Molecular docking studies of N-(benzo[d]thiazol-2-ylcarbamothioyl)-2/4-substituted benzamides as an antibacterial inhibitor for E. coli dihydroorotase

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Abstract. In pharmacological studies, it is common that drug molecules fail in the final stages of testing. Human, as well as animal trials, have serious regulatory limitations. An alternate option to test energetically suitable binding conformations of synthesized ligands in a dynamic site cavity of a target receptor is to accomplish a molecular docking study. After carrying out the synthesis, characterization, and biological evaluation of molecular properties experimentally, to strengthen and investigate the findings further, we have carried out a molecular docking study to confirm binding of ligand N-(benzo[d]thiazol-2-ylcarbamothioyl)-2/4-substituted benzamides against the protein E. coli dihydroorotase (PDB ID 2eg7). Among the synthesized compounds 3a, 3b, 3c, and 3e are found to have promising antibacterial activity. Moreover, the compound 3a is identified as a potential lead molecule with the lowest binding affinity value.

Keywords. Benzothiazole, Benzamide, Benzoylisothiocyanate, Molecular docking, CADD

1. Introduction

The drug discovery process is finding a new chemical entity that can be useful in the treatment and cure of diseases. The process itself is multifaceted which involves identifying candidates, synthesis, characterization, screening, and efficiency analysis. So, it requires multidimensional efforts to design a successful drug [1].

Owing to the high costs of laboratory research and human clinical studies, drug research and production is a costly process. But within the past few decades, the strategies of drug design have altered significantly. It has been enhanced and improved because of the advancement of computing software and procedures. Computational research is considered as one of the most recent and rapid evolving drug development practices. The use of computers in the field of pharmaceutical research is known as computer-aided drug design (CADD). It helps to reduce the time required for tedious processes that include details about molecular properties, bioactivity, and drug likeness of the synthesized product. It has emerged as an efficient tool for pharmaceutical companies and academic researchers to shorten the expense and period necessary for the recognition and optimization of proficient principal compounds with minimum side effects in drug discovery [2], [3]. If we need to find how to control diseases or infections at the molecular and physiological level on the target entities Computer Aided Drug Design software can help extensively. One of the important pillars of computer-assisted drug design is Molecular docking. It is a structure-based drug design method that pretends the molecular interaction and helps to find the binding modes and affinity between protein and ligands. By studying the molecular
interactions modeling between ligands and target macromolecules it is easy to identify and optimize the lead drug candidate [4], [5], [6], [7], [8]. The invention of antibiotics brought an incredible revolution in the field of medicinal chemistry. But in recent past years due to its improper use, the problem of drug resistance is very common. Therefore, it is worthwhile to invent new antibiotics with simple composition and high reactivity. In medicinal chemistry, the importance of benzothiazole containing heterocycles is well known and established. The presence of benzothiazole ring imparts pharmaceutical value to many active drugs because of its potent and significant myriad spectrum of biological activities like antimicrobial [9], anticancer [10], antidiabetic [11], anti-Inflammatory [12], antimalarial [13], antitumor [14], antiviral [15], anthelmintic [16], anticonvulsant [17], etc. Benzothiazole is an important pharmacophore in the field of pharmaceutical chemistry so, many benzothiazole derivatives are used in different marketed drugs [18], [19], [20], [21], [22] (Figure 1).

![Figure 1](image)

**Figure 1** Structures of some marketed drugs containing active benzothiazole moiety

Our research group has already reported synthesis, characterization and in vitro antibacterial activity of N-substituted benzamides containing benzothiazole moiety [23], [24]. The results of the antibacterial activity study, obtained molecular properties results from molinspiration, and Osiris property explorer motivated us to carry out molecular docking study with suitable protein to further investigate the effects of the synthesized molecule on the target protein. The present work incorporates the molecular docking of N-(benzo[d]thiazol-2-ylcarbamothioyl)-2/4-substituted benzamides 3(a-g) against protein E. coli dihydroorotase (PDB ID 2eg7).

2. Experimental

2.1. General procedure for the synthesis
Scheme I: Synthesis of N-(benzo[d]thiazol-2-ylcarbamothioyl)-2/4-substituted benzamides 3(a-g)

2.2. Antibacterial activity
In the current work, in vitro antibacterial activity of the compounds synthesized was studied thoroughly opposite to the gram-positive and gram-negative bacteria. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Proteus aeruginosa* bacteria were used for the study. The agar well diffusion method was used for antibacterial screening study [25], [26]. The N-substituted benzamides and standard drug were dissolved in Dimethylformamide (DMF) to make the stock solution. We have created a stock solution of the standard drug similarly for comparison. Then agar plate was immunized with the microbial inoculums. The 0.05 mL of the sample along with the required concentration of a standard solution was introduced in the well of diameter 6 millimeters. Dispersal of the anti-bacterial agent gave a concentration gradient and also displayed the inhibition of the microbial strain growth. After incubating the plates at 37°C for 24 hours’ the dimensions of the inhibition zones were measured in mm. The Antibacterial activities were repetitive for at least three times and means of three determinations are enlisted in Table 1.

Table 1. Antibacterial activity of compounds 3(a-g)

| Test Compounds | (Zone of inhibition in mm at 100 µg/mL) |
|----------------|----------------------------------------|
|                | *S. aureus*  | *B. subtilis* | *E. coli*  | *P. aeruginosa* |
| 3a             | + +          | +            | ++         | + +            |
| 3b             | + +          | ++           | ++         | --             |
| 3c             | ++           | ++           | +++        | ++ +           |
| 3d             | +            | --           | ++         | +              |
| 3e             | + +          | ++           | +++        | +              |
| 3f             | --           | +            | +          | +              |
| 3g             | --           | --           | +          | +              |
| **Amp**        | + + +        | +            | + + +      | + + +          |

Key to symbols: Amp - Ampicillin
Inhibition zone antibacterial activity: Highly active = + + + (inhibition zone >15 mm); moderately active = + + (inhibition zone 10-15 mm); slightly active= + (inhibition zone 5-10 mm); Inactive = -- (inhibition zone <5 mm).

2.2. Molecular docking studies

2.2.1. Protein preparation
For present docking study, after a sufficient literature survey, E. coli dihydroorotase (PDB ID 2eg7) was chosen as the target protein [27], [28]. The structure (three-dimensional) of protein complexed with 2-oxo-1,2,3,6-tetrahydropyrimidine-4,6-dicarboxylic acid (HDDP) was downloaded from the protein data bank having a resolution of 2.0 A° [29] (https://www.rcsb.org/structure/2EG7). The Accelrys Discovery Studio 3.5 software is used for refinement of the protein crystal structure. The software is used for the removal of water molecules and a complexed ligand that is bound to it. Auto dock tools (ADT) 1.5.6 was used to add polar water and Gasteiger charges to the protein structure before actual docking exercise. For the protein binding sphere (26.85, 40.56,73.05) was selected by using the binding site tools. The protein .pdb file is then saved to .pdbqt format which is ready for docking study.

2.2.2. Ligand Preparation
ChemDraw Ultra 19.0, was used to draw the 2D molecular structure of synthesized ligands (3a-g) and was converted to the 3D (.pdb) format for energy minimization. The energy-minimized molecules were imported for further molecular docking in Auto Dock 4.2. The ligand’s .pdb file is also saved to .pdbqt format which is also ready for docking.

The target grid box was set to cover most of the protein structure as the exact centre and binding sphere radius was unknown. It was earlier decided to find suitable binding sphere parameters through literature but later dropped the idea to avoid settling to the local minima. The main idea was to allow the Auto Dock Vina to find the best active site cavity for the energetically promising binding where the ligand (3a-3g) binding poses onto the receptor (2eg7). The default algorithm of Vina was used for docking. For molecular docking study, the protein is kept as a rigid while the ligands are flexible. Automatically random number of docking runs were carried out. Various commands were run in the DOS and Windows based platform to get the docking results using AutoDock Vina. All the parameters were recorded and minimum binding energy values were considered for final selection of the best compound. To minimize the local minima problems various runs of the docking were performed and then the best lead compound was selected.

| Ligand | Binding affinity (kcal/mol) | Ligand efficiency | rmsd | No. of hydrogen bonds | Bonding residues (Bond length) |
|--------|----------------------------|------------------|------|----------------------|--------------------------------|
| 3a     | -7.4                       | -0.35            | 0.00 | 3                    | ARG A:20, ASN A:44, PRO A:105, HIS A:139, ASP A:250, ALA A:252, HIS A:254, ALA A:266, TYR A:79, TYR A:104, PRO A:105, HIS A:139, GLU A:141, LEU A:222, ALA A:266, ALA A:106, SER A:118, VLA A:119, VLA A:142, PHE A:156, SER A:159, VLA A:119 |
| 3b     | -7.2                       | -0.33            | 0.00 | 1                    | |
| 3c     | -7.1                       | -0.32            | 0.00 | 1                    | |
| 3d     | -7.0                       | -0.30            | 0.00 | 1                    | |
3. Results and Discussion

3.1. General

The synthesis (Scheme I), characterization (see supporting information), and biological evaluation of ligands \( \text{N-[(benzo[d]thiazol-2-ylcarbamothioyl)-2/4-substituted benzamides 3(a-g)} \) used in the present study were already reported by our research group. Characterization of synthesized compounds was done using spectroscopic and analytical techniques confirms the given structure of compounds. The synthesized compounds were virtually evaluated using bioinformatics tools like molinspiration calculator and Osiris property explorer. Then after virtually screened compounds were evaluated experimentally for their antibacterial activity against gram-positive (\( \text{Staphylococcus aureus and Bacillus subtilis} \)) and gram-negative (\( \text{Escherichia coli and Proteus aeruginosa} \)) bacteria. The obtained results show moderate to potent antibacterial activity compared to the standard drug. The structure and activity correlation of the compounds using various physicochemical properties concerning Lipinski’s rule of five, drug-likeness, and oral bioavailability is thoroughly discussed using relevant tools. The results are consistent with RO5 results and confirm the drug-likeness and oral bioavailability of the compounds. The bioactivity score shown by all compounds in these tests establishes its drug-like activity similar to the standard drug. The synthesized compounds were evaluated virtually (using bioinformatics tools) and practically (well diffusion method). The synthesized compounds were studied theoretically for bioactivity predictions. The structure and activity correlation of the compounds using various physicochemical properties for Lipinski’s rule of five, drug-likeness, and oral bioavailability is thoroughly discussed using relevant tools. The obtained results are consistent with RO5 results and confirm the drug-likeness and oral bioavailability of the compounds. The bioactivity score shown by all compounds in these tests establishes its drug-like activity similar to the standard drug. Data obtained from Osiris calculations are consistent with the results obtained using molinspiration. After antibacterial screening, when compared with the standard drug it was noticed that compounds \( 3a, 3b, 3c, 3d, 3e \) are moderate to high active except \( 3f \) and \( 3g \) [24]. The promising results obtained from our previous study inspired us to execute a molecular docking study. It helped us to understand the ligand-protein interactions and to find out the lead compound.

3.2. Molecular docking

To perform a docking study, \( \text{E. coli dihydroorotase (PDB ID 2eg7)} \) was complexed with \( 2\text{-oxo-1,2,3,6-tetrahydroprymidine-4,6-dicarboxylate (HDDP)} \) (https://www.rcsb.org/structure/2EG7) was selected as the target protein. A zinc metalloenzyme (Dihydroorotase), that functions along the line with the biosynthesis of pyrimidine nucleotides which also catalyzes the rescindable inter-conversion of dihydroorotate and carbamoyl aspartate [28] [30]. To develop novel antibiotics this enzyme may represent a valuable target. The Autodock Vina [31] is used to dock synthesized products and the standard drug Ampicillin. The obtained results are shown in (Fig. 2) and tabulated in (Table 2) revealed

| Compound | Bioactivity Score | Lipinski's Rule of Five |
|----------|------------------|------------------------|
| 3e       | -7.2             | 4                      |
| 3f       | -7.0             | 2                      |
| 3g       | -6.9             | 3                      |
| Amp      | -7.4             | 3                      |

ASN A:44, TYR A:79, TYR A: 104, PRO A: 105, HIS A: 139, GLU A: 141, ALA A: 252, ALA A: 266, GLY A: 267

PHE A: 205, ARG A: 216, PRO A: 217, HIS A: 218, LEU A: 219, LEU A: 336, ALA A: 337, GLU A: 339

LEU A:219, VAL A: 333, PRO A: 334, LEU A:336, ALA A: 337, GLU A: 339

HIA A: 18, TYR A: 79, TYR A: 104, PRO A: 105, ASN A: 107, THR A: 110, HIS A:139, ALA A: 252
that all the synthesized molecules 3(a-g) showed good binding affinity (-7.4 to -6.9) and ligand efficiency (-0.35 to -0.30) values. The obtained data depicted that all the docked molecules are showing interaction with more than one amino acid in the receptor active pockets.

The compounds 3a, 3b, 3c, 3e are showing binding affinity values -7.4, -7.2, -7.1, -7.2 kcal/mol respectively. The binding energy values revealed that most of the compounds had a good binding affinity toward the dihydroorotase receptor. So they may be projected as a good inhibitor for dihydroorotase. Among all, 3a is having the lowest value of binding affinity (-7.4 kcal/mol). It is having 3 hydrogen bonds with ARG A:20, ASN A:44, ALA A:266 with bond length 2.03Å, 2.72Å, 3.58 Å respectively and interact with other residues PRO A: 105, HIS A: 139, ASP A: 250, ALA A:252, HIS A: 254 in the binding pocket. It was noticed that the mainly oxygen and the sulphur atoms present in the ligands were responsible for hydrogen bond interactions. These remarkable docking reports are in strong support with the in-vitro antibacterial screening results. So, finally we may come to an end that compound 3a can be a good inhibitor against E. coli dihydroorotase. The molecular docking findings provide substantial understanding about ligand-protein interactions to develop new lead antibacterial agent to target dihydroorotase of E. coli. All modes of molecular docking results of 3a are presented in Figure 3.
Figure 2. Interaction of ligands with protein, E. coli dihydroorotase (PDB ID 2eg7)
4. Conclusion
To validate the results obtained from the in-vitro antibacterial study of synthesized N-(benzo[d]thiazol-2-ylcarbamothioyl)-2/4-substituted benzamides molecular docking simulation has been performed. All the synthesized ligands showed favorable interactions with amino acid residues of dihydroorotase. The results provided in the present work evidence that N-substituted benzamides containing benzothiazole moiety may be helpful to inhibit the action of the tested enzyme. Among all tested compounds 3a with the lowest binding energy (−7.4) could be a novel efficient inhibitor against the protein E. coli dihydroorotase (PDB ID 2eg7).

Supplementary Data
Table of Contents
1. General procedure for the synthesis 3(a-g)
2. Copies of the FTIR, $^1$H, $^{13}$C NMR and mass spectra data for compound 3a

General procedure for the synthesis
**Aminobenzothiazole 1**

A saturated solution of ammonium thiocyanate (0.12 mole, 30g) in water (60 ml) was added slowly to the warm mixture of aniline (0.25 mole, 24 g) and conc. HCl (0.25 mole, 25 ml) with shaking. The solid obtained (phenyl thiourea) was filtered, washed with water, dried, and crystallized from distilled water to get a pure compound. To the phenyl thiourea (0.1 mole, 15.22g) sufficient amount of chloroform (20-25 ml) was added to get slurry and brominated using 5% bromine solution till to get orange-red color. The obtained solid hydrobromide was filtered and washed several times with chloroform till the disappearance of the orange-red color. It was dissolved in alcohol and basified with 10% NH₄OH. The solid 2-aminobenzothiazole was filtered, washed with water, dried, and recrystallized from ethanol (m.p 127-128°C) [32].

**2/4-substituted benzoyl isothiocyanates 2(a-g)**

To a solution of ammonium thiocyanate (0.1 mole, 7.6g) substituted benzoyl chloride (0.1 mole) was added dropwise in dry benzene (25ml) with continuous stirring. It was refluxed for two hours. The obtained mixture was cooled and filtered. The filtrate is separated as benzoyl isothiocyanates 2(a-g) [33].

**N-(benzo[d]thiazol-2-ylcarbamothioyl)-2/4-substituted benzamides 3(a-g)**

2-Aminobenzothiazole 1 (0.01mole, 1.5g) and substituted benzoyl isothiocyanates (0.01mole) 2(a-g) were refluxed in a mixture of dry benzene (25ml) and 2-propanol (5ml) for 3 hours. The solid N-benzothiazolyl benzamides 3(a-g) obtained was filtered, washed with dil HCl, dried, and recrystallized from benzene.

![Figure S1. FTIR spectrum of N-\{(1,3-benzo[d]thiazol-2-yl)carbamothioyl\}benzamide (3a)](image-url)
Figure S2. $^1$H NMR spectrum of N-{$(1,3$-benzo[d]thiazol-2-yl)carbamothioyl}benzamide (3a)

Figure S3. $^{13}$C NMR spectrum of N-{$(1,3$-benzo[d]thiazol-2-yl)carbamothioyl}benzamide (3a)
Figure S4. Mass spectrum of N-[(1,3-benzo[d]thiazol-2-yl)carbamothioyl]benzamide (3a)

5. References

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Conflict of interest

The authors declare that there are no conflicts of interest.