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Pharmacophore Modeling and Binding Analysis for targeting *Mycobacterium tuberculosis* MurC ligase

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Introduction

Tuberculosis: The Problem

- ⇒ 1.7 billion people affected worldwide
- ⇒ 8 million new cases annually
- ⇒ 3 million deaths per year
- ⇒ Treatment is difficult and requires long courses of multiple antibiotics
- ⇒ Multidrug resistant TB (MDR-TB) populations emergence within the TB species, remains unaffected by traditional TB treatments
- ⇒ Prevention relies on screening programs and vaccination, usually with Bacillus Calmette-Guerin vaccine
- ⇒ Targeting cell wall biosynthesis is a prime area for anti-mycobacterial drug research

- ♦ Chemotherapy
- ♦ First line drugs (INH, Rifampicin)
- ♦ Second line drugs (Streptomycin ,PAS)
- ♦ Combination therapy (DOTS)

Therapy:

- ♦ Development of Resistance
- ♦ Nephrotoxicity and Hepatotoxicity
- ♦ Ototoxicity
- ♦ Ocular toxicity

Targeting MurC ligase

Drawbacks of Current Therapy:

- ⇒ Member of Mur amide Ligase super family (MurA-X).
- ⇒ Catalyses ligation of L-alanine (Ala) and UDP *N*- acetyl-muramic acid (UNAM) to form UDP-*N*-acetylmuramoyl-L-alanine (UMA).
- ⇒ Essential enzyme for survival in 7 species of mycobacterium according to Database of Essential Genes (DEG).

Materials and Methods

(a) Structure of Molecules

- 50 molecules belonging to 4 different chemical classes were chosen from Literature.
- Structures were drawn using Sybyl7.1 installed on Silicon Graphics Fuel workstation.
- All molecules were minimized with Powell's conjugate gradient method using MMFF94 force field.

(b) Pharmacophore mapping

- ♦ 3D-QSAR ligand based pharmacophore model was generated using transition state inhibitors and pulvinone series reported in literature.
- ♦ HipHop Pharmacophore model was developed using Catalyst module of Accelrys Discovery Studio 2.5.
- ♦ 50 molecules were divided into 3 classes: Active, Moderately active and least active.
- ♦ Training set constituted of 10 molecules and remaining molecules were assigned to test set.
- ♦ The model was then validated using test set and the model was used for virtual screening of Zinc database and Maybridge database.
- ♦ The hits obtained from above were subjected to molecular docking studies.

(c) Molecular Docking

- ♦ Molecular docking was performed using Glide 4.5 module of Maestro 8.5.207.
- ♦ Poses which are closest to the bound ligand in template protein crystal structure (1P31) were selected for the analysis.

Figure 2– Molecule series chosen for Pharmacophore mapping (from left to right) 1) *N*-Acyl Hydrazones 2) Pulvinic acid 3) *N*-Benzyl Rhodanine and 4) Phosphinate series.

Results and Discussion

- ⇒ The Pharmacophore mapping of MurC ligase inhibitors yielded a map with following features: one ring aromatic, two acceptors, one hydrophobe with a fit value of 4 and a score of 130.14 (Fig. 3).

Figure 3 –Pharmacophore map of MurC ligase inhibitors

- ⇒ The ligands were aligned with the Pharmacophore map and correlation between the IC₅₀ values and fit values were established.
- ⇒ Table 1 displays ligands with the best fit values and their corresponding pIC₅₀ values.

- ⇒ Validation of Pharmacophore model: A good correlation was found to exist between best fit values and pIC₅₀ values

Table 1 Relation between pIC₅₀ and Fit values

S. No.	Name of compound	pIC ₅₀	Fit value
1	Pulvinamide	8.6513	4
2	Pulvinic acid	8.48	3.985
3	Vulpinic acid	7.5826	3.976
4	Pulvinone_11a	7.2130	2.815
5	Disubstituted Pulvinone_18b	6.1934	2.723
6	Disubstituted Pulvinone_20d	5.9120	2.658
7	Nbenzylidene sulfonohydrazide_11h	4.9870	2.423
8	Disubstituted Pulvinone_20b	3.9306	2.207

♦ Molecular Docking

- ⇒ Reported inhibitors were docked in the developed homology model of MurC ligase enzyme, within a distance of 10 Å of the active site as predicted by LigPlot of PDBsum software.
- ⇒ Most of the inhibitors were found to undergo interactions with 3 key residues i.e. Gly125, Lys126 and Arg332
- ⇒ There was a satisfactory correlation between IC₅₀ and Glide Score (GScore) of Docking

Figure 4: Best GScore inhibitor showing interaction with important MurC cavity residues.

Table 2– Best 6 inhibitors with their Glide docking scores

Molecule	GScore	H-bonding residues
NBenz_5c	-7.4623	Gly125, Lys126, Glu170, Arg332
NAcylhyd_19	-5.8512	Gly125, Lys126, Glu170
NBenz_5c	-5.0450	Gly124, Lys126, Glu170
NBenz_11c	-4.7091	Gly125, Gly126, Arg331
Pulvinone_20b	-4.4805	Gly125, Lys126, Glu170
Pulvinamide	-4.1000	Gly125, Lys126, Arg332

Conclusions

- ⇒ MurC ligase is a potential target for the treatment of TB infection.
- ⇒ Pharmacophore mapping of MurC ligase inhibitors yielded essential features for a lead to act against the enzyme.
- ⇒ Molecular Docking studies with the designed ligands revealed important ligand interacting residues i.e. Gly125, Lys126, Glu170 and Arg332.
- ⇒ These results can aid in further drug development against *Mycobacterium tuberculosis* infections.

Bibliography

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