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Research Article

IN SILICO STUDIES FOR VARIOUS ANTIBACTERIAL BENZIL AND ITS SUBSTITUTED ANALOGS

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ABSTRACT

Objective: The antibacterials have moved on to low levels by more challenges toward antibacterial discovery of drug over an earlier period of 30 years. The resistance pathogens such as *Staphylococcus aureus*, *Mycobacterium tuberculosis* (MTB), and *Streptococcus pneumoniae* are nowadays facing difficulty in effective treatment. This leads to the necessary for the new discovery of drugs for antibacterial activity. The foremost disease in the world among all the infectious disease is found to tuberculosis (TB) which causes high proportions of mortality. Hence, we have decided on identifying the leads for the target of enzymes of infectious disease TB.

Methods: The new leads for MTB have been discovered using computer-aided drug design docking tool. The new compounds identified were made to dock into the enzyme active site retrieved from protein data bank.

Results: After three different docking strategies, the score was found to be 4.558 kcal mol^{-1} for the compound 2'-chloro-4-methoxy-3-nitro benzyl in structure activity relationship and docking studies.

Conclusion: The molecule shows valuable interactions and also it is found to be surrounded by non-polar amino acids. On further analyzing the compound it is found to be potent to antibacterial drug discovery.

Keywords: Antibacterials, Docking, Resistance, Absorption, Distribution, Metabolism, Excretion study.

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INTRODUCTION

The demand for novel therapeutic interventions such as antimycobacterial and antibacterial drugs is increasing daily since there are an increased drug resistant bacterial in the world market. *Mycobacterium tuberculosis* (MTB) which is found to be most pathogenic bacteria is a causing agent for human TB leading to a world level bacterial killer [1]. The bacterial strains for drug resistant and also multidrug-resistant existence occur using this bacteria. There are various side effects the may be caused during the treatment due to the usage of more antibiotics for curing the disease. The antibacterial activity on these pathogenic bacteria is identified by doing some preliminary studies using small dosage to reduce the side effects.

The activity was found to be superior, and it shows that benzyl and its derivatives are good antibacterial agents. As a result, the activity of pathogenic bacteria's such as *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli,* and *Staphylococcus epidermidis* gets reduced. In this study [2], the binding on specific sites for target molecules such as proteins has been carried out by a computational method, namely, virtual screening. The future point of view of the compound could also lead to the pharmacokinetic properties

METHODS

Computational details

The computational details for this study were carried out in an Intel Core I5 capacity processor with a memory of 4GB RAM running with the Windows 7 operating system. The virtual screening options for high throughput virtual screening (HTVS), standard precision (SP), and Glide extra precision (XP) docking were all checked to be executed. The module Glide XP of Schrodinger 9.3 (Glide, version 5.7, Schrodinger, LLC, New York, NY, 2015) was utilized to perform docking studies. Suitable bonding and the charges were added to the hetero atoms, and the corresponding hydrogen atoms were added to all the atoms [3].

Protein preparation

The protein file was prepared on protein preparation wizard, and the energy minimization was performed. About 500 cycles of steepest descent and 5000 cycles of conjugate gradient methods with optimized potential for liquid simulations (OPLS) 2005 force field using Schrodinger suite version 9.3 were employed. Grid-A rectangular box surrounding the active site of the protein was located using receptor grid generation panel. The "Write XP descriptor information" option was selected and "Compute RMSD" option was enabled, and rest of the parameters was kept as default. The XP Glide scoring function was used to order the best-ranked compounds, and the important interactions such as π-cation and π-π stacking were analyzed using XP visualizer in Glide module. The input RMSD of the crystal ligand was also ascertained [4].

Preparation of ligands

The synthesized molecules were processed through the Lipinski filters to enable the drug property. Ligand preparation was performed for the synthesized molecules using LigPrep module available in the software (LigPrep v2.2, Schrodinger LLC, New York, NY) and Epik (Epik v1.6, Schrodinger, LLC, New York, NY) to expand protonation and tautomeric states at 7.0±2.0 pH units. Conformational sampling was also performed for all database molecules using the ConfGen search algorithm. ConfGen with OPLS 2005 force field was applied for the generation of conformers with duplicate poses eliminate if the RMSD was <2.0 Å. A distance-dependent dielectric constant of 4 and maximum relative energy difference of 10 kcal mol^{-1} were applied [4].

Molecular docking

Docking studies for the synthesized compounds were performed using Glide module of Schrodinger, LLC, 2015. Primarily, using Glide

module [5,6] (Grid based ligand docking with energetics), we examined for important interactions based on the reference ligand and the protein of interest in the flexible mode docking. The Glide module with three modes of docking, HTVS, SP, and XP mode was employed sequentially. The XP mode was used for exhaustive sampling and advanced scoring, resulting in even higher enrichment.

Finally, the shortlisted hit molecules were selected based on the visual inspection of amino acid interaction, docking score and the active site cavity [7].

Absorption, distribution, metabolism, and excretion (ADME) prediction

All the synthesized compounds for our study were selected, and the molecules were subjected to ADME predicted analysis using QikProp module of Schrodinger. The important properties such as octanol water coefficient (LogP), human oral absorption, Lipinski's rule of five, blood brain barrier (BBB) coefficient, HERG property, and Caco-2 permeability property were predicted for the synthesized compounds, and also the predicted results were checked for any violations to determine the nature of the compounds.

RESULTS AND DISCUSSION

Synthesized compounds have taken for docking studies to establish the structure activity relationship using crystal structure of MTB cocrystallized with inhibitor thiazole benzamide (protein data bank ID:4WYC) [1]. Analysis of crystal structure of 4WYC revealed with hydrogen bonding interactions with nonpolar interaction like Trp398. The inhibitor is well associated with hydrophobic amino acids Met61, Trp398, Trp64, Tyr407, and Phe402 [8]. To validate the active site pocket the reference ligand was re-docked, and the docking score was found to be −6.032 kcal/mol. Re-docking results showed that the compound exhibited similar interactions as that of crystal structure and showed a RMSD of 1.02 Å. Further, the compounds synthesized were screened based on three different docking strategies [9]. The ligand interaction with protein was depicted in Fig. 1. The docking score and the ligand interactions for the compounds were tabulated in Table 1.

Docking studies and structure activity relationship for the compound 2'-chloro-4-methoxy-3-nitro benzil

The compound 2'-chloro-4-methoxy-3-nitro benzil was found to inhibit the pathogenic bacteria's *S. aureus*, *K. pneumonia,* and *E. coli* at a distance of 10 mm using disc diffusion method when compared to other compounds. The compound after three different docking strategies reveals that the score was found to be −4.558 kcal mol^{-1}. On the closer analysis of this molecule, the molecule showed similar stacking interaction like the reference molecule; the compound has also found to be surrounded by nonpolar amino acids which make this molecule potent toward antibacterial drug discovery. The binding analysis and ligand interaction diagram for the compound 2'-chloro-4-methoxy-3-nitrobenzil were depicted in Fig. 2.

Docking studies and structure activity relationship for the compound 4,4'-dibromo benzil

Based on our docking studies, it has confirmed that the structure changes in the compounds series were found to be well correlated with *in vitro* antibacterial results. The compound 4,4'-dibromo benzil possessed high docking score of −5.225 kcal/mol with the stacking interaction with amino acid Phe402. The activity of this compound also found to be well correlated with the reference ligand. The close analysis of this compound revealed that the compound is well packed with nonpolar interactions which make this compound more active against the pathogenic bacteria. The binding analysis and ligand interaction diagram for the most active compound 4, 4'-dibromo benzil are shown in Fig. 3. The docking score and its ligand interaction with the synthesized compounds are tabulated in Table 1.

Docking studies and structure activity relationship for the compound 2,2'-dichloro benzil

The compound 2,2'-dichloro benzil was found to inhibit the bacteria at a distance of 8 mm. This is quite lesser than the other molecules. This makes this molecule more effective binding, and the docking score was found to be −4.523 kcal mol^{-1}. The binding analysis of this compound reveals that the compound well fitted into the active site pocket and

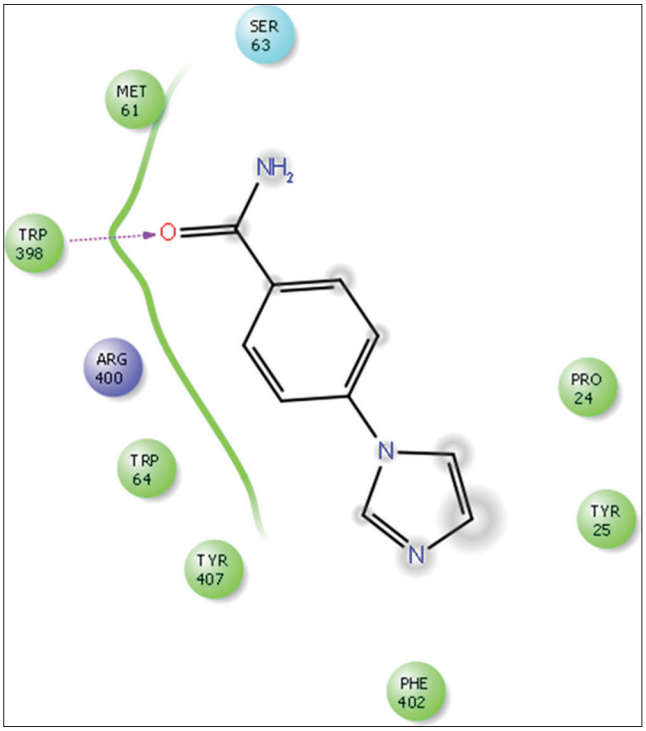

Fig. 1: Reference ligand interaction with protein

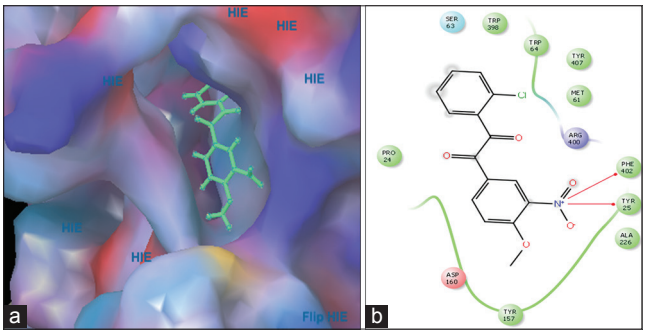

Fig. 2: (a and b) Binding analysis and ligand interaction diagram for the most active compound 2'-chloro-4-methoxy-3-nitro benzil

Table 1: Docking score and ligand interaction results for the synthesized compounds

S. No.	Compound name	Docking score kcal/mol	Ligand interaction
1.	2'-chloro-4-methoxy-3- nitro benzil	−4.558	Phe402, Tyr25
2.	4'4-dibromo benzil	−5.225	Phe402
3.	2,2'-dichlorobenzil	−4.523	Trp64, Arg400
4.	Benzil	−3.140	Trp64, Arg400

Table 2: ADME prediction for the synthesized compounds

Compound name	QPlog Po/w[a]	QPlog HERG[b]	QPP Caco[c]	QPlog BB[d]	Percent human oral absorption[e]
2'-chloro-4-methoxy-3-nitro benzil	3.112	−5.016	1545.995	−0.513	100
2,2'-dichlorobenzil	4.143	−2.686	314.014	−0.359	98.291
4,4'-dibromo benzil	4.153	−3.285	202.689	−0.363	96.543
Benzil	2.98	−3.193	202.314	−0.652	82.365

[a]Predicted octanol/water partition coefficient logP (acceptable range: −2.0-6.5). [b]Predicted IC_{50} value for blockage of HERG K+channels (below −5). [c]Predicted apparent Caco-2 cell permeability in nm/s (<25 poor; >500 great). [d]Predicted brain/blood partition coefficient (−3.0-1.2). [e]Percent human oral absorption (<25% is poor and>80% is high). [f]Rule of 5 violation (mol_MW<500, QPlogPo/w<5, donorHB≤5, accptHB≤10). ADME: Absorption, distribution, metabolism, and excretion

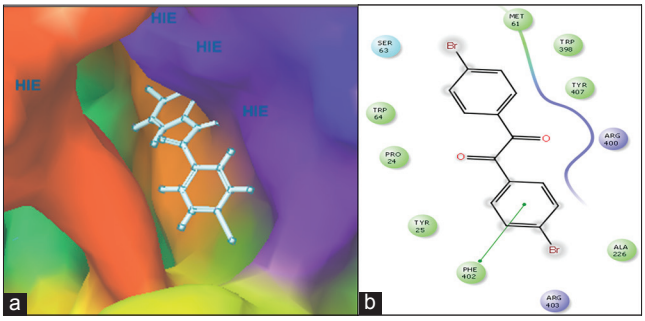

Fig. 3: (and b) Binding analysis and ligand interaction diagram for the most active compound 4,4'-dibromo benzil

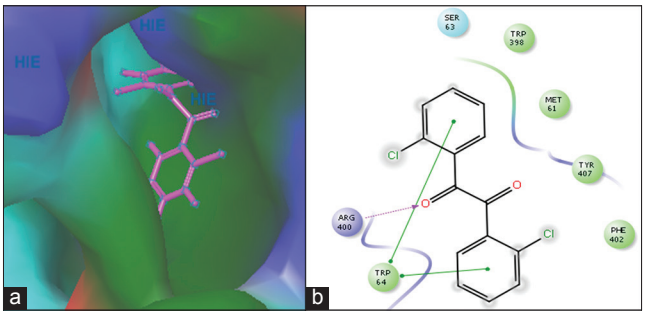

Fig. 4: (a and b) Binding analysis and ligand interaction diagram for the most active compound 2,2'-dichloro benzyl

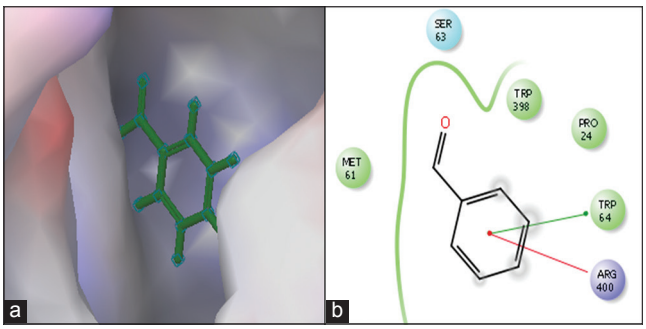

Fig. 5: (a and b) Binding analysis and ligand interaction diagram for the most active compound benzil

the group phenyl chloride was found to interact with nonpolar amino acids Trp64 and Arg400 which reveals that there are two stacking interactions making this compound more stable for further processing as better drug compound [10]. The binding analysis and ligand interaction for the compound 2,2'-dichloro benzil was depicted in Fig. 4.

Docking studies and structure activity relationship for the compound benzil

The compound benzil was found to inhibit the bacteria *K. pneumoniae* at a distance of 12 mm. This compound after *in silico* screening analysis was found to possess good docking score −3.140 kcal mol^{-1}. On the closer analysis of this compound reveals that the molecule has well fitted into the active site pocket of the protein; also their ligand interaction shows that the molecule was surrounded by nonpolar amino acid and it is found to be interact with an amino acid Trp64 and Arg400 which is an important interaction of original ligand [11,12]. The binding analysis and ligand interaction diagram for the compound benzil were depicted in Fig. 5.

ADME prediction

To further account for the potential of the compounds to act as efficient drug candidates, their ADME properties were also calculated in silico using Qikprop. The obtained values for molecular logP, HERG property, Caco accessibility, BBB, and human oral absorption; it is also used to assess violation of Lipinski's rule of five if any. All the compounds were shown to correlate well with the human oral absorption. BBB separates the human brain from the direct contact of the circulatory system, thus protecting the brain for unwanted solute particles. Both the predicted compounds were shown to be BBB negative ensuring their administration safe for the brain. The ADME predictions for the synthesized compounds were tabulated in Table 2.

CONCLUSION

The structured based drug design strategy had been carried out by medicinal chemistry tools. The new scaffold molecules have been identified using docking studies. This strategy revealed hitherto unknown binding pockets and inhibitor binding modes distinct from the earlier reported inhibitors and will be exploited successfully in further antimycobacterial drug development process. The compound 2'-chloro-4-methoxy-3-nitro benzil was found to be most active in both *in silico* and *in vitro* antibacterial analysis. Further, these compounds will be carried out for their antimycobacterial property as these are small molecule leads could easily cross the cell barrier systems in mycobacteria.

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