RESEARCH ARTICLE

Identification of High Affinity Non-Peptidic Small Molecule Inhibitors of MDM2-p53 Interactions through Structure-Based Virtual Screening Strategies

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Abstract

Background: Approaches in disruption of MDM2-p53 interactions have now emerged as an important therapeutic strategy in resurrecting wild type p53 functional status. The present study highlights virtual screening strategies in identification of high affinity small molecule non-peptidic inhibitors. Nutlin3A and RG7112 belonging to compound class of Cis-imidazoline, MI219 of Spiro-oxindole class and Benzodiazepine derived TDP 665759 served as query small molecules for similarity search with a threshold of 95%. The query molecules and the similar molecules corresponding to each query were docked at the transactivation binding cleft of MDM2 protein. Aided by MolDock algorithm, high affinity compound against MDM2 was retrieved. Patch Dock supervised Protein-Protein interactions were established between MDM2 and ligand (query and similar) bound and free states of p53. Compounds with PubCid 68870345, 77819398, 71132874, and 11952782 respectively structurally similar to Nutlin3A, RG7112, MI219 and TDP 665759 demonstrated higher affinity to MDM2 in comparison to their parent compounds. Evident from the protein-protein interaction studies, all the similar compounds except for 77819398 (similar to RG 7112) showed appreciable inhibitory potential. Of particular relevance, compound 68870345 akin to Nutlin 3A had highest inhibitory potential that respectively showed 1.3, 1.2, 1.16 and 1.26 folds higher inhibitory potential than Nutlin 3A, MI 219, RG 7112 and TDP 1665759. Compound 68870345 was further mapped for structure based pharmacophoric features. In the study, we report Cis-imidazoline derivative compound; Pubcid: 68870345 to have highest inhibitory potential in blocking MDM2-p53 interactions hitherto discovered.

Keywords: MDM2-p53 interactions - virtual screening - molecular docking

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Introduction

Since the discovery of p53 in 1979 (Lane et al., 1979; DeLeo et al., 1979; Linzer et al., 1979); it has surfaced as a most important tumor suppressor protein central to regulation of cell cycle, apoptosis, DNA repair, senescence, and angiogenesis (Vousden et al., 2002; Fridman et al., 2003; Teodor et al., 2007). The prominent role of p53 is reflected in nearly 50% cases of malignancies wherein p53 gene is either mutated or under expressed (Feki et al., 2004; Srivatanakul et al., 2004; Xu et al., 2012; Tian et al., 2013). However, the pioneering studies by Oliner et al. (1992) provided a convincing evidence of involvement of other regulatory proteins that encourages abnormal cell division. Involvement of MDM2 (Murine Double Minute 2) as an ancillary protein in sarcomas (Oliner et al., 1992, Yang et al., 2014) provided a fresh

avenues in oncological research which offered a clue in understanding the pathophysiological rationale behind the cancers wherein wild type status of p53 was retained.

MDM2 gene is known to be transcribed by p53 itself (Momand et al., 1992; Jun Liu et al., 2011), once expressed, MDM 2 binds to the p53 transactivation domain and inhibits its transcriptional activity (Momand et al., 1992; Chen et al., 1993) and exports p53 out of the nucleus. The event is followed by proteasome-mediated degradation of p53 that makes its degradation and rendering it inaccessible to the target genes (Freedman et al., 1998; Haupt et al., 1997). The process can be thought of feedback loop mechanism wherein both p53 and MDM2 is maintained in the basal levels in the normal cell (Wu et al., 1993). However, in certain malignancies the over expression of MDM2 renders p53 inactive which involves synergistic MDM2-p53 interactions (Momand et al., 1998).

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Approaches in disruption of MDM2-p53 interactions have now surfaced as an important therapeutic strategy in resurrection of p53 function. Highly potent and specific small-molecule inhibitors with desirable pharmaceutical properties to inhibit MDM2-p53 interactions such as Nutlin-3 (Vassilev et al., 2004) and MI-219 (Shangary et al., 2008) are now available. Studies using these inhibitors in preclinical models have already provided strong evidence that targeting the MDM2-p53 interaction using small-molecule inhibitors is a promising cancer therapeutic approach (Vassilev et al., 2004; Patton et al., 2006; Tovar et al., 2006; Sarek et al., 2007). Nevertheless, as every drug suffers setbacks, Nutlin-3 and MI-219 in spite being potential inhibitors have shown limited tumor volume reduction (Ray et al., 2012, Stakleff et al., 2012). Therefore in the view of above, through virtual screening approaches, we sought to identify a novel compound as a substitute to the customary drugs anticipated having higher affinity against MDM2 protein and possibly having commendable potential in disrupting MDM-p53 interactions.

Materials and Methods

Selection of inhibitors and structure similarity search

Nutlin3A and RG7112 belonging to compound class of cis-imidazoline, MI219 of spiro-oxindole class and benzodiazepine derived TDP 665759 served as query molecules for shape similarity search.

Structure similarity search was performed by NCBI's Pubchem search with a threshold of 95%. All the similar molecules were screened with Lipinski filters.

Ligand receptor docking

Molecular docking program Molegro Virtual Docker (MVD, 2010.4.0.0) which includes highly efficient PLP (Chanda Sinha et al., 2015) and MolDock scoring function (Yang et al., 2004) provided flexible platform for molecular docking (Bandaru et al., 2014). The optimized structures of Nutlin3A, MI-69, MI-217, RG7112 and TDP 7115 were docked into the p53 binding cleft of MDM2 protein. Docking parameters were set to 0.20 Å as grid resolution, maximum iteration of 1500 and maximum population size of 50. Energy minimization and hydrogen bonds were optimized after the docking. Simplex evolution was set at maximum steps of 300 with neighborhood distance factor of 1. Binding affinity and interactions of inhibitor with protein was evaluated on the

basis of the internal ES (Electrostatic Interaction), internal hydrogen bond interactions and sp²-sp² torsions. Post docked ligand-receptor complex energy was minimized using Nelder Mead Simplex Minimization (using non-grid force field and H bond directionality) (Nelder et al., 1965).

Protein-protein docking studies

Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain was retrieved from Protein Databank with (PDB accession number: 1YCR). Protein - Protein docking was executed through object recognition and image segmentation algorithm embedded in Patchdock server (Schneidman et al., 2005). Default parameter was set as clustering RMSD at 4.0.

Lipinski filters toxicity screening and bioactivity prediction of compounds

All the similar compounds retrieved were screened for its drug ability by lipinski filters. The toxicity screening was achieved by using LAZAR toxicity prediction server (Maunz et al., 2013). Biological activity score of the ligands was predicted using Molinspiration webserver (©Molinspiration Cheminformatics, 2014). LC 50 was predicted using T.E.S.T. Version 4.1 (2012, U.S. Environmental Protection Agency) software. The complete ADMET properties was calculated using admetSAR (Cheng et al., 2012).

Solvent accessible surface area (SASA) and interface property calculation

Solvent accessible surface area of the complexes were calculated by GETAREA server (Ahmad et al., 2004), protein interfaces calculated by Aquaprot (Reichmann et al., 2008) and interface property was calculated using 2P2I inspector (Basse et al., 2013) online server.

Results and Discussion

Evident from rerank scores, all the akin compounds identified against parent compound had higher binding affinity against MDM2 protein in comparison to parent compounds. Further, compound PubCid: 71132874 akin to MI 219 showed highest affinity against the MDM2 receptor amongst the compounds undertaken in this study. The docking scores of top selected compounds belonging to each lead compound are provided in Table 1.

The compounds were further screened for their in silico ADMET profile, compound PubCid: 11952782

Table 1. MolDock Algorithm Aided Docking of Compounds in p53 Transactivation Binding Cleft of MDM2 Protein. PubCid: 71132874 Akin to MI 219 Showed Highest Affinity Against the MDM2 Receptor

Compound	MolDock Score	Rerank Score	Internal	H Bond
MI219	-135.95	-72.1997	-29.8749	-2.45121
TDP 665759	-113.4	-71.5204	4.99076	0
RG7112	-117.551	-61.9914	2.64802	-1.37174
NUTLIN 3A	-109.209	-61.9045	-9.8754	-1.0106
PubCid: 71132874 (MI 219 SIMILAR)	-165.95	-106.647	-26.9939	-2.63359
PubCid: 77819398 (RG 7112 SIMILAR)	-164.829	-104.109	2.92253	-2.5
PubCid: 11952782 (TDP 665759 SIMILAR)	-123.099	-84.7467	39	0
PubCid: 68870345 (NUTLIN SIMILAR)	-275.21	-99.681	5.2	-2.34

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akin to TDP 665759 and compound 77819398 akin to RG 7112 showed toxicity being mutagenic and carcinogenic respectively. The ADMET profile of the compounds is represented in Table 2.

As evident from protein-protein docking studies,

the MDM2-p53 interactions are efficiently inhibited in presence of inhibitors as compared to ligand free MDM2, thus implying there occurs a declined MDM2-p53 interactions in presence of inhibitors. It is interesting to note that, except for compound PubCid:77819398 akin to

Table 2a. ADMET Prediction of Virtually Screened Compounds by ADMETSAR Server. Additionally Toxicity was Calculated by LAZAR Toxicity Prediction Server. Compound PubCID 68870345 and 71132874 respectively Similar to Nutlin 3A and MI 219 were Predicted to be Safe. Compound (PubCID 11952782) akin to TDP 7115 was mutagenic while Compound (PubCid: 77819398) akin to RG7112 was Predicted to be a Carcinogen

Model	PubCid: 71132874 (MI 219 SIMILAR)		PubCid: 77819398 (RG 7112 SIMILAR)	
	Result	Probability	Result	Probability
Absorption				
Blood-Brain Barrier	BBB-	0.547	BBB+	0.7325
Human Intestinal Absorption	HIA+	1	HIA+	1
Caco-2 Permeability	Caco2-	0.6511	Caco2-	0.5494
P-glycoprotein Substrate	Substrate	0.9166	Substrate	0.8089
P-glycoprotein Inhibitor	Non-inhibitor	0.5104	Non-inhibitor	0.5604
Renal Organic Cation Transporter	Non-inhibitor	0.8754	Non-inhibitor	0.5437
Distribution & Metabolism				
CYP450 2C9 Substrate	Non-substrate	0.803	Non-substrate	0.6844
CYP450 2D6 Substrate	Non-substrate	0.7822	Non-substrate	0.7846
CYP450 3A4 Substrate	Substrate	0.7543	Substrate	0.7369
CYP450 1A2 Inhibitor	Non-inhibitor	0.7047	Non-inhibitor	0.7395
CYP450 2C9 Inhibitor	Non-inhibitor	0.592	Non-inhibitor	0.57
CYP450 2D6 Inhibitor	Non-inhibitor	0.7918	Non-inhibitor	0.8051
CYP450 2C19 Inhibitor	Non-inhibitor	0.5183	Non-inhibitor	0.5664
CYP450 3A4 Inhibitor	Inhibitor	0.9146	Inhibitor	0.6919
CYP Inhibitory Promiscuity	Low	0.6003	Low	0.6134
Excretion & Toxicity				
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9026	Weak inhibitor	0.6894
AMES Toxicity	Non toxic	0.6328	Non toxic	0.563
Fish Toxicity	High FHMT	0.9989	High FHMT	0.9868
Acute Oral Toxicity	III	0.6288	III	0.5731
HoneyBee toxicity	Low	0.8123	Low	0.7411

Model	PubCid: 11952782 (TDP 665759 SIMILAR)		PubCid: 68870345 (NUTLIN SIMILAR)	
	Result	Probability	Result	Probability
Absorption				
Blood-Brain Barrier	BBB+	0.9635	BBB+	0.7745
Human Intestinal Absorption	HIA+	0.9861	HIA+	1
Caco-2 Permeability	Caco2-	0.5428	Caco2-	0.5082
P-glycoprotein Substrate	Substrate	0.7175	Substrate	0.7877
P-glycoprotein Inhibitor	Non-inhibitor	0.7525	Inhibitor	0.5522
Renal Organic Cation Transporter	Non-inhibitor	0.6984	Non-inhibitor	0.52
Distribution & Metabolism				
CYP450 2C9 Substrate	Non-substrate	0.8062	Non-substrate	0.7524
CYP450 2D6 Substrate	Non-substrate	0.7567	Non-substrate	0.8536
CYP450 3A4 Substrate	Substrate	0.6762	Substrate	0.6647
CYP450 1A2 Inhibitor	Non-inhibitor	0.596	Non-inhibitor	0.8176
CYP450 2C9 Inhibitor	Non-inhibitor	0.6678	Non-inhibitor	0.5564
CYP450 2D6 Inhibitor	Non-inhibitor	0.8903	Non-inhibitor	0.8724
CYP450 2C19 Inhibitor	Inhibitor	0.5692	Non-inhibitor	0.6094
CYP450 3A4 Inhibitor	Inhibitor	0.8028	Inhibitor	0.7239
CYP Inhibitory Promiscuity	Low	0.508	Low	0.6664
Excretion & Toxicity				
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.8601	Weak inhibitor	0.5113
AMES Toxicity	Non toxic	0.594	Non toxic	0.6423
Fish Toxicity	High FHMT	0.9785	High FHMT	0.9161
Acute Oral Toxicity	III	0.6793	III	0.6601
HoneyBee toxicity	Low	0.9301	Low	0.8658

Table 2b. ADMET prediction of virtually screened compounds by ADMETSAR server. Additionally toxicity was calculated by LAZAR toxicity prediction server. Compound PubCID 68870345 and 71132874 respectively similar to Nutlin 3A and MI 219 were predicted to be safe. Compound (PubCID 11952782) akin to TDP 7115 was mutagenic while compound (PubCid: 77819398) akin to RG7112 was predicted to be a carcinogen.

Compound	DSSTox Carcinogenic Potency DBS MultiCellCall	DSSTox Carcinogenic Potency DBS Mutagenicity	DSSTox Carcinogenic Potency DBS Rat	DSSTox Carcinogenic Potency DBS Hamster	DSSTox Carcinogenic Potency DBS Mouse
PubCid: 68870345 (NUTLIN 3A SIMILAR)	non-carcinogen	non- mutagenic	non-carcinogen	non-carcinogen	non-carcinogen
PubCid: 71132874 (MI 219 SIMILAR)	non-carcinogen	non- mutagenic	non-carcinogen	non-carcinogen	non-carcinogen
PubCid: 11952782 (TDP 665759 SIMILAR)	non-carcinogen	mutagenic	non-carcinogen	non-carcinogen	non-carcinogen
PubCid: 77819398 (RG 7112 SIMILAR)	non-carcinogen	non- mutagenic	non-carcinogen	carcinogen	non-carcinogen

Table 3. MDM2 and p53 protein docking scores evaluated by surface patch matching (Patch Dock Server)

PROTEIN 1	PROTEIN 2	Score	Transformation
MDM2 bound to :	p53 TRANSACTIVATION DOMAIN		
PubCid: 68870345 (NUTLIN SIMILAR)		5490	-0.36 -0.56 -3.11 40.78 -50.82 11.99
PubCid: 71132874 (MI 219 SIMILAR)		5560	3.13 -0.72 -1.54 -4.20 -5.68 4.29
PubCid: 11952782 (TDP 665759 SIMILAR)		5842	1.35 0.45 -1.09 8.77 0.92 1.44
Mi 219		5914	-0.52 -1.20 -1.68 43.65 -12.35 21.52
NUTLIN 3A		6290	-0.18 -1.14 2.61 23.66 -54.55 12.43
RG7112		6394	-2.79 -0.16 2.45 51.31 -25.60 -15.44
PubCid: 71132874 (MI 219 SIMILAR)		6394	(-3.11 -0.64 -1.18 -5.16 -16.32 -3.05)
TDP665759		6926	2.43 0.75 2.88 60.62 -11.85 -21.74
MDM2 UNBOUND		10134	-2.40 0.25 1.94 42.18 -50.34 -37.45

Table 4. Interface property calculations in ligand free and ligand bound states of MDM2-p53 Complex

	Total Interface Area (A2)	Gap volume (A3)	Gap Index	SASA	Buried atoms	Surface atoms
MDM2 UNBOUND STATE	1481.8	783	0.52841	5794	652	758
MDM2 BOUND TO:						
MI 219	628.7	1242.6	1.97646	6005	335	483
NUTLIN 3A	698.2	1210	1.73303	5930	353	465
RG 7112	722.2	1121.6	1.55303	5970	330	488
TDP 665759	1340.6	721.2	0.53797	5823	340	478
PubCid: 77819398 (RG7112 SIMILAR)	852.4	1009.6	1.18442	5797	336	482
PubCid: 11952782 (TDP 665759 SIMILAR)	521	1421.2	2.72783	6005	335	483
PubCid: 71132874 (MI 219 SIMILAR)	410.1	1655.2	4.03609	6040	336	482
PubCid: 68870345 (NUTLIN 3A SIMILAR)	322.2	1828.7	5.67567	8582	328	490

Table 5. Predicted LC 50 and bioactivity of compounds

BIOACTIVITY	LC 50 96 hour	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
PubCid: 71132874 MI 219 similar	26.36	0.24	-0.14	-0.03	-0.04	0.19	0
PubCid: 77819398 RG 7112 similar	22.28	-0.51	-1.44	-1.04	-1	-0.19	-1.01
PubCid: 11952782, TDP 665759 similar	14.55	0.05	-0.28	-0.19	-0.21	-0.12	-0.2
PubCid: 68870345 NUTLIN 3A similar	12.24	0.03	-0.56	-0.14	0.04	0.07	-0.24

RG7112 all the similar compounds had superior inhibitory potential than their parent compounds. In particular compound with PubCid: 68870345 akin to Nutlin 3A had highest inhibitory potential among all the compounds (parent and their respective similar). PubCid: 68870345

showed showed 1.14 folds better inhibitory potential than its parent compounds Nutlin 3A. The patch dock scores (protein-protein docking scores) of MDM2-p53 interaction in presence of inhibitors and their respective akin is shown in Table 3.

The MDM2-p53 docking results were further complemented by interface property calculations. Interface properties of MDM2-p53 complex in presence and absence of inhibitors is shown in Table 4. From extensive interface properties calculated it is quite apparent that all the similar compounds had well inhibitory potential than their respective parent compound. As mentioned afore, PubCid :68870345-a Nutlin 3A akin compound was superior among the undertaken compounds in preventing the MDM-p53 interactions (as revealed from patch dock scores),the interface properties calculations further asserts this observation. The concurrence between patch dock results and MDM2-P53 interface property calculation is supported by the fact that the total interface area in MDM-p53 complex was 2.1 folds declined in

presence of compound PubCid : 68870345 compared to the complex harboring its parent compound Nutlin 3A. Further the comparative analysis of the number of surface and buried atoms of the MDM2 -p53 complexes has unveiled that PubCid:68870345 bound MDM2 -p53 complex had considerable increase in the number of surface atoms and a decrease in buried atoms implying a weak interaction between p53 and MDM2. Likewise, gap index and SASA was highest between MDM2-p53 in presence of PubCid :68870345 indicating it to be the powerful and potential inhibitor undertaken in the existing study. In addition to the analyses, the LC 50 values and bioactivity of the compounds were predicted. The LC 50 values at 96 hour interval were predicted to be least lethal (Table 5). In addition the similar compounds identified against their parents show enhanced bioactivity providing a clue for target specificity.

Figure 1. The Potential of Compounds in Blocking the MDM-p53 Interaction Followed the Descending Order of 68870345 > 71132874 > 11952782 > Mi219 > Nutlin 3A > RG7112 > 77819398 > TDP 665759 > MDM2 Unbound. Evident from extensive interface analysis; Nutlin 3A similar compound had highest inhibitory potential in the present study. There was 1.44 folds increase in the SASA when MDM bound to Nutlin 3A similar to Nutlin 3A bound counterparts (Δ SASA Nutlin 3A sim-Nutlin 3A=2651.98, folds=1.44). In addition, there was considerable decrease of interface area in the interactions involving Nutlin 3A similar (Δ IA Nutlin 3A-Nutlin 3ASim=376, folds=2.16). Further gap volumes testifies the inhibitory potential of Nutlin 3A Similar than any other compound undertaken in the study (Δ GVNutlin 3A Sim-Nutlin 3A=618.7, folds=1.51)

Figure 2. Electrostatic Surfaces of MDM2 (Solid) and p53 (mesh) in MDM2-p53 Complex. Compound PubCid: 68870345 Nutlin 3A similar (solid yellow) is bound between the interfaces

Figure 3. Compound PubCid 68870345 (Stick Model) Bound at the p53 Transactivation Domain Binding Site (Pink Mesh) in MDM2 Protein. The prior occupied high affinity inhibitor at the binding site of p53 transactivation domain in MDM2 falls short to interact with the p53 transactivation domain, thereby detains MDM2-p53 interactions

Figure 4. Interactions of Nutlin 3A Similar Compound PubCid 68870345 with MDM2. Residues circled in green participate in vanderwaals interaction while residues in pink forms electrostatic interactions. Hydrogen bonds are shown as green arrows

Solvent accessible surface area of the MDM2 -p53 complex in the presence of the parent and respective akin with that of the ligand free MDM2-P53 complex, Nutlin 3A akin bound MDM2-P53 complex showed highest total solvent accessibility surface area which gives a clue towards decreased MDM2 -P53 interaction. It is also imperative to note that the total solvent accessibility surface area of Nutlin 3A akin bound MDM2-P53 complex has increased by 1.45 folds when compared to the Nutlin3A bound MDM2-P53 complex.

In conclusion, the together with molecular docking analysis, protein-protein patch docking, interface property and SASA analysis has put forth that the Nutlin 3A akin compound demonstrated and brought about the weakest interaction between MDM2 and p53, thereby making p53 available for carrying out its tumor suppressing actions in the cells.

our observations.

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