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ORIGINAL ARTICLE

TB Drug Potency of a Structure Modified Derivative of Ethambutol : A Docking Based and Quantum-Chemical Comparison Study

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ABSTRACT
A structure modified derivative of Ethambutol is designed by virtual screening using molinspiration and named as Pyrobutol. Drug potency of Pyrobutol as a TB drug is tested through comparison with Ethambutol using quantum computational techniques in density functional approach, molecular docking using Docking Server and bio-activity calculations using molinspiration and Chemosophia packages. It is observed that according to bio-activity predictions, Pyrobutol may be a diversely active drug. It is also predicted to be less toxic compare to Ethambutol according to relative toxicity calculation. Metabolic path of Pyrobutol may be same as Ethambutol. Probability of metabolism by cytochrome P450 3A4 is 98.8 % and 94.4 % for Ethambutol and Pyrobutol respectively. Predicted physical properties like Log P, polar surface area, molar volume, pK_b, etc, of Pyrobutol also in favor of its applicability as a potential TB drug.
Key Words: *Ethambutol, Pyrobutol, Docking, Bioactivity, Quantum Computation, Relative toxicity*

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INTRODUCTION

Search for a new TB drug is one of the important area of researches today due to the high capacity of acquired drug resistance [1] of this pathogen. It is world's leading infectious disease at present [2]. According to World Health Organization (WHO) report, in 2014, out of 9.6 million TB affected persons 1.5 had died [3]. This is a threat for under developed and developing countries.

Ethambutol is one of the five basic or "first line" TB (tuberculosis) drugs [4] which are used for the treatment of new patients. Other four first line TB drugs are Isoniazid, Rifampicin, Pyrazinamide and Streptomycin. Except these five TB drugs, there are near about twenty more drugs which are used for the treatment of TB which caused by *Mycobacterium tuberculosis*. Most of these drugs were developed some years ago. TB, which is caused by *Mycobacterium tuberculosis,* is an intracellular parasites which is capable of living and reproducing either inside or outside host cells. Primarily it is a pathogen of the mammalian respiratory system. In human body it infects the lungs. For new patients, according to the World Health Organisation (WHO) TB drug treatment should have been prolonged for six months. First two months "intensive" treatment phase followed by a four months "continuation" phase. Ethambutol is used in the "intensive" treatment phase along with Isoniazid, Rifampicin and Pyrazinamide [5]. A patient should take all recommended drugs as prescribed and continue at least six months without any break. It is essential to take several TB drugs together because if only one or two TB drug is taken at a time, then the patient will very quickly become resistant to those drugs. If the treatment is interrupted or stopped before the schedule date of termination or being fully cured, then also TB bacteria that a patient has, develops resistance to the TB drugs. It is because TB bacteria use a waxy coat for its survival against any unlikely surroundings. Among 3959 genes [6] of TB bacteria, 250 genes involved in fatty acid metabolism including 39 genes for the metabolism of polyketide which generates the waxy coat. TB bacteria isolated from the lungs of infected mice were shown to preferentially use fatty acids over carbohydrate substrates

[7]. These bacteria can also grow on the lipid cholesterol as a sole source of carbon, and the genes involved in the cholesterol use pathway(s) have been validated as important during various stages of the infection lifecycle of TB bacteria, particularly during the chronic phase of infection when other nutrients are not available [8]. The change of pathways of livelihood of TB bacteria is one of the reasons of its high capacity of drug resistant.

Ethambutol is a bacteriostatic drug. It does not kill the bacteria but stop its reproduction by obstructing the formation of cell wall inhibiting the activity of arabinosyltranferase which form mycolyl-arabinogalactan-peptidoglycan complex in the cell wall. Disruption of the arabinogalactan synthesis inhibits the formation of this complex and leads to increased permeability of the cell wall [5]. Ethambutol also target the biosynthesis of lipoarabinomannan (LAM) which is equally essential component of the unique cell envelope of this pathogen. Biosynthesis of LAM requires three types of arabinosyltranferase namely EmbA, EmbB and EmbC. Recent works conclude that mutations in EmbC reduce the activity of arabinosyltranferase [9]. In the same works, it is also confirmed that EmbC is one of the targets of Ethambutol. The resistance to Ethambutol has been reported as 4% of the total clinical isolates of TB and it is prevalent among multidrug resistant strains [10]. Some mutant proteins from EmbA, EmbB and EmbC have been isolated from Ethambutol resistant TB [11] where the sequence similarity is nearly 57% among the proteins in this family. The mutations which are responsible for the resistance to Ethambutol are located near the polypeptide chain of 390aaof the C-terminal region [5].

In the present work, a new compound is modeled by substitutions and modifications of Ethambutol and characterized. Its potency as a TB drug is tested and found that it may be an alternative to Ethambutol. This modeled compound is (2R)-3-fluoro-2-({1-[(2s)-1-fluoro-3-hydroxypropan-2-yl]-1,6-dihydropyridine-3-yl}(fluoromethyl)amino)propan-1-ol. For simplicity, we may name this new compound as PYROBUTOL. This compound is not previously reported (confirmed by SciFinder search). Pyrobutol has some structural and chemical similarity with Ethambutol except one dihydropyridin ring and three fluoride substitutions. Bioactivity of Pyrobutol is predicted through different insilico tests. Binding affinity is calculated through molecular docking. Docking calculations are well known and frequently applied in pharmaceutical researches for nearly two decades. Very recently docking calculations of Ethambutol is studied by Salgado-Moran *et. al.* [5] to the C-terminal domain of arabinosyltranferase from Mycobacterium tuberculosis. Thus, docking study of Pyrobutol is also done at the same domain. Bio-activity and other properties of Pyrobutol are compared with that of Ethambutol calculated and estimated at the same level of theory. It is found that Pyrobutol may be an alternative drug to Ethambutol. With respect to tuberculosis dihydrofolate activity Pyrobutol is a better drug compare to Ethambutol.

COMPUTATIONAL DETAILS

Docking

Docking calculations were carried out using DockingServer [12]. The MMFF94 force field [13] was used for energy minimization of ligand molecule (*Pyrobutol*) using DockingServer. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on C-terminal of *arabinosyltranferase* protein downloaded from protein data bank (PDB code : 3pty, web – http://www.rcsb.org). Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [14]. Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Autogrid program [14]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [15]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Bio-activity and property calculations

For bio-activity prediction two different packages were used which work online; molinspiration [16] and Chemosophia [17]. Important physical properties, LogP and PSA, are calculated using molinspiration. LogP calculation is performed in molinspiration using group continuation methodology. The polar surface area (PSA) calculation in molinspiration use topological PSA methodology developed by Etrl *et. al.* [18]. LogP, pk_b, water solubility and toxicity are also calculated using Chemosophia. Relative toxicity (γ) of Pyrobutol with respect to Ethambutol is also calculated using bag's method [19].

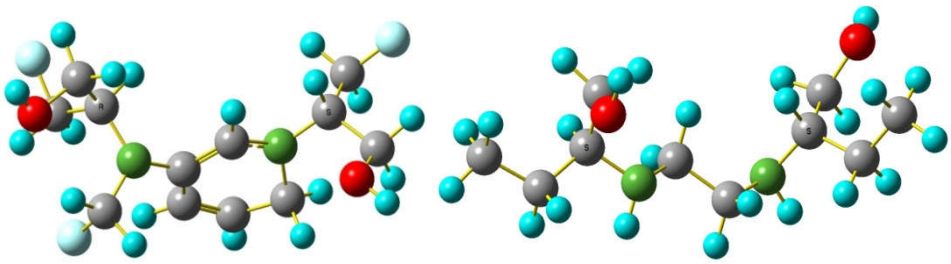
Quantum Computation

Geometry optimizations are done using Gaussian 09 package [20]. Optimization is done without any symmetry constraints. Minimum energy structures are confirmed by the harmonic vibrational frequency calculation with all positive mode of vibrational frequency. The convergence thresholds we set to 0.000015 Hartree/Bohr for the forces, 0.00006 Å for the displacement and 10^6 Hartree for the energy change. Calculations are performed with the density functional theory (DFT) with unrestricted Becke's three parameter hybrid exchange functional [21] combined with exchange component of Perdew and Wang's 1991 functional [22-25], abbreviated as B3PW91. We have used cc-pVDZ [26] basis for these calculations which is available in Gaussian 09 package.

RESULTS AND DISCUSSIONS

Quantum computational results

Geometry of Ethambutol and Pyrobutol were optimized using Gaussian 09 package with B3PW91 functional and cc-pVDZ basis. Optimized geometries are presented in Figure-1. Electric dipole moment and reactivity descriptors ( ) [27, 28] were calculated at these optimized geometries. Condensed Fukui functions [29, 30] were also calculated to find the primary center for electrophilic (f_{ep}) and nucleophilic (f_{np}) attacks. From the charge distribution analysis electron density over two oxygen atoms are considered to compare the hydrogen bonding capacity of these two compounds. Absolute electronegativity (χ) [31] is also calculated for these two compounds for comparison of hydrogen bonding capacity with the enzyme. Calculated results are presented in Table-1.



(a) *(b)*

Figure 1 : Optimized geometries of (a) Pyrobutol and (b) Ethambutol

Table -1 : Computed properties of Ethambutol and Pyrobutol

Compound	Charge density over oxygen atoms	χ	ω	Highest electrophilic center (f_{ep})	Highest nucleophilic center (f_{np})	Calculated dipole moment (D)	Relative toxicity (γ)
Ethambutol	-0.264, -0.270	0.079	0.023	O(23) 0.223 O(37) 0.181	N(1) 0.191 N(9) 0.182	1.72	1.0
Pyrobutol	-0.264, -0.270	0.099	0.063	C(14) 0.239 C(12) 0.197	C(12) 0.146 C(14) 0.141	3.93	0.3

Relative toxicity [19] of Pyrobutol is calculated (using bag's method) with respect to Ethambutol. As structural similarity between these two compounds is more than 50% (62%, calculated as total similarity/total events) relative toxicity relation would be applicable for these compounds. It is observed that Pyrobutol is less toxic compared to Ethambutol. Toxicity of Pyrobutol is nearly 1/3rd of the toxicity of Ethambutol. Both reactivity index (ω) and absolute electronegativity (χ) of Pyrobutol is higher (0.063 and 0.099 respectively) than that of Ethambutol (0.023 and 0.079 respectively). This suggests that Pyrobutol would be more reactive and would form more stable enzyme-inhibitor complex than Ethambutol. Thus, it is expected that IC_{50} of Pyrobutol would be less than that of Ethambutol. From the chemical structure it is expected that molecular volume of these two compounds would be nearly same. As dipole moment of Pyrobutol is more than double of Ethambutol, surface potential of Pyrobutol would be higher than that of Ethambutol and hence polar interaction between enzyme-inhibitor would be more favorable for Pyrobutol. Charge densities over two oxygen atoms were analyzed for both these compounds to compare the hydrogen bonding capacity of them. But, surprisingly, it is observed that charge density over oxygen atoms is exactly same for both compounds. Still it may be concluded that Pyrobutol should form more number of hydrogen bonds and stronger hydrogen bonds with enzyme due to the presence of three highly electronegative fluorine atoms. To compare the electrophilic and nucleophilic substitutions or covalent bond formation condensed fukui functions were calculated for these two compounds. It is

observed that, for Ethambutol, two oxygen atoms are favorable centers for electrophilic attack where two nitrogen atoms are centers for nucleophilic attack. But for Pyrobutol, two carbon atoms (C_{14}, attach to one nitrogen atom, and C_{12}, opposite to C_{14}) of dihydropyridin ring are preferable centers for both electrophilic as well as nucleophilic attack due to the presence of conjugate double bond and some short of aromatic ring character. From the calculated values it may be concluded that electrophilic attack would be more favorable for Pyrobutol but nucleophilic attack would be favorable for Ethambutol.

Bio-activity results

Bio-activities of Ethambutol and Pyrobutol are calculated using molinspiration. Bio-activity score prediction in molinspiration uses the Bayesian statistics [32]. The numerical values range from -3 to +3. In this scale, binding probability values of the corresponding ligands range from -0.5 to 1.5 with a maximum near at 0.5 (see different plots presented on the web of molinspiration). An analysis of about 100000 known drugs show that an activity score greater than 0.2 implies drug like behavior and a score greater than 0.5 implies very good drug activities. The higher the activity value of a compound, higher is the probability to be active as a drug. Bio-activities of these two compounds for six different types of drugs were calculated using molinspiration and presented in Table-2.

Table -2: Bio-activity score of Ethambutol and Pyrobutol using molinspiration

Compounds	GPCR ligand	Ion Channel modulator	Kinase inhibitor	Nuclear receptor	Protease inhibitor	Enzyme inhibitor
Ethambutol	-0.30	-0.16	-0.44	-0.68	-0.23	-0.08
Pyrobutol	0.46	0.30	0.22	0.39	0.26	0.54

According to bio-activity score, Pyrobutol may be a very good drug for enzyme inhibitor. It may be a good drug for other five classes also. On the other hand, Ethambutol is expected to has some drug like behavior as enzyme inhibitor. Thus, it is expected that Pyrobutol may be a better TB drug compare to Ethambutol. Encouraged by bio-activity study, we performed molecular docking on C-terminal of arabinosyltranferase with these two compounds using Docking Server. We have also studied tuberculosis dihydrofolate reductase inhibitor activities of these two compounds using Chemosophia bio-activity prediction software which works online. Bio-activity results using Chemosophia package are presented in Table -3.

Table -3: Bio-activities of Pyrobutol and Ethambutol using Chemosophia

Compound	Tuberculosis dihydrofolate reductase inhibitor activity	Probability of metabolism by cytochrome P450 3A4	Probability of metabolism by cytochrome P450 2D6
Ethambutol	51.9 %	98.8 %	0.6 %
Pyrobutol	75.6 %	94.4 %	37.4 %

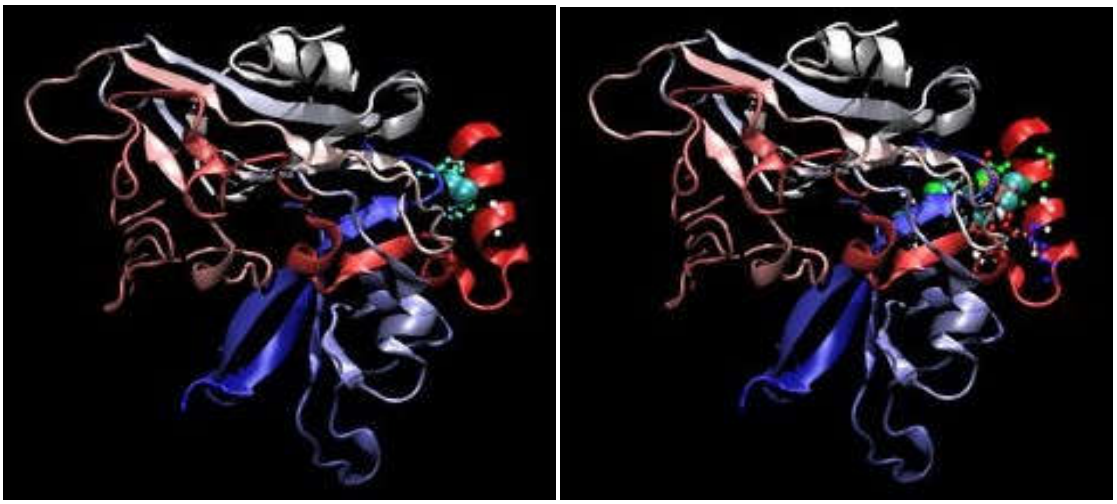
From the computed results, it is observed that tuberculosis dihydrofolate reductase inhibitor activities of Pyrobutol are nearly 1.5 times of Ethambutol. Both these two compounds have high metabolic probability by cytochrome P450 3A4. But Pyrobutol has significant chance of metabolism by cytochrome P450 2D6 also.

Docking results :

Table -4 : Binding free energy and binding constant (Ki) of Pyrobutol and Ethambutol

Compound	Binding free energy (kcal/mol)	Binding constant (Ki) (mM)	Binding frequency	Interacting amino acids
Ethambutol	-2.69	10.59	10 %	Hydrogen bonding : ASN 740 Polar : ASP 1051, ASP 754 Hydrophobic : LEU 744, LEU 751, LEU 1049 Other : LYS 747, ALA743
Pyrobutol	-2.59	12.60	10 %	Halogen bonding : ASN 740, SER 739, TRP 1057 Hydrogen bonding : ASP 1051 Polar : ASP 754 Hydrophobic : LEU 744, LEU 751, LEU 1049, ALA 743 Other : ARG 1055

Results of molecular docking using docking server are presented in Table-4. Binding free energy of Pyrobutol is marginally (0.1 kcal/mol) higher than that of Ethambutol; also the binding constant (2.01 mM). But, predicted binding frequency is same (10 %) for both compounds. Most of the interacting aminoacids are common for both the compounds. But for Pyrobutol more number of aminoacids are interacting. Major difference is about halogen bonding. Due to the presence of three fluorine atoms Pyrobutol form halogen bonding with ASP 740, SER 739 and TRP 1057 which is absent in case of Ethambutol. Polar and hydrophobic interactions are nearly same. The image of 3D structure simulated using visual molecular dynamics (VMD) [33] of docking of these two compounds are presented in Figure 2.



(a) Ethambutol *(b) Pyrobutol*

Figure 2 : Binding of arabinosyltranferase with (a) Ethambutol and (b) Pyrobutol

Properties

We have computed few important properties, like logP, PSA, pK_b, molar volume, etc., for these two compounds which are very important for the drug potency of a compound. Results are presented in Table -5. Log P values of these two compounds are nearly same, for Pyrobutol marginally higher. Pk_b value of Pyrobutol is significantly lower than Ethambutol which implies Pyrobutol is a stronger base than Ethambutol. Molar volume of these two compounds are nearly same which means both of them should fit to the same binding site of an enzyme. Polar surface area (PSA) of Pyrobutol is less than that of Ethambutol which may be the reason of less interaction energy of Pyrobutol in spite of number of interacting amino acids is more for this compound. From the physical properties of these two compounds we may conclude that Pyrobutol may act as a drug as effectively as Ethambutol.

Table -5 : Physical properties of Ethambutol and Pyrobutol

Compound	Log P (molinspiration)	pk_b (Chemosophia)	PSA (molins.)	Molar volume (molins.) (cc)
Ethambutol	0.35	10.06	64.51	221.1
Pyrobutol	0.63	7.83	46.93	247.5

CONCLUSIONS

Pyrobutol, structure modified derivatives of Ethambutol which is obtained by virtual screening using molinspiration, is characterized using Density functional theory (DFT), molecular docking, qualitative structure activity relationship (QSAR) and relative toxicity relation. It is observed that, Pyrobutol shows promising drug potentiality. Its activity as tuberculosis dihydrofolate reductase inhibitor is remarkably higher than the FDA approved and commonly used TB drug, Ethambutol. Though, the molecular docking based calculations using arabinosyltranferase show marginally lower binding free energy of Pyrobutol than Ethambutol, other physical properties like LogP, pKb, PSA and molecular volume etc, suggests that it may be a very good drug for TB. As we have found that Pyrobutol has the capacity of forming halogen bonding with the protein along with the high capacity of hydrogen bond formation, polar interactions (as dipole moment of this compound is higher than that of Ethambutol) and hydrophobic interactions (as hydrophobicity of Pyrobutol is higher than that of Ethambutol reflected from the LogP values), it may be a

good drug for other diseases also. From the bio-activity score calculated using molinspiration, it is predicted that this compound may act as multifunctional drug. In TB treatment, as we have discussed earlier, five different drugs are used at a time to stop the chance of development of drug resistant TB. As it is predicted from QSAR study using molinspiration that Pyrobutol has drug-like behavior for all six classes of drugs, it may be a replacement of all five first line TB drugs by a single drug. In other words we can say it may be a resistance proof TB drug. Comparison of Pyrobutol with other four first line TB drugs would be effective to justify our claim.

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