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Study of pyridine based triazole derivatives as *Mycobacterium Tuberculosis* TMPK Inhibitors

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ABSTRACT : The re-emergence of tuberculosis (TB) as a global health problem over the past few decades, accompanied by the rise of drug-resistant strains of Mycobacterium tuberculosis, emphasizes the need for discovery of new therapeutic drugs against this disease. 1,2,3-triazoles are an important class of heterocyclic compounds due to their wide range of applications as pharmaceutical agents. Literature describes triazoles as anti-microbial agents, for e.g. N-substituted phenyl–1,2,3-triazoles derivatives show activity against mycobacterium tuberculosis. This work describes theoretical study of series of triazole derivatives of pyridine hydrochlorides as possible inhibitors of thymidine monophosphate kinase (TMP kinase) by molecular docking method. TMP kinase is an attractive target for many drugs as per literature. Reference compound (thymidine monophosphate) has been used to bind with active sites of enzyme and compared with the pyridine based triazole derivatives. The studied compounds inhibit the desired enzyme as they act in the same pattern as the reference. These designed inhibitors make several interactions with the amino acid residues that conform the active sites. The "G value of all the compounds is obtained.

Keywords : Triazoles, autodock, trimidine monophoshate kinase, anti-mycobacterium, tuberculosis

INTRODUCTION

Despite the availability of antitubercular agents, tuberculosis remains primary cause of comparatively high mortality worldwide. The statistics shows that around three million people throughout the world die annually from tuberculosis [1,2]. Incidences of tuberculosis have been increasing during the last 20 years; it is now the first cause of mortality among infectious diseases in the world. Once nearly vanquished by antibiotics, at least in the developed world, TB resurged in the late 1980's and now kills more than 2 million people a year. Thirty million people are at risk of dying from TB in the next 10 years, most of them living in Africa [3]. Mycobacterium tuberculosis, the causative agent of this disease is a slow-growing bacillus, primarily transmitted via the respiratory route, mostly causing pulmonary TB. In HIV infected patients, TB has become the main cause of death. At the moment one out of three HIV infected patients is co-infected with TB and 40% of them develop the active disease, rendering the HIV-problem countries (most of them lying in Africa) also the regions with the highest TB incidence in the world. Although it is a leading cause of HIV-related morbidity and mortality, only little attention is paid to TB in HIV/AIDS programs so far [4]. Thymidine monophsophate kinase (TMPK, ATP:dTMP phosphotransferase, thymidylate kinase) belongs to the large super family of nucleoside monophosphate kinases (NMPK's). It catalyses the reversible phosphorylation of thymidine monophosphate (dTMP) to thymidine diphosphate (dTDP) utilising ATP as its preferred phosphoryl donor [5]. The position of TMPK at the junction of the *de novo* and salvage pathways of the thymidine triphosphate (dTTP) metabolism, as the last specific enzyme for dTTP synthesis, makes it an interesting target for new drug design. Using a *cdc*8 mutant of *Saccharomyces cerevisiae*, deficient in TMPK, Jong et al. proved that yeast TMPK (TMPKy) is essential for DNA replication and thus for cellular growth [6-7]. Furthermore, the thymidylate kinase activity of yeast shows to be a cell-cycle-regulated: enzyme activity is barely detectable in non-proliferating tissues, whereas in growing tissues, thymidylate kinase activity is increased considerably. A correlation between thymidylate kinase and the rate of DNA synthesis during rapid growth has been documented [8]. In the case of the herpes simplex virus (HSV), the most successful antiviral drug (acyclovir) available on the market is directed against thymidine kinase. Acyclovir is phosphorylated by several viral or host kinases into acyclovir triphosphate, which terminates DNA synthesis when incorporated into the viral DNA [9, 10]. Azoles (imidazoles and triazoles) are the most widely studied and currently used class of antimycobacterial agents. The crystallographic data indicated that the above compound has binding pockets. TMPK from *M. tuberculosis* is a homodimer with 214 amino acids per monomer [11]. The X-ray structure has been recently solved at 1.95-Å resolution as a complex with TMP, thereby making it possible to initiate structure-based drug design studies [12]. To validate the docking studies, the thymidine monophosphate was docked with the protein structure of thymidylate kinase as reference [13]. The TMP kinase backbone is characterized by nine solvent-exposed alpha-helices surrounding a central beta-sheet made of five beta-strands, typical of the so-called Rossmann-fold [14 – 18]. Therefore, there is a greet demand to identify and discover new compounds with high binding affinity in same binding pocket. The AutoDock4 program was used for molecular docking. AutoDock is available for academic use from the Scripps Research Institute for free of charge [19-21]. In the docking studies, the Lamarckian Genetic algorithm (LGA) parameter is used [22-23].

MATERIAL AND METHODS

Molecular modeling is performed on Compaq AMD athlon laptop with windows vista operating system. Atomic coordinates for the three dimentional protein model (complex of ligand with protein; PDB code1g3u [24]) were obtained from brookhaven protein data bank. Latest version of autodock is used for docking studies as its algorithm allows full flexibility of small ligands. The ligand and all the water molecules were removed from protein database file and hydrogens were added to the protein molecule. The resulting 3D structure of the protein was saved as PDB file. All the ligands used for docking were drawn by the online demonstration of CORINA for generating 3D coordinates program [25]. The MOPAC2009 program was used to optimize the structures of designed ligands. The optimization of each ligand was repeated many times. It has been shown that it successfully reproduces many crystal structure complexes and includes an empirical evaluation of the binding free energy. The enzyme structure was first cleaned of its water molecules and cocrystallized ligands. The preparation of protein and ligand input structures and the definition of the binding sites were carried out under a GRID-based procedure. A rectangular grid box was constructed over all the protein ($126 \times 126 \times 126$ Å^3) with grid points separated by 0.375 Å under blind docking procedure.Taking initial population of 300 randomly placed individuals and a maximum number of energy evaluation(1.0×10^7), all docking simulations were carried out by using the hybrid Lamarckian Genetic Algorithm. The resulting docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound is considered and used for further analysis. Consequently a population of 10 docked configurations was produced for each inhibitor. All other parameters were maintained at their default settings. All the docking result visualizations are achieved by using autodock4. The structures of the compounds used in study are listed in Table given.

Table 1 : Calculated free energy of binding (Kcal/mol) between the protein and the ligand, with the rank of difference derivative used.

S. No.	Structure ID	R2	R1	"G_{BIND}	Rank
1	1a	H	H	– 6.20	7
2	1b	H	Cl	– 6.62	2
3	1c	Cl	H	– 6.12	8
4	1d	Cl	Cl	– 6.47	4
5	1e	H	CH_3	– 6.57	3
6	1f	CH_3	H	– 6.02	10
7	1g	CH_3	CH_3	– 6.21	6
8	1h	CH_3	Cl	– 6.35	5
9	2	H	H	– 6.09	9
10	3	H	H	– 7.33	1

RESULTS AND DISCUSSION

In order to propose new inhibitors, we designed a series of different triazole derivatives. The geometrical optimization of all the derivatives has been done with MOPAC2009 software which uses the semiemprical molecular orbital calculation method for geometrical optimization [26].

Analysis of docking

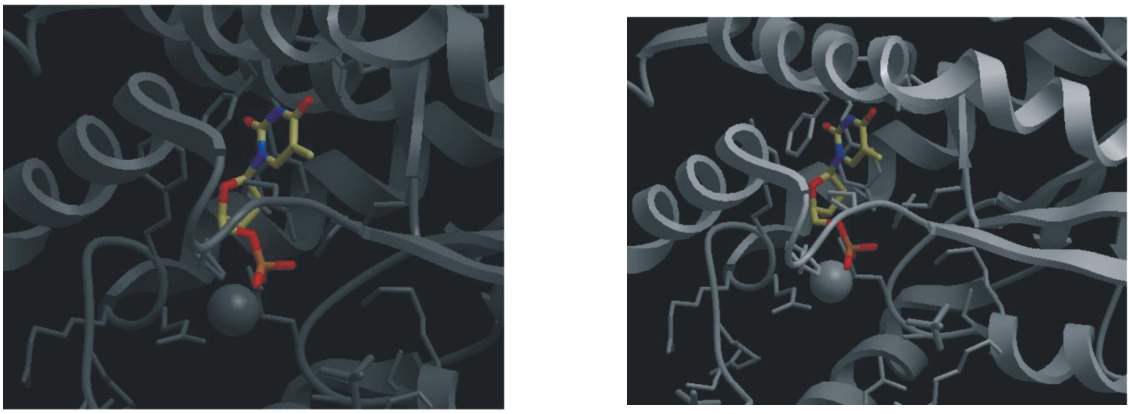
According to the procedure adopted for the molecular docking, all designed triazole compounds (**1a-3**) (Fig. 1-3) and reference were characterized by a similar docking mode in the binding pocket of the thymidylate kinase from *M. tuberculosis.* For computational drug design, there are two key requirements for accurate molecular docking.

(*i*) The generation of optimized conformations of docked ligands, and (*ii*) the accurate prediction of binding affinity of the ligand with the crystallographic structure of the inhibitor.

To check whether our procedure complied with requirement (*i*), we modeled and docked thymidine monophosphate, for which the crystallographic structure (obtained from Protein drug database) in complex with TMPK is available, as a reference system. This was done by removing the inhibitor from the enzyme allosteric site, building a new molecular model for this compound, applying the conformational procedure. The best docked structure, which is the configuration with the lowest docking energy in a prevailing cluster, was then compared with the corresponding crystal structure. Fig. 1(*a*) and (*b*) show a comparison between the co-crystallized conformation of thymidine monophophate into the allosteric binding site of the thymidylate kinase from *M.tuberculosis* and the docked conformation obtained upon application of the computational strategy adopted in this work. A comparative study between the crystallographic data and the structural data obtained by docking revealed that the docking results matched those obtained by crystallography.

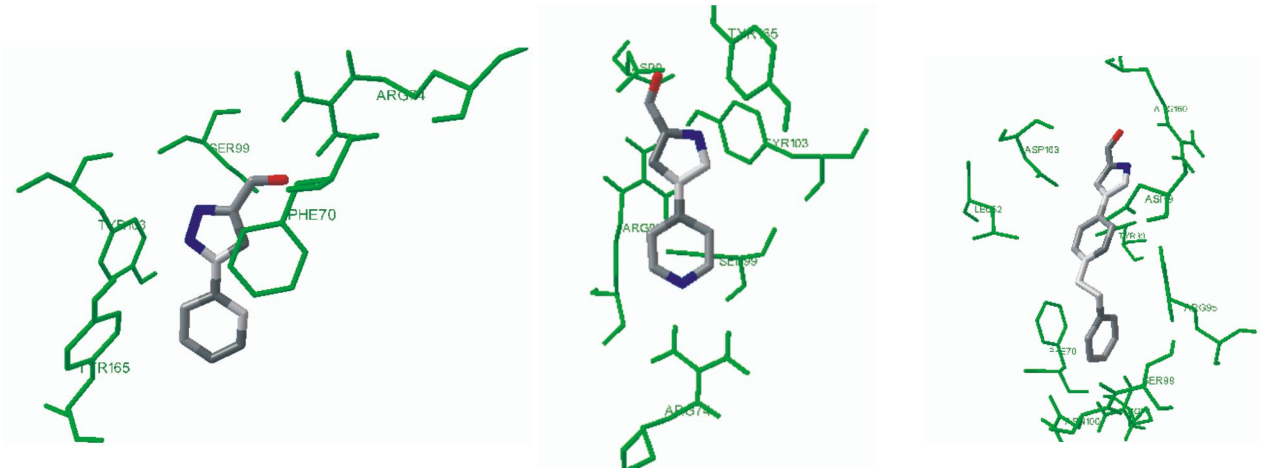
For the analysis of the binding mode of the different inhibitor residues, the best docked conformation of each molecule is taken. In binding mode of all the inhibitors, the triazole ring and the pyridyl ring are in same plain. Further, rings show interactions with the side chain residues for e.g. in first compound, triazole ring is interacted with SAR99, PHE70 and the pyridyl ring is interacted with TYR103 which is further attached with TYR165. In the second compound there is clear attachment between the pyridyl ring with SER99 and bonded and non-bonded interaction of triazole ring with ARG95, TYR103, TYR155, ASP9. The substituted group of the various compounds undergoes various types of bonded and non-bonded interaction with various residues depending on the type of group present on designed inhibitors. The molecular docking of different molecules shows that the predicted binding free energies are quite good, without any reparametrization (Table).

The docking results indicates that the compound **3** (Fig. 5) has minimum free binding energy (ΔG = – 7.33), among all the designed compounds. So, the compound 4-aminoazobenzene shows the best docking results. There are bonding and nonbonding interaction of TYR39 and ASP163 with the triazole ring, and SER99, ARG95, PHE70, ASP9 and LEU52 residues with the benzene rings. The compound **1b** also shows good free binding energy (ΔG = – 6.62) in which chlorine is attached at 1 position. Analysis of the data reveals that the investigated triazoles either show interactions with same amino acids as is shown by the reference compound or they show interaction nearby amino acid residues. Consequently these may prove to be as good inhibitors as the reference.



(a) **(b)**

Fig.1(a). The co-crystallized conformation of reference into the active site from *M. tuberculosis* and **(b)** the corresponding docked conformation of reference in the same enzyme pocket obtained upon application of the computational strategy adopted in this work.



(a) **(b)** **(c)**

Fig.2(a). Structure of compound 1(*a*) after docking in pocket of protein.

Fig.2(b). Structure of compound 2 after docking in pocket of protein docking in protein pocket.

Fig.2(c). Structure of compound 3.

CONCLUSION

For our goal to propose new inhibitors, we designed a series of different pyridine based triazole derivatives. The general structure of different derivatives used is given in Fig. 3, 4, 5. About 10 compounds are docked and their binding free binding energy are compared. All the compounds show good binding affinity with acceptable free binding energy indicates that the designed compounds give good stable complexes. So the docking studies can further be used to design newer compounds with better activity targeting thymidylate kinase. This study also reveals that thymidylate kinase can also be targeted by antituberculosis drugs having triazole moiety. A similar approach might lead to potent antitubercular agents.

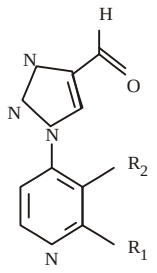


Fig.3. General structure of the compounds used for 1(a-h).

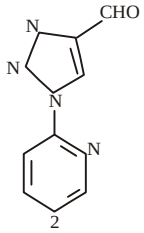


Fig.4. Structure of compound 2.

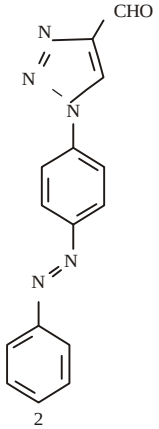


Fig.5. Structure of compound 3.

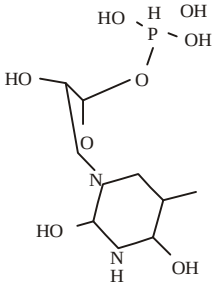


Fig.6. Thymidine monophosphate (reference).

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