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Designing of Novel Inhibitors of *Mycobacterium Tuberculosis* H37Rv by Pharmacophore based Drug Designing and its Evaluation

Faizan Ahmad[1]*, Kaushal K. Chandrul[2], Huma Naz[3] and Neeraj Tandan[4]

[1]Department of Biotechnology, Shri Venkateshwara University Gajraula, Amroha, UP, India
[2]School of Pharmaceutical Science, Shri Venkateshwara University Gajraula, Amroha, UP, India
[3]Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, U.P., India
[4]Scientific & Applied Research Centre, Meerut, UP, India

Corresponding author

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A B S T R A C T

The growing frequency of drug-resistant tuberculosis, which is resistant to valuable multiple antibiotic, presents a major global health warning. Filamenting temperature-sensitive mutant gene-Z (FtsZ) a tubulin homologue involved in bacterial cell division is considered an attractive target for the development of effective antibiotics against tuberculosis. In this study, we attempted to identify novel ZINC compounds that specifically target the M. tuberculosis *H37Rv*, FtsZ protein and docked on the FtsZ protein crystal Structure (PDB Id: 1RLU, resolution 2.08 Å). We have developed a pharmacophore model based on the already existing drugs and drug candidates with PHASE module of Schrodinger. This pharmacophore was used to screen against the prepared fragment-like and drug-like natural compound dataset (ZINC database) with altogether different scaffold using high throughput virtual screening and docking studies. Finally, we have reported eight top scoring compounds which possess high affinity for active site of FtsZ protein as well as showed the highest docking score and H-bond interaction and thus, can be considered as potential FtsZ inhibitors for the treatment of tuberculosis.

Introduction

Tuberculosis (TB) is communicable disease caused by Mycobacterium tuberculosis (Mtb) and associated species that is most commonly observed as a chronic pulmonary infection (World Health Organization (WHO), 2013).

TB, once the leading cause of infectious disease mortality, was nearly eradicated from industrialized nations in the 20th century through a combination of public health measures and the introduction of antibiotics (Centers for Disease Control and

Prevention (CDC), 1999). However, in conjunction with the spread of HIV infection, (Nunn *et al*., 2005) tuberculosis is today amongst the worldwide health threats, which encouraged the WHO to declare TB a global public health emergency. Present efforts to carry TB under manage are paying attention on development of new antibiotics, improved diagnostics, and vaccines (NIH, 2014) ultimately, success in each area is required to control TB. As resistant strains of Mycobacterium tuberculosis have slowly emerged, treatment failure is too often a fact, (Frieden *et al*., 2003) especially in countries lacking the necessary health care organization to provide the long and costly treatment adapted to patients. Pitiable chemotherapeutics and inadequate local-control programs contribute to the incapability to manage TB and lead to the emergence of drug resistant strains of Mtb. (Raviglione *et al*., 2000) Treating drug-resistant TB is much more complex, which takes 18 to 24 months if the patient responds to the second line treatment procedure and is around 20 times more costly than treating totally drug susceptible TB. The currently existing second-line drugs are less efficient, and have more side-effects (Tuberculosis Fact sheet, 2005), thus, warranting the identification of novel drug targets and development of novel chemotherapeutics.

In eukaryotic pathogens, as well as in higher eukaryotic cells, cell division has been a dynamic area for finding drugs that fight infection or uninhibited cell propagation (Kreuter *et al*., 2004; Falchetti *et al*., 2004; Fingar *et al*., 2004), although the bacterial cell division apparatus has remained largely vacant for curative purposes (Margalit *et al*., 2004). Recently, FtsZ, a tubulin homologue involved in bacterial cell division, has received significant consideration and has been considered as an attractive target to develop innovative anti- TB drugs, as well as new wide-ranging antibacterial agents. Filamenting temperature-sensitive transformed Z (FtsZ), a tubulin homologue, is a extremely preserved and ever-present bacterial cell division protein. In the 1960s, genes designated "Fts" (filament-forming temperature-sensitive genes, *fts*), were recognized by mutational analysis in *Escherichia coli.* The gene products of the Fts-genes are known to be involved in septum development (Hirota *et al*., 1968; Van De Putte *et al*., 1964). While the participation of Fts-proteins in cell division was known, it was not discovered until 1991 that FtsZ was implicated in Z ring creation and the initiation of cell division (Bi *et al*., 1991). FtsZ is one of a number of genes required for cell division identified in *E. coli*. Other genes include *ftsA*, *ftsQ*, *ftsN*, *ftsL*, *ftsK*, *ftsW*, *ftsI*, and *zipA* (Lutkenhaus *et al*., 1997).

In the present study, a more extended version of the protein-protein interaction network of proteins in *M. tuberculosis H37Rv* was derived from the STRING database (Mering *et al*., 2003) in which FtsZ protein has protein-protein interaction with FtsW, FtsQ and SepF. FtsW is a polytopic membrane protein that is present in almost all bacteria that have a peptidoglycan cell wall (Ikeda *et al*., 1989; Henriques *et al*., 1998). It is required for cell division in *E. coli* (Khattar *et al*., 1994; Ishino *et al*., 1989) two functions have been attributed to FtsW: stabilization of the FtsZ cytokinetic ring (Boyle *et al*., 1997) and facilitation of septal peptidoglycan synthesis by recruitment of FtsI (PBP3) to the division site (Mercer *et al*., 2002). The initial topological model of FtsW based on computational methods and investigational data has recently been planned for the FtsW of *Streptococcus pneumonia* (Gerard *et al*., 2002). So that this functional relationship between them makes FtsZ an essential protein.

Similar to the process of microtubule formation by tubulin, FtsZ polymerizes in a GTP-dependent manner, forming a extremely dynamic cytokinetic organization, selected as the Z-ring, at the intermediate of the cell. The enrollment of the additional cell division proteins leads to Z-ring contraction and outcome in septum formation (Awasthi *et al*., 2010). The requirement of FtsZ in mycobacterial cytokinesis, and inhibition of FtsZ is a incredibly hopeful target for innovative anti-microbial drug discovery because of its essential role in cell division and its known biochemical activity FtsZ would not be affected by known drug resistance mechanisms caused by the use of current anti-TB drugs. Specifically, we reported the pharmacophore model and docking study of novel zinc compounds against M.tb *H37Rv* cell division protein FtsZ for development of next generation mycobacterium inhibiters with activity against difficult to treat clinical strains.

Materials and Methods

Preparation of ligand dataset for pharmacophore modeling

Three dimensional structures of sixteen different Tuberculosis inhibitors were retrieved from Drugbank: Amikacin, Azithromycin, Capreomycin, Ciprofloxacin, Cycloserine, Ethambutol, Ethionamide, Fluconazole, Getifloxacin, Isoniazid, Kanamycin, Livofloxacin, Moxifloxacin, Ofloxacin, Pyrizinamide and Streptomycin. Pharmacophore modeling was carried out using Phase module of Schrodinger. The ligands were prepared using Prepare Ligands step of the Develop Pharmacophore Model panel in maestro. The preparation steps involved conversion of structures from 2D to 3D, addition of hydrogen atoms and counter ions, elimination of crystal water molecules, production of stereoisomers followed by energy minimization. A conformational investigation was run for the cleaned up ligands to generate a set of conformers for each ligand.

Development of pharmacophore model

Once the ligands were cleaned and conformations were generated, a set of pharmacophore features was used to create pharmacophore sites (site points) for all the ligands. It was performed using the Create Sites step of the Develop Pharmacophore Model panel. Phase stores a built-in set of six pharmacophore features: hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively charged group (N), positively charged group (P), aromatic ring (R).

Now using the Find Common Pharmacophore feature, pharmacophores from all conformations of the ligands were examined.

All pharmacophores containing equal sets of features with extremely related spatial arrangements were then clustered together. If a specified group was found to contain at least one pharmacophore from each ligand, it gave rise to a common pharmacophore, explaining how ligands bind to the receptor. These common pharmacophores were examined by applying a scoring method, which ranked all the hypotheses and identified the pharmacophore that yielded the best alignment of the chosen ligands. The highest scoring hypothesis was then used to search for matches in a dataset of known natural drug-like compounds.

Preparation of a phase database from a dataset of natural compounds

A large data set consisting of natural chemical compounds ware downloaded from ZINC database (Silman *et al*., 2005). The

database was created and structures were added to it using Generate Phase Database panel. The natural compounds were transformed to all-atom structures with reasonable 3D geometries. A complete set of conformations was generated for each compound with addition of site points to the structures for a given set of pharmacophore features.

Database screening using the selected pharmacophore hypothesis

The phase database organized was screened for structures that match the hypothesis of the model. The search process consisted of two steps: finding and fetching. Firstly, the database was searched for 3D arrangement of pharmacophoric sites with similar site types and intersite distances in comparison to the selected hypothesis. After finding such a structure, its information was written to a match file. In the fetch step, the most related conformers, called hits, were retrieved from the database with the help of the match file and were then aligned to the hypothesis. All the hits above a certain fitness score were fetched and analyzed further.

Interaction analysis of the hits generated using in-silico docking studies

The hits generated were further investigated for their binding affinities and mode of interaction with Tuberculosis FtsZ protein using extra precision (XP) docking protocol of Glide, Schrodinger.

The crystal structure of FtsZ of Tuberculosis origin was downloaded for Protein Data Bank (PDB ID: 1RLU) at resolution 2.08A, (Rarey *et al*., 1997) Crystal water molecules and all non-bonded heteroatoms, including the docked ligand were removed from the protein structure using Schrodinger. The protein was prepared for docking studies using Schrodinger's protein preparation wizard. Hydrogen bonds were added and optimized to the structure. Other preparation steps involved removal of bad contacts, optimization of bond lengths, creation of disulfide bonds, capping of protein terminals and conversion of selenomethionine to methionine. A grid was generated at the active site of the prepared protein structure using the Glide docking module of Schrodinger (Friesner *et al*., 2004; Halgren *et al*., 2004). The matches found in the database of natural compounds were then virtually screened against the prepared protein at desired grid coordinates using Glide model's XP docking protocols.

Results and Discussion

Preparation of ligand dataset for pharmacophore modeling

The 3D structures of sixteen known Tuberculosis inhibitors were retrieved from Drug bank. These structures were cleaned to remove counter ions and water molecules. Explicit hydrogen atoms were added to each inhibitor to ensure that all of them were all-atom structures, followed by energy minimization. Since most of the ligands are flexible, considering a range of different conformations for each molecule is an important requisite for developing a pharmacophore so as to get a structure similar to the real experimental molecular orientation. These preparation steps were completed using prepare ligands step of the develop pharmacophore model panel in maestro.

Development of pharmacophore model

Different site points or pharmacophoric features were defined for each conformer of each ligand. The six in built pharmacophore features of phase, hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic

group (H), negatively charged group (N), positively charged group (P) and aromatic ring (R) were used. A list of 150 variants was generated keeping 5 as maximum number of sites and 5 as the minimum number of sites; give 5 to be the number of ligands that must be matched. This list reflected the 16 possible combinations of features that could give rise to common pharmacophores. Then, all these variants were selected to find the common pharmacophore among the tuberculosis inhibitors. The common pharmacophore with maximum survival score of 3.72 was selected for finding relevant matches in the drug-like natural compound database. Fig.1

(A) illustrates the selected hypothesis consisting of 5 pharmacophore features with (B) intersite distances and (C) angles, respectively. The alignment of 5 ligands-Ciprofloxacin, Getifloxacin, Livofloxacin, Moxifloxacin and Ofloxacin resulting in the development of common pharmacophore is shown in Fig. 2.

Table.1 The best three common pharmacophore hypotheses with survival active scores.

Hypothesis	Survival active
AHNRR.10	3.722
HHNRR.11	3.509
AHNRR.10	3.498

Table.2 Hits Taken from ZINC Database for Selected Hypothesis AHNRR.10.

ZINC Database	No of Structures	No of Hits	No of Hits Taken
Standard Fragments Like Molecules	953566	5647	41
Standard Lead Like Molecules	7096188	73881	233
TOTAL	**8049754**	**79528**	**274**

Table.3 Molecular Docking Scores.

Ligand Name	GScore	LipophilicEvdW	PhobEnHB	Electro	Rotpenal	HBond
Kanamycin	-9.96	-1.42	0	-3.3	0.12	-6.88
Capreomycin	-8.34	-2.24	0	-3.5	0.15	-3.07
ZINC27199796	-8.19	-1.42	0	-2.58	0.09	-4.05
ZINC67665159	-7.92	-1.24	0	-2.79	0.15	-2.74
ZINC33753243	-7.89	-1.22	0	-2.89	0.16	-2.62
ZINC03789759	-7.75	-2.00	0	-2.13	0.14	-3.48
ZINC19594549	-7.66	-0.78	0	-2.96	0.05	-2.62
ZINC06385314	-7.54	-1.44	0	-2.83	0.25	-2.24

GScore - Total Glide Score; sum of XP terms. LipophilicEvdW - Lipophilic term derived from hydrophobic grid potential at the hydrophobic ligand atoms. PhobEnHB - Reward for hydrophobically packed H-bond. Electro - Electrostatic rewards; includes Coulomb and metal terms. RotPenal –Rotatable bond penalty. H-Bond - ChemScore H-bond pair term.

Fig.1 The best generated pharmacophore model AHNRR.10, developed using Phase module. The selected hypothesis consisting of 5 pharmacophoric features with (A) Hypothesis (B) Intersite distances, (C) Intersite angles.

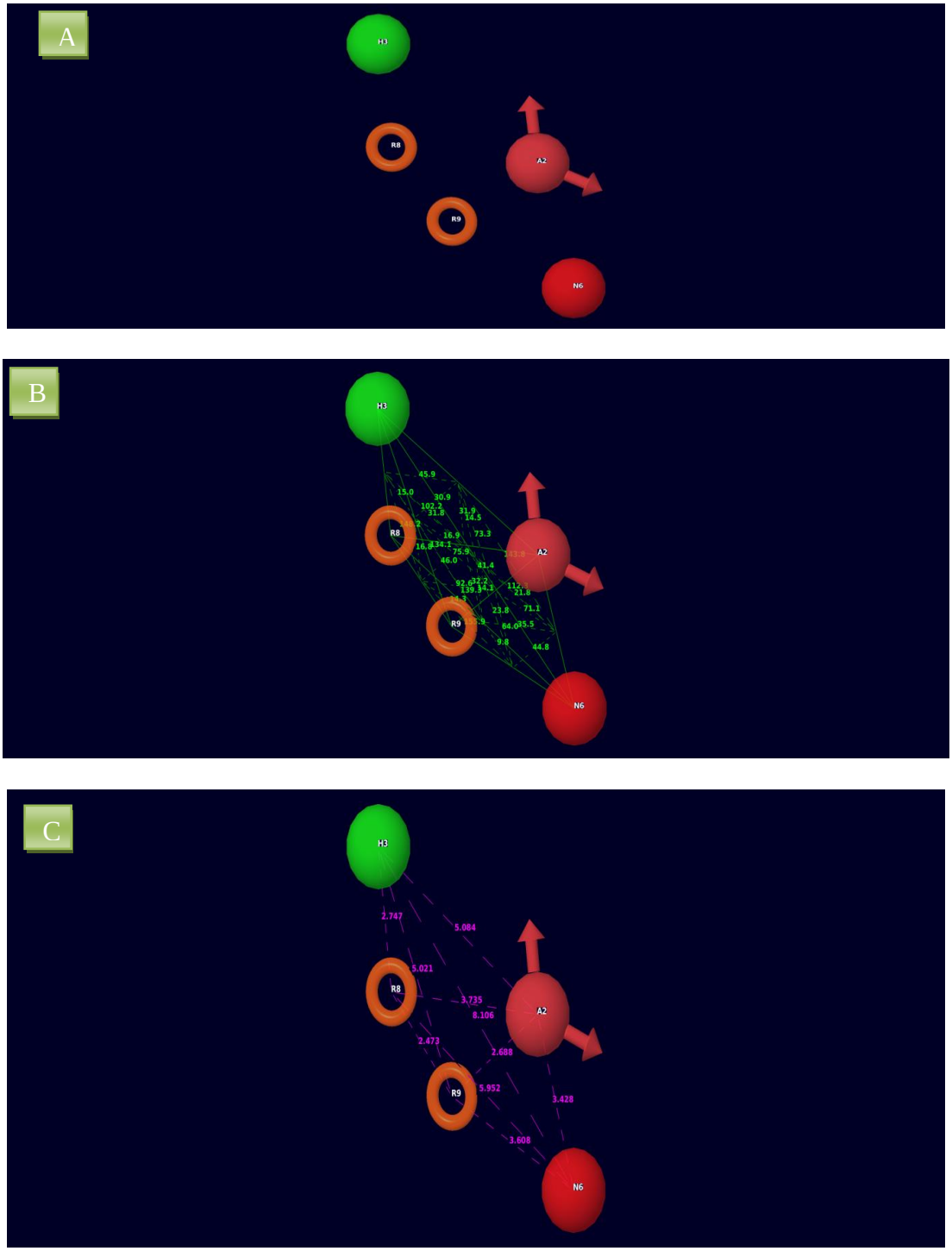

Fig.2 AHNRR.10 with best fit drug Livofloxacin and rest four fit drugs

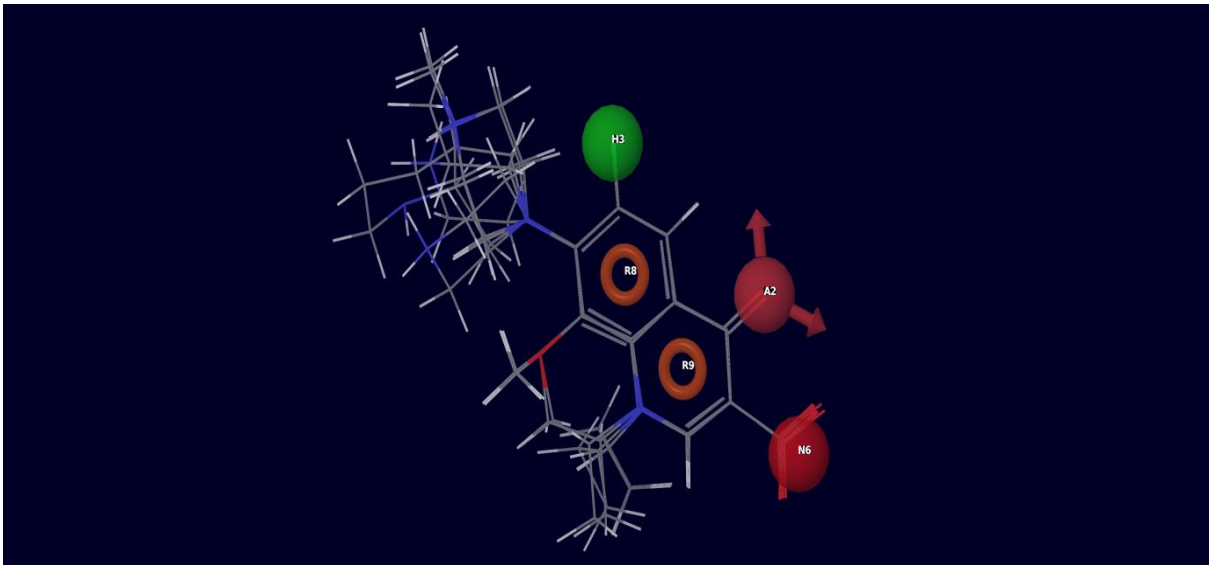

Table.4a Standard Fragments Like Molecules.

File Name	No of Structures	No of Hits	No of Hits Taken
2_P0.0	211785	1249	12
2_P0.1	212010	1126	5
2_P0.2	211159	1049	13
2_P0.3	211203	1550	7
2_P0.4	1527	6	0
2_P1.0	105882	667	4
TOTAL	**953566**	**5647**	**41**

Table.4b Standard Lead Like Molecules.

File Name	No of Structures	No of Hits	No of Hits Taken
1_P0.0	147812	1824	7
1_P0.1	147934	2095	8
1_P0.2	147921	1531	1
1_P0.3	147957	1586	4
1_P0.4	147781	1382	9
1_P0.5	147368	1419	12
1_P0.6	147355	1559	4
1_P0.7	147842	1585	2
1_P0.8	147924	1469	5
1_P0.9	147723	1228	1
1_P0.10	147626	1400	5
1_P0.11	147432	1368	7

1_P0.12	147650	1084	4
1_P0.13	147350	1616	7
1_P0.14	147538	1516	7
1_P0.15	147730	823	3
1_P0.16	147526	1527	1
1_P0.17	147475	1384	5
1_P0.18	147544	1746	10
1_P0.19	147547	1427	2
1_P0.20	147660	1328	9
1_P0.21	147623	1478	3
1_P0.22	147256	1581	6
1_P0.23	147075	908	0
1_P0.24	147466	1521	3
1_P0.25	147321	986	2
1_P0.26	147253	932	6
1_P0.27	147351	1071	0
1_P0.28	147527	1550	4
1_P0.29	147625	1534	6
1_P0.30	147391	1493	3
1_P0.31	147918	1870	3
1_P0.32	147477	1328	5
1_P0.33	147558	1260	2
1_P0.34	147638	1707	7
1_P0.35	147693	1780	3
1_P0.36	147368	1849	3
1_P0.37	147421	1602	2
1_P0.38	147391	924	2
1_P0.39	147667	1653	6
1_P0.40	147636	1713	5
1_P0.41	2920	36	0
1_P1.0	140253	1739	8
1_P1.1	140732	2099	3
1_P1.2	140986	1614	6
1_P1.3	140801	1928	5
1_P1.4	142069	2533	8
1_P1.5	140963	1913	5
1_P1.6	140804	1681	12
1_P1.7	56310	701	2
TOTAL	**7096188**	**73881**	**233**

Table.5 Hydrogen Bond Analysis.

Ligand Name	No of H-Bond	Amino Acid name	H-bond Distances (Å)
Kanamycin	7	Gly18	1.978
		Asn41	2.043
		Asp43	1.662
		Thr130	2.006
		Asn163	1.945
		Glu136	1.929
		Glu136	2.472
Capreomycin	8	Asp43	1.890
		Gly107	2.099
		Asn22	2.206
		Asn22	1.881
		Arg140	1.909
		Asp184	1.642
		Phe180	2.160
		The130	2.173
ZINC27199796	5	Thr106	2.243
		Ala68	1.933
		Ala70	1.865
		Arg140	2.325
		Glu136	1.732
ZINC67665159	5	Thr106	2.144
		Ala68	1.866
		Ala68	1.924
		Ala70	2.005
		Glu136	2.099
ZINC33753243	7	Glu136	1.892
		Ala70	2.127
		Gly68	2.189
		Ala68	2.150
		Thr106	2.014
		Thr106	2.209
		Thr106	2.295
ZINC03789759	4	Glu136	2.007
		Gly18	2.393
		Ala68	2.117
		Gly69	2.185
ZINC19594549	6	Glu136	2.164
		Arg140	2.240
		Gly69	1.993
		Ala68	1.946
		Thr106	1.758
		Thr106	2.058
ZINC06385314	5	Gly69	2.132
		Ala68	2.090
		Thr106	1.913
		Thr106	2.351
		Thr106	2.229

Preparation of the phase database

A dataset consisting of small-molecule of natural compounds was downloaded in (SDF) format from ZINC database. It was used as an input in the Generate phase database panel of phase. The structures were cleaned and different conformations were generated for each compound along with defining the pharmacophore sites points for each. The database prepared was then used to screen potential tuberculosis inhibitors.

A fragment subset (9, 535, 66 compounds) and the lead like subset (70, 961, 88 compounds) of the ZINC small-molecule database are screened to best fit the pharmacophore model, which is constructed earlier for FtsZ target. This will reduce the time in screening the compounds which are more suitable for the active site binding. Computer aided screening is thus a useful tool in identifying the potential compounds which can inhibit the target molecule. Using this method, the collective ligand set has been brought down to 274 compounds, which have the best pharmacophoric properties with the selected hypothesis AHNRR.10.

Virtual screening of database using the developed pharmacophore model and interaction analysis of the hits generated using in-silico docking studies

To find novel molecules comprising the required special arrangement of pharmacophore sites, the selected AHNRR.10 hypothesis was screened against the prepared fragment-like and drug-like natural compound dataset (ZINC database). 274 hits matching the selected hypothesis were obtained. AHNRR.10 hypothesis and the 274 best fit compounds are further used for molecular docking studies (Table: 2 & 4).

These compounds were further investigated for their binding affinities and mode of interaction with FtsZ Protein using extra precision docking protocol of Glide. Multiple hydrogen bonds were observed in the docked FtsZ Protein. The FtsZ residues involved in this interaction were Gly 18, Asp 43, Thr 106, Ala 68, Glu136, Arg 140, Gly69 (Table: 5).

Many other amino acids, namely Glu 136, Asn 22, Ala 70, Thr 106, Ala 68 and other were further contributing in stabilizing FtsZ molecules within the active site of the protein with the help of hydrophobic and van der Wall interactions. Many compounds with XP docking score above (-7.0) were obtained. Finally, the following eight top scoring compounds (Kanamycin, Capreomycin, ZINC27199796, ZINC 67665159, ZINC33753243, ZINC03789759, ZINC19594549 and ZINC06385314) were then investigated to get insights of their binding mode in the active cavity of FtsZ which has high glide score (Table: 3) are selected for further exploratory studies.

Conclusion

Drug resistance is always a major concern in the development of targeted agents; FtsZ is an important protein in the process of cell division of tuberculosis. In this study, we designed a pharmacophore model based on the already known tuberculosis inhibitors. It was then used to screen a large virtual database of natural compounds (ZINC database) has resulted in new scaffolds for developing tuberculosis inhibitors. Eight compounds including two known inhibiters (Kanamycin, Capreomycin, ZINC27199796, ZINC67665159,ZINC33753243,ZINC03789759, ZINC19594549 and ZINC06385314) were shortlisted from the huge list of hits using extra precision docking of these small molecules against the active site of FtsZ.

These compounds were shown to possess high binding affinity for the FtsZ active site. Thus, these compounds can be considered as potential inhibitors for the treatment of tuberculosis.

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