vander waals energy
2-[5-(hydroxymethyl)thiophen-2-yl]-5-[5-(methoxymethyl)-3-propylthiophen-2-yl]-1*H*-pyrrol-1-ol	9.26624	14.0594	-6,804.43	-682.215
5-({[(2*R*,3*R*)-3-hydroxy-5-(hydroxymethyl)-2,3-dihydrothiophen-2-yl]methoxy}methyl)-2-(hydroxymethyl)thiophene-3-ol	-5.50232	21.4598	-682.215	-6,804.43

PHARMACOPHORE

The docked compound with binding pocket of receptor can be easily visualized on four feature of pharmacophore model. Aromatic ring features (yellow), hydrophobic region feature (blue), hydrogen bond acceptor feature (red). Hydrogen bond donor feature (green).

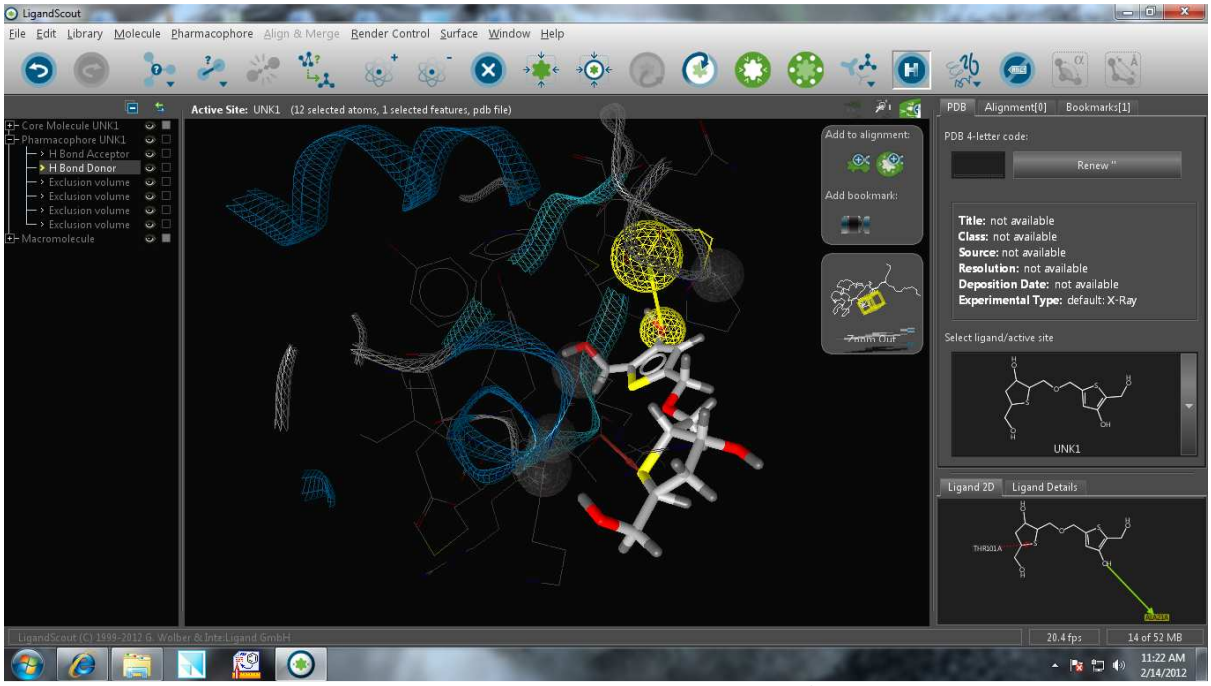


Figure 3: The designed compound with binding pocket of target receptor.

DISCUSSION

The interactions between designed potent inhibitor and receptor were studied by using various computational methods. Based on binding energy , and hydrogen bond formed, docking results were analyzed to find out the best ligand which can inhibit the target receptor 1zim protein. Based on these observations, the ligand 5 ({[(2*R*,3*R*)-3-hydroxy-5-(hydroxymethyl)-2,3-dihydrothiophen-2-yl]methoxy}methyl)-2-(hydroxyl methyl)thiophene-3-ol has high values to inhibit the target among the all ligands. Thus the *in silico m*ethod adopted in the present study helped in identifying the ligands using the commercial software and online tools for the treatment of cancer. This method reduces the time and cost in designing a drug as well as in analyzing the drug likeliness before it enters the clinical trials. The further studies were carried out by pre-clinical trials.

CONCLUSION

The drug we developed that is 5-({[(2*R* ,3*R*)-3 –hydroxy -5- (hydroxymethyl)- 2,3-dihydrothiophen- 2-yl] methoxy} methyl)-2- (hydroxymethyl) thiophene- 3-ol. The above drug molecule is binding with Mdm2 ,acting as Mdm2 antagonist , inhibiting its role to interact with P53 protein and there by P53 is freely available and can induce apoptosis and can regulate cell cycle progression in the case of damaged DNA and in the case of mutation.

After the all research by using Insilco tools we can conclude that the above drug can be the probable drug for inhibiting Mdm2 protein.

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REFERENCES

[1] Vogelstein, B. et al.Surfing the p53 network. Nature 408, 307–310
[2] Levine , A.J. (**1997**) p53, the cellular gate keeper for growth and division. *Cell* 88, 323–331
[3] Harris, S.L. and Levine, A.J. (**2005**) *Oncogene* 24, 2899–2908
[4] Oren, M. (**2003**) *Cell Death Differ*. 10, 431–442.
[5] Moll, U.M. et al . (**2005**) *Curr. Opin. Cell Biol*. 17, 631–636
[6] Vousden , K.H. and Lu, X. (**2002**) *Nat. Rev. Cancer* 2, 594–604
[7] Hainaut, P. And Hollstein, M. (**2000**) *Adv. Cancer Res.* 77, 81–137
[8] Bullock, A.N. and Fersht , A.R. (**2001**) *Nat. Rev. Cancer* 1, 68–76
[9] Fojo, T. (**2002**) p53 as a therapeutic target: unresolved issues on the road to cancer therapy targeting mutant p53. Drug Resist. Updat. 5 , 209–216
[10] Lane, D.P. (**1999**) *Br. J. Cancer* 80 (Suppl 1), 1–5
[11] Fakharzadeh, S.S. et al. (**1993**) *Genomics* 15,283–290
[12] Oliner, J.D. et al. (**1992**) *Nature* 358, 80–83
[13] Freedman, D.A. et al. (**1999**) *Cell. Mol. Life Sci.* 55, 96–107
[14] Momand , J. et al. (**1998**) *Nucleic Acids Res*. 26, 3453–3459
[15] Michael , D . and Oren, M. (**2003**) . *Semin. Cancer Biol*. 13, 49–58
[16] Momand, J. et al. (**1992**) *Cell* 69, 1237–1245
[17] Honda, R. et al. (**1997**) *FEBS Lett.* 420, 25–27
[18] Haupt, Y. et al. (**1997**) *Nature* 387, 296–299
[19] Kubbutat, M.H. et al. (**1997**) *Nature* 387, 299–303
[20] Jones, S.N. et al. (**1995**) *Nature* 378 , 206–208
[21] Montes de Oca Luna, R. et al. (**1995**) *Nature* 378, 203–206
[22] Bond, G.L. et al. (**2005**) *Curr. Cancer Drug Targets* 5 , 3–8
[23] Vousden, K.H. and Prives, C. (**2005**) *Cell* 120, 7–10