Design, Molecular Docking and In-Silico Analysis of Novel thiazole-azetidinone hybrids as Potential Antitubercular Agents

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ABSTRACT

The recent emergence of extensively drug-resistant tuberculosis has become a cause of concern for the management of tuberculosis globally. Shikimic acid pathway seems to be a potential and favorable target for the drug design of new anti-infective agents. This work aims to change the focus from traditional cell approaches to the target-based design of novel thiazolyl-azetidinone derivatives with Shikimate kinase as the drug target for antitubercular activity. Thiazazole and azetidinone derivatives were methodically reprised to design a series of 3-chloro-4-(aryl)-1-(5-sulfanyl-1,3,4-thiadiazol-2-yl)azetidin-2-one derivatives (AZ1-AZ12). Molecular docking studies were performed on a crystal model of Mycobacterium tuberculosis Shikimate kinase (MtSK) using Vlife MDS 4.4 suite to evaluate their antitubercular potential. Further, drug-likeness properties and ADMET prediction were performed by molinspiration and admetSAR software to better describe the designed molecules as prospective candidates. 3-chloro-4-(4-nitrophenyl)-1-(5-sulfanyl-1,3,4-thiadiazol-2-yl)azetidin-2-one (AZ3) was found to have better dock score when compared with the natural substrate, Shikimate. Docking studies confirmed that the molecules showed significant binding in the active site region of Shikimate kinase. Strong hydrogen bonding and hydrophobic interactions with amino acid residues and other parameters further explicate their effectiveness for inhibition of MtSK. Also, the physiochemical properties and drug scores for the designed compounds obtained by in silico studies were found to be satisfactory, signifying the overall potential of the designed molecules to be drug candidates. Thus these molecules could be explored as a lead for further anti-tubercular studies with Mycobacterium tuberculosis Shikimate kinase as the drug target.

INTRODUCTION

Tuberculosis (TB) is a communicable disease caused by the bacillus Mycobacterium tuberculosis (MTB). Recently, the rising numbers of drug-resistant TB have significantly threatened to jeopardize global...
efforts to control TB, especially in HIV endemic regions. Despite recent advances, TB continues to be a global epidemic and calls for an urgent global response. With 1.2 million deaths in 2018, TB is among the top ten reasons of fatality. Globally, an estimated 10 million people developed TB disease in 2018. Drug-resistant TB continues to be a public health threat. India, China, and the Russian Federation account for almost half of the world’s global TB burden. (World Health Organization, 2019). With this scenario, it is imperative to broaden effective new anti-tubercular agents to combat TB, which includes MDR-TB and XDR-TB, and to curtail the six-month regimen of currently recommended treatment for better acquiescence by patients. (Lienhardt et al., 2012; Tiberi et al., 2018). Cutting-edge anti-tubercular drugs more often target cell approaches concerned in bacterial progression. Their mechanism of action involves mostly inhibition of cell wall synthesis, nucleic acid synthesis, protein synthesis, and ATP synthesis. However, newer drugs with novel targets are being exploited to cope up with the problems of multidrug tolerance and dormant TB populations.

The shikimate pathway involves biosynthesis of chorismate from phosphoenolpyruvate (from glycolysis) and erythrose-4-phosphate (from HMP shunt) in a series of seven steps. This chorismate further acts as a precursor in the biosynthesis of aromatic amino acids and folic acid essential for the survival of microorganisms. (Herrmann and , 1999) It is a potential target for the design of new antimicrobial agents as it is exclusively present in microorganisms whilst being absent from mammals. Hence it allows for the recognition of targets that can possibly reduce the toxicity of drug candidates. It is remarkable to note that Shikimic pathway is critical for the survival of MTB despite the supply of exogenous supplements, which highlights its importance as a drug target. Consequently, inhibition of vital enzymes involved in this pathway seems to be an attractive target for the development of new anti-infective agents. (Davies et al., 1994; Bentley and Haslam, 1990) A detailed structural study of these enzymes further aids to propose and design inhibitors of enzymes involved in the pathway (Arcuri et al., 2010).

Shikimate kinase (SK), involved in the fifth step of the pathway, is responsible for catalyzing the ATP dependent phosphorylation of shikimic acid to form shikimate-3-phosphate. In M. tuberculosis, the aroK gene codes for M. tuberculosis Shikimate kinase (MtSK) which catalyzes the SK reaction and is essential for the subsistence of M. tuberculosis. Shikimate kinase presents an alpha/beta-fold and includes five significant parallel beta-sheets, flanked by means of alpha-helices. SK is a member of the nucleoside monophosphate kinase (NMP) family, which comprise of three domains- NMP-binding, CORE, and LID domains. The shikimate binding region is similar to the NMP-binding area of NMP kinases. (Li et al., 2007) This comprises of α-2 and α-3 helices and N-terminal region of an α-4 helix and follows β-2 strand. The lipophilic region is lined with Pro11 and highly conserved residues Phe49, Phe57, Gly79, Gly80, and Leu119. Ring stacking interaction is liable due to phenyl rings of Phe49 and Phe57. (Dhaliwal et al., 2004) The shikimate binding site is described by a hydrophobic surface along with hydrophilic charged residues which infiltrate the cavity. The shikimate binding in the cavity presents essential residues that build possible interactions of ligand to its protein, as depicted in Figure 1.

![Figure 1: Key interactions of Shikimate with favourably conserved residues inside the binding pocket of Shikimate kinase.](image)

The crystal structure of M. tuberculosis Shikimate kinase (MtSK) structure complexed with shikimate presents crucial data for the design of specific SK inhibitors, which can either target the shikimate-binding site only or the shikimate- and ATP-binding sites both. (Gordon et al., 2015) aroK and aroL genes which encode for all SKs include Asp34, Arg58, Glu61, Gly79, Gly80, Gly81, and Arg136 as residues involved directly or indirectly in the binding of shikimate. (Pereira et al., 2004) The site of these residues is well preserved in proteins encoded by aroK gene. In MTB, the SK reaction is catalysed by aroK gene. The deletion of aroK gene, which codes for the shikimate kinase and results in cell death of Mtb, has been shown to be crucial for the viability of Mtb, thus making it an attractive target for drug design of new
anti-tubercular molecules. (Li et al., 2007).

In drug discovery, molecular docking is a widely used structure-based drug design approach for analysis of molecular interactions, conformational changes, and binding energies. Virtual screening approaches are used for the selection of drugs which not only have good activity but also balanced with complimentary pharmacokinetics including absorption, distribution, metabolism, and excretion, and low toxicity profile to avoid failures in the late stages of drug development. (Vyas et al., 2013) In this work, we have investigated important structural features required for inhibition of MtSK by in silico analysis for de novo design of selective inhibitors.

Previous docking studies have reported that a triazole or tetrazole heteroaromatic ring with a mercapto group is fundamental for inhibition of MtSK (Segura-Cabrera and Rodríguez-Pérez, 2008). The sulphur group being electron rich alters the electron density while improving lipophilicity and contributes to hydrogen binding interactions with the receptor. It was reported that the best scoring compounds, including ASXE1, contained mercapto group and triazole ring in the scaffold. (Gordon et al., 2015) Further isosterism can be explored as a tool to alter the pharmacological profile of a compound. Bioisosterism can be employed to enhance lipophilicity and ameliorate the pharmacological properties of the compound. It was systematically planned to first replace the triazole ring of ASXE1 by its bioisostere i.e., thiaodiazole ring system, to impart lipophilicity for better cell permeation, good oral absorption leading to improved bioavailability. (Serban et al., 2018) Also, the heterocycle, 2-azetidinone, a four-membered ring, is widely used in medicinal chemistry. Its derivatives show a diverse range of biological activities for example, antitubercular (Thaker et al., 2003), anti-inflammatory (Kumar and Rajput, 2009), anti-tumor (Veinberg et al., 2004), anti-HIV (Sperka et al., 2005), antiparkisonian (Kumar et al., 2012) and antidiabetic (Wang et al., 2008). Kagthara et al. (2000) have reported antmycobacterial activity of some 2-azetidinone derivatives and found that compounds with ortho-chlorophenyl and 2,4-dichlorophenyl substituents (Compound 1) showed the highest activity against M.Tuberculosis. Compound 1 showed 97 % MTB inhibition at concentration of 0.031 μg/ml. (Kagthara et al., 2000). In this light, molecules were designed by molecular hybridization of 2-azetidinones and 1,3,4-thiadiazoles as bioisosteres of 1,2,4-triazoles, as depicted in Figure 2.

**MATERIALS AND METHODS**

**Docking Studies**

**Protein Preparation**

Docking was performed by means of the BioPredicta module of VLifeMDS 4.4 suite from VLife Technologies Pvt. Ltd., Pune, India. The computational work was carried out on Dell PC with Pentium IV processor and Windows 7 operating system. The X-ray crystal structure of MtSk co-crystallized with shikimate (PDB ID: 2IYQ) and resolution of 1.8 A° was imported from the Protein Data Bank (Available from http://www.rcsb.org/). The ligand enclosed in the protein structure was extracted from the binding site and saved in a new file. Further tasks were performed, such as inserting missing atoms and incomplete residues, removal of water and ADP, and addition of hydrogens and amino acids. Docking simulation was then performed on an optimized receptor saved as .mol file.

**Co-Crystallized Ligand Preparation**

Hybridization states and bond orders were assigned for the extracted Shikimate and saved as .mol file.

**Ligand Preparation**

The designed molecules, as depicted in Table 1, were drawn as 2D structures using CHEMDRAW ULTRA 12+ SERIES and then transformed into 3D structures using VLifeMDS 4.4 software. The 3D structures were then subjected to energy minimization, and other parameters maintained as RMS gradient 0.01 kcal/mol A° with Merck Molecular Force Field (MMFF) and saved as .mol file. Consequently, conformers were generated by systematically selecting suitable rotatable bonds. Only optimized least energy ring conformation per ligand were selected.
Table 1: Dock Score of designed molecules

| Compound            | R       | Dock Score |
|---------------------|---------|------------|
| AZ1_1_3D_opt_P3     | 4-OH    | -50.2244   |
| AZ2_5_3D_opt_P5     | 4-CH3   | -52.1494   |
| AZ3_6_3D_opt_P23    | 4-NO2   | -62.4095   |
| AZ4_7_3D_opt_P7     | 4-Cl    | -51.3739   |
| AZ5_8_3D_opt_P24    | 4-Br    | -51.0667   |
| AZ6_9_3D_opt_P21    | 4-NH2   | -47.0369   |
| AZ7_10_3D_opt_P5    | 4-CH3   | -53.1522   |
| AZ8_11_3D_opt_P15   | 2-Cl    | -52.4574   |
| AZ9_12_3D_opt_P1    | 2-F     | -49.2094   |
| AZ10_2_3D_opt_P5    | 2-NO2   | -55.5049   |
| AZ11_3_3D_opt_P10   | 4-F     | -48.5432   |
| AZ12_4_3D_opt_P19   | 3,4-diOCH3 | -54.6588 |
| Shikimate_opt_P6    | -       | -54.2207   |

Figure 4: 4 (a) and 4(b) : Hydrogen bonding interactions with GLY80, ARG58 in the docked molecule of Shikimate (4a) and with LYS15, ARG58, GLY80 in the docked molecule of AZ3 (4b) in the active site of MtSK (PDB Code: 2IYQ) respectively (Shown in green dotted lines)
Figure 5: 5(a) and 5(b): Hydrophobic bonding interactions with LEU119, PRO11, GLY80, GLY81, ARG117 in the docked molecule of Shikimate (5a) and PRO11, LYS15, ARG117 in the docked molecule of AZ3 (5b) in the active site of MtSK (PDB Code: 2IYQ) respectively. (Shown in cyan blue dotted lines)

Figure 6: 6(a) and 6(b): Vander Waal's interactions with GLY81, LYS15, ARG58, ARG117, ARG136, PRO11, GLY80, LEU119 in the docked molecule of Shikimate (6a) and PRO11, LYS15, ASP34, ARG58, GLY80, GLY81, ARG117, LEU119, ARG136 in the docked molecule of AZ3 (6b) in the active site of MtSK (PDB Code: 2IYQ) respectively. (Shown in pink dotted lines)
for further study.

**GRIP Batch Docking Method**

The docking study was performed on a crystal structure of Shikimate kinase co-crystallized with shikimate and ADP (PDB ID: 2IYQ) using the GRIP batch docking methodology. (Tripathi, 2017).

All the optimized conformers were virtually docked in the defined cavity of the receptor using the exhaustive method and Dock Score as the Scoring function. The number of placements and rotation angle were fixed to a value of 30. The best placement, which also gives the lowest energy pose, is highlighted. The best poses were further analyzed for their interaction with the amino acid residues in the active site of the receptor. These interactions involve hydrogen bonding, van der Waal’s interaction, aromatic/pi stacking, and other charge interactions. The more negative the energy is given by Dock score, the better is the affinity of the ligand for the target protein.

**Validation of the docking protocol**

For this, the optimized co-crystallized ligand, which was previously extracted from the protein, was redocked into the binding site. The lowest energy pose predicted by Dock Score was compared to the experimental X-ray crystallographic conformation, and binding interactions were analyzed.

**In-silico ADMET properties and drug-likeness prediction**

ADMET implies Absorption, Distribution, Metabolism, Excretion, and Toxicity. admetSAR, a comprehensive source and tool was used to predict ADMET properties of the designed substituted 1,3,4-thiadiazolyl-azetidin-2-one derivatives (www.lmmd.ecust.edu.cn, accessed on 21st April 2018). (Cheng et al., 2012) The structures were drawn using ACD labs Chemsketch v 12.0 and SMILES notation data was generated. Molecular descriptors were analyzed using Molinspiration software v2011.06 (www.molinspiration.com), which is based on Lipinski’s rule of five to assess the drug-likeness properties of the designed molecules and predict bioactivity scores for different targets. (Lipinski et al., 1997). The ADMET properties and the bioactivity scores of the designed molecules were compared to the standard drug Isoniazid. (Veber et al., 2002; Li et al., 2007).

**RESULTS AND DISCUSSION**

**Designing Novel Substituted Thiadiazolyl-Azetidinone Derivatives**

We attempted to utilize the conclusions reported from previous docking simulations of shikimate kinase inhibitors and antimycobacterial activity of azetidinone derivatives for the design of novel substituted 1,3,4-thiadiazolyl-azetidin-2-one derivatives. In doing so, the main strategies in the design of these new compounds were molecular hybridization, bioisosterism, and molecular simplification. The azetidinone ring from Compound 1 was maintained in the new compounds, as also was the substituted phenyl ring. The 1,2,4-triazole ring from ASXE1 was replaced with 1,3,4-thiadiazole bioisostere. The mercapto group was retained. This approach permitted the design of new compounds by molecular hybridization of 2-azetidinones and 1,3,4-thiadiazoles as bioisosteres of 1,2,4-triazoles, as depicted in Figure 2. The design principle was aimed at combining the pharmacophores of 2-azetidinone derivatives and 1,3,4-thiadiazole with mercapto moiety to gain synergism and obtain molecules with better anti-tubercular activity, as shown in Figure 3.

**Docking studies**

In an attempt to rationalize the anti-tubercular activity, docking studies were carried out with the designed molecules (AZ1-AZ12) to explore the potential interactions with Shikimate kinase from *Mycobacterium tuberculosis* (PDB Code: 2IYQ). The compounds fit well into the cavity of Shikimate kinase. The compounds AZ3 and AZ10 have shown better Dock scores in comparison to Shikimate, probably due to hydrogen binding and hydrophobic interactions due to more lipophilicity. The dock score of the molecules (AZ1-AZ12) is presented in Table 1, and their interactions with the amino acids in the active site of MtSK are listed in Table 2.

The relevant moieties, azetidinone ring and thiadiazole ring with mercapto group, most likely interact with LYS15, ARG58, GLY80 residues and mimic the key interactions of the enzyme-substrate (MtSK-Shikimate) complex, specifically the interaction of these residues with the carboxylic moiety of shikimate, suggestive of the possible stabilization by CH-electron interactions. Hydrophobic forces were seen with residues PRO11, LYS15 (P-loop), and ARG117 (lid domain). The azetidinone ring interacted with PRO11, LYS15, and ARG117 through hydrophobic forces. The interaction with ARG117 is important for phosphoryl transfer reaction catalyzed by MtSK. Additionally, hydrogen bonding with residues ARG58 (NMP-binding domain), GLY80 (Walker-B motif), and LYS15 (P-loop) were observed similar to those reported for the natural substrate, Shikimate. The oxygen atom of the azetidinone is aligned in the direction of the polar region of the
Table 2: Interactions of designed molecules and Shikimate in the active site of MtSK

| Compound | Hydrogen Bond | Charge Interaction | Hydrophobic Interaction | Van der Waal’s interaction |
|----------|---------------|--------------------|-------------------------|---------------------------|
| AZ1      | GLY80, ARG117 | NIL                | ARG117                  | PRO11, LYS15, ASP34, GLY80, GLY81, ARGL17, LEU119 |
| AZ2      | ARG58         | NIL                | PRO111, LYS15, GLY80, ARG117 | PRO11, LYS15, ASP34, GLY80, GLY81, ARGL17, LEU119 |
| AZ3      | LYS15, ARG58, GLY80 | NIL | PRO111, LYS15, ARG117 | PRO11, LYS15, ASP34, ARG58, GLY80, GLY81, ARG117, LEU119, ARG136 |
| AZ4      | NIL           | NIL                | GLY80, ARG117           | PRO11, SER13, LYS15, ASP34, ARG58, GLY80, GLY81, ARGL17 |
| AZ5      | LYS15, ARG117 | NIL                | PRO111, LYS15, SER16, ARG117 | PRO11, LYS15, SER16, ASP34, GLY80, ARG117, LEU119 |
| AZ6      | GLY80, ARG117 | NIL                | ARG117                  | PRO11, LYS15, ASP34, GLY80, GLY81, ARG117 |
| AZ7      | GLY80         | NIL                | ARG117                  | PRO11, LYS15, ASP34, ARG58, GLY80, GLY81, ARG117, LEU119 |
| AZ8      | ARG136        | NIL                | GLY80, ARG117           | PRO11, LYS15, ASP34, GLY80, GLY81, ARG117 |
| AZ9      | LYS15, GLY80, ARG117 | NIL | ARGL17                | PRO11, LYS15, ASP34, GLY80, GLY81, ARGL17 |
| AZ10     | GLY80, GLY81, ARG117 | PHE49 (Aromatic Interaction) | PRO111, LYS15, GLY80, ARG117 | PRO11, LYS15, ASP34, GLY80, ARGL17, LEU119 |
| AZ11     | ARG58, ARG117 | NIL                | PRO111, LYS15, ASP34, GLY80, ARG117 | PRO11, ASP34, LYS15, ARG58, GLY80, ARG117, LEU119 |
| AZ12     | ARG58, GLY80, ARG81 | NIL | PRO111, LYS15, ARG117 | PRO11, LYS15, ASP34, ARG58, GLY80, ARG117 |
| Shikimate | GLY80, ARG58 | LYS15, ARG117 | LEU119, ARG117    | PRO11, GLY80, ARG117 |

enzyme and interacts with LYS15, ARG58, GLY80 with increased hydrogen bonding in comparison to the reference compound Shikimate. Per se, shikimate kinase is crucial in the shikimate pathway and catalyzes the phosphorylation of shikimate to shikimate-3-phosphate in the presence of ATP for their ultimate conversion to chorismate, which is further used in the synthesis of aromatic amino acids and specialized metabolites. Deficiency of SK has proved to be critical for the survival of M. tuberculosis, thus rendering it an appealing target for the drug design of new molecules. In this light, it can be proposed that molecule AZ3 has an affinity to go and bind with MtSK and may provide understanding in designing novel anti-tubercular agents.

**In-silico ADMET properties and drug-likeness prediction**

Currently, several computational techniques have been developed to identify drug-like molecules ranging from simple counting schemes like Lipinski’s rule of five to structural descriptors such as topological indices or molecular fingerprints and similarity searching. admetSAR was used for the prediction of chemical ADMET properties of substituted 1,3,4-thiadiazolyl-azetidin-2-one derivatives. admetSAR analysis revealed that all molecules except AZ1, AZ6, AZ8 showed better Human Intestinal Absorption (HIA) score than the standard drug Isoniazid (INH) indicating better bioavailability and absorption by the
**Table 3: Various ADMET parameters of the designed molecules obtained from admetSAR tool**

| Compound Code | HIA   | BBB   | P-glycoprotein Substrate/Inhibition* | Carcino-genicity* | Acute Oral Toxicity | LD50 in rats |
|---------------|-------|-------|-------------------------------------|-------------------|---------------------|--------------|
| AZ1           | 0.9888| 0.6552| NS/ NI                             | NC                | 0.5628              | 2.3938       |
| AZ2           | 0.9973| 0.8569| NS/ NI                             | NC                | 0.6093              | 2.4057       |
| AZ3           | 0.9969| 0.7598| NS/ NI                             | NC                | 0.5896              | 2.5162       |
| AZ4           | 0.9898| 0.9062| NS/ NI                             | NC                | 0.6371              | 2.5187       |
| AZ5           | 0.9930| 0.9027| NS/ NI                             | NC                | 0.6683              | 2.5216       |
| AZ6           | 0.9889| 0.8464| NS/ NI                             | NC                | 0.6541              | 2.5013       |
| AZ7           | 0.9941| 0.8813| NS/ NI                             | NC                | 0.6306              | 2.4544       |
| AZ8           | 0.9870| 0.9092| NS/ NI                             | NC                | 0.5789              | 2.5225       |
| AZ9           | 0.9938| 0.9257| NS/ NI                             | NC                | 0.5909              | 2.5656       |
| AZ10          | 0.9953| 0.7791| NS/ NI                             | NC                | 0.6365              | 2.4930       |
| AZ11          | 0.9951| 0.9226| NS/ NI                             | NC                | 0.6234              | 2.5584       |
| AZ12          | 0.9949| 0.7533| NS/ NI                             | NC                | 0.6269              | 2.4219       |
| Isoniazid     | 0.9892| 0.9895| NS/ NI                             | NC                | 0.8032              | 2.0713       |

*S-Substrate, NS-Non-substrate, NI-Non-inhibitor, NC- Non-carcinogenic

**Table 4: Molecular properties of the designed molecules calculated using Molinspiration software**

| Compound Code | Mol. Wt. | Total no. of Atom | H-Bond Donor O/NHN | H-Bond Acceptor O/N | TPSA | Total no. of rotating bonds | Log P | N Violation |
|---------------|----------|------------------|--------------------|---------------------|------|-----------------------------|-------|------------|
| AZ1           | 313.79   | 19               | 1                  | 5                   | 66.32 | 2                           | 2.15  | 0          |
| AZ2           | 327.82   | 20               | 0                  | 5                   | 55.33 | 3                           | 2.69  | 0          |
| AZ3           | 342.79   | 21               | 0                  | 7                   | 91.92 | 3                           | 2.59  | 0          |
| AZ4           | 332.24   | 19               | 0                  | 4                   | 46.09 | 2                           | 3.31  | 0          |
| AZ5           | 376.69   | 19               | 0                  | 4                   | 16.09 | 2                           | 3.44  | 0          |
| AZ6           | 312.81   | 19               | 2                  | 5                   | 72.12 | 2                           | 1.71  | 0          |
| AZ7           | 311.82   | 19               | 0                  | 4                   | 46.09 | 2                           | 3.08  | 0          |
| AZ8           | 332.24   | 19               | 0                  | 4                   | 46.09 | 2                           | 3.26  | 0          |
| AZ9           | 315.78   | 19               | 0                  | 4                   | 46.09 | 2                           | 2.75  | 0          |
| AZ10          | 342.79   | 21               | 0                  | 7                   | 91.92 | 3                           | 2.54  | 0          |
| AZ11          | 315.78   | 19               | 0                  | 4                   | 46.09 | 2                           | 2.79  | 0          |
| AZ12          | 357.84   | 22               | 0                  | 6                   | 64.56 | 4                           | 2.28  | 0          |
| Isoniazid     | 137.14   | 10               | 3                  | 4                   | 68.01 | 1                           | -0.97 | 0          |

Molecule AZ9 showed highest Blood-Brain Barrier penetration but did not cross the standard drug INH (0.9257 versus 0.9895, resp.). The penetration through the Blood-Brain Barrier (BBB) came out to be best for AZ9 but was not higher than the control molecule (0.9257 versus 0.9895, resp.). All molecules were found to be non-substrate and non-inhibitor of P-glycoprotein similar to INH, decreasing their rate of transport out of the cells and thus decreased chances of therapeutic failure. Furthermore, all molecules showed lower acute oral toxicity and higher LD50 compared to INH and were non-carcinogenic with respect to AMES test data. Table 3 exemplifies the ADMET parameters evaluated using admetSAR.

Molecular properties and bioactivity of the series was calculated using Molinspiration, to evaluate their drug-likeness. In the current study, all the compounds conformed to Lipinski’s rule of five with respect to partition coefficient (Log P ≤ 5), molecular weight (≤ 500kDa), number of H-bond donors (≤ 5), number of H-bond acceptors (≤ 10) as shown in...
Table 5: Bioactivity scores of the designed molecules using Molispiration software

| Compound Code | GPCR ligand | Ion Channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---------------|-------------|------------------------|-----------------|------------------------|-------------------|-----------------|
| AZ1           | -0.82       | -0.76                  | -0.60           | -0.80                  | -0.41             | -0.24           |
| AZ2           | -0.85       | -0.87                  | -0.63           | -0.90                  | -0.44             | -0.34           |
| AZ3           | -0.91       | -0.81                  | -0.69           | -0.92                  | -0.48             | -0.40           |
| AZ4           | -0.88       | -0.82                  | -0.68           | -1.00                  | -0.48             | -0.34           |
| AZ5           | -1.00       | -0.92                  | -0.72           | -1.12                  | -0.58             | -0.40           |
| AZ6           | -0.82       | -0.75                  | -0.52           | -1.06                  | -0.33             | -0.20           |
| AZ7           | -0.93       | -0.91                  | -0.71           | -1.02                  | -0.51             | -0.38           |
| AZ8           | -0.90       | -0.87                  | -0.81           | -0.97                  | -0.56             | -0.41           |
| AZ9           | -0.88       | -0.87                  | -0.63           | -0.98                  | -0.58             | -0.37           |
| AZ10          | -0.88       | -0.76                  | -0.81           | -0.88                  | -0.52             | -0.40           |
| AZ11          | -0.86       | -0.83                  | -0.62           | -0.95                  | -0.47             | -0.33           |
| AZ12          | -0.73       | -0.82                  | -0.52           | -0.78                  | -0.36             | -0.32           |
| Isoniazid     | -1.39       | -1.45                  | -1.05           | -2.33                  | -1.23             | -0.66           |

Table 4. Thus the designed molecules (AZ1-AZ12) are likely to exhibit drug-like physicochemical properties for better pharmacological activity and pharmacokinetics in the human body.

The bioactivity scores of the designed compounds and the standard drug, Isoniazid, were calculated with reference to diverse parameters including G-protein coupled receptor binding, ion channel modulation, kinase inhibition, protease inhibition, and enzyme-based inhibition as shown in Table 5. The bioactivity score above 0.0 indicates that the molecule is anticipated to be biologically active. While molecules having a bioactivity score between -5.0 and 0.0 are expected to be moderately active and those with scores less than -5.0 are implied to be inactive. As seen from Table 5, the bioactivity scores for all the designed molecules were between -5.0 and 0.0, which is an indicator of moderate activity. Further, the bioactivity scores are more towards 0.0 for enzyme inhibition in comparison to the other parameters, which substantiates our design principle involving inhibition enzyme activity in MTB. Molecule AZ6 was found to be a potential candidate based on the bioactivity scores.

CONCLUSIONS

In summary, a rational stratagem for the design of a series of substituted 1,3,4-thiadiazolyl-azetidin-2-one derivatives using systematic iteration based on the molecular framework of thiadiazole and azetidinone has been illustrated. The docking study revealed that thiadiazolyl-azetidinone derivatives showed significant binding by interacting with the amino acid residues in the active site of Mycobacterium tuberculosis Shikimate kinase. The *in silico* method adopted in the present ADMET studies demonstrated that these molecules are likely to be orally bioavailable and could be easily transported, diffused, and absorbed. Hence, it was concluded that thiadiazolyl-azetidinone series is likely to be a potential lead for the development of novel MtSK inhibitors and may exert interesting anti-tubercular activity. In the future, the series will be synthesized and evaluated for its anti-tubercular activity.

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