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# Design and *In silico* Studies of 2,5-Disubstituted 1,2,4-Triazole and 1,3,4-Thiadiazole Derivatives as Pteridine Reductase 1 Inhibitors

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors RS, DP and SP have contributed equally to the conception and design, critical review of the manuscript, Statistical analysis, administrative and technical support as well as final approval of manuscript. Authors RS and DP have also supervised the process of article preparation. Drafting of manuscript, data acquisition, interpretation, communication and correspondence done by author SP. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Design and *in silico* studies of 2,5-disubstituted triazole and thiadiazole derivatives as Pteridine Reductase 1 inhibitors. With a view to develop effective agents against Leishmaniasis, 2-substituted-5-[(1H-benzimidazol-2yl) methyl] azole derivatives (A1-A12) were designed against the target enzyme Pteridine reductase 1.
**Methodology:** The series was designed by targeting Pteridine reductase 1 which is an enzyme responsible for folate and pterin metabolism. Based on thorough study of the enzyme structure and structural features of ligands required for optimum interaction with the enzyme, a series of 12 compounds consisting of 2,5-disubstituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives was designed. *In silico* studies were carried out which included docking studies (using V Life software)

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to understand binding of the compounds with enzyme PTR1, ADMET studies, drug likeness studies for physicochemical properties and bioactivity studies to understand the possible mechanism of action of the compounds. These studies were undertaken using online softwares, molinspiration and admetSAR web servers.

**Results:** Compounds A10 and A12 gave the best docking scores of -59.9765 and -60.4373 respectively that were close to dihydrobiopterin (original substrate). All the compounds complied with Lipinski's rule of five. Most of the compounds displayed favorable ADMET properties.

**Conclusion:** The 2,5-disubstituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives exhibited good binding affinity for PTR1 enzyme (PDB code: 1E92). The docking scores indicated that enzyme binding may be governed by the nature and size of the substituents on the azole ring. The compounds display well-defined drug-like and pharmacokinetic properties based on Lipinski's rule of five with additional physicochemical and ADMET parameters. Bioactivity studies suggested the possible drug mechanism as enzyme inhibition. Hence, this study provides evidence for consideration of valuable ligands in 2,5-disubstituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives as potential pteridine reductase 1 inhibitor and further in vitro and in vivo investigations may prove its therapeutic potential.

*Keywords: Leishmaniasis; pteridine reductase 1 inhibitor; 2,5-disubstituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives; in silico studies.*

## 1. INTRODUCTION

Leishmaniasis is a vector borne disease transmitted through phlebotomine sand fly. Around 20 species of the protozoal parasite *Leishmania* (family *trypanosomatidae*) infect humans. This disease is a serious health threat in developing countries majorly in the continents of Africa and Asia. Around 1 million new cases are reported each year worldwide. World Health Organization (WHO) has categorized the disease as neglected tropical disease (NTD). The disease occurs in three different forms namely visceral, cutaneous and mucocutaneous leishmaniasis [1]. Visceral leishmaniasis (VL) also known as Kala-azar is most fatal out of the three forms of the disease and is a form that predominantly occurs in India. VL is associated with fever and enlargement of liver and spleen. Worldwide around 50, 000 to 90,000 new cases of VL occur annually. Development of resistant strains has made the conditions more severe [2]. Drugs used for treatment of leishmaniasis are associated with drawbacks like toxicity and high cost. These two factors are major contributors for development of resistance. New agents that selectively affect the parasite, which are inexpensive and effective against the emerged resistant strain are urgently needed [3-5].

Toxicity of the existing drugs can be attributed to action of the drugs on both the parasite as well as host system. Effective therapy against leishmaniasis can be developed by targeting the enzymes and systems that are unique in life processes of parasite. *Leishmania* genome sequencing has helped the researchers to focus on such targets.

Pteridine reductase 1 (PTR1) is one such enzyme which is essential for survival of organism. PTR 1 is an oxido-reductase enzyme. It is an essential broad spectrum enzyme responsible for pteridine salvage in trypanosomatids [6]. Pterins and folates are essential for the growth of the parasites, however, these organisms do not possess the mechanism for synthesis of pterins and folates. The parasites depend on the host system for these essential components. The parasites acquire folates and unconjugated pterins from the mammalian host which are required in their reduced form by the parasite. Within the parasite the bifunctional enzymes dihydrofolate reductase (DHFR) – thymidylate synthase (TS) facilitate conversion of folates to their active reduced form. The DHFR-TS reduce folates to dihydrofolates (DHF) and tetrahydrofolates (THF). While PTR1 brings about reduction biopterins to dihydrobiopterins ($H_2B$) which further is reduced to tetrahydrobiopterins ($H_4B$). In addition to the ability of PTR1 to reduce biopterin to tetrahydrobiopterin, it also has the ability to reduce folates to tetrahydrofolates. Thus this enzyme acts as a bypass for parasites that facilitates uninterrupted supply of folates and pterins in absence of DHFR-TS.

In the course of treatment for leishmaniasis generally a DHFR inhibitor is included in the drug regime. However, it has been observed that these agents fail to control the disease. This is

because even in absence of DHFR leishmanial parasite is able to obtain the reduced folates along with pterins required for its survival by the action of PTR1. Thus PTR 1 is considered as a key factor for antifolate drug resistance and for the failure of conventional therapies against the trypanosomatids. Hence it is important to understand that targeting the leishmanial parasite through the folate biosynthesis pathway must include inhibition of DHFR-TS as well as PTR1 simultaneously [7-10].

The present work attempts to design and study novel PTR1 inhibitors. The enzyme PTR1 has been extensively studied. Crystallographic analysis of PTR1 from different *leishmania* species are reported. The crystal structure of *Leishmania major* pteridine reductase 1 (*Lm*PTR1) shows a substrate binding site as a well-defined cleft. In majority of the crystal structures, the pteridine binding sites were identified as π-stacking interactions to Phe 113 and the nicotinamide part of NADPH; an essential hydrogen bond with oxygen atoms of cofactor phosphate group; optional hydrogen bonds to either the hydroxyl group of Ser 111, Tyr 194, or the ribose part of the cofactor. Four pharmacophore features have been identified as key features involved in the inhibitor–PTR1 interaction, which are two H-bond donors, one hydrophobic aromatic feature and one ring aromatic feature. From the studies reported on interactions of 1, 3, 4-thiadizol-2-yl-amine derivatives with PTR1, the hydrophobic region has enough space to accommodate various substituted aromatic ring systems. Increasing the hydrophobic quotient by adding a benzene ring to give benzothiazole showed better overlap with the hydrophobic region when compare to the thiadiazole ring system. Scaffolds, such as aminobenzimidazole and aminobenzothiazole, show selective binding to *Lm*PTR1. Thus, a structure containing an aromatic ring system, a hydrophobic group, and H-bond acceptors would act as a good substrate for the receptor [9,11-15]. A number of heterocyclic compounds like aminobenzimidazole and amino-benzothiazole [12], 2,4-diaminopteridine and 2,4-diaminopyrimidine [16], 1,3,4-thiadiazole [13], oxadiazole and triazoles [17-19], pyrrolopyrimidine and pyrimido[1,2-b] pyrimidinone [20] have been explored for anti-leishmanial activity via PTR1 inhibitory mechanism.

The present work consists of *in silico* studies for development of PTR1 inhibitors against leishmaniasis. This work is based on the results of our previous work on 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl)methyl] azole derivatives [21]. The findings of docking studies and *in silico* ADMET studies of the our previous series were used for structural modifications that would improve receptor binding and ADMET profiles. In present work we have designed 2,5-disubstituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives. General structure of 2-substituted-5-[(1H-benzimidazol-2yl)methyl]azole derivatives is depicted in Fig. 1.

*In silico* modeling has been effectively utilized and explored for drug designing and simulations. It helps the medicinal chemists and drug discovery scientists to design drug-like candidates. These studies include molecular docking, in *silico* ADMET studies, bioactivity prediction studies. Such studies are a logical approach in drug discovery process [22].

This study thus aims at designing PTR1 inhibitors, investigating the relationship between the designed scaffold, its substituents and the receptor binding affinity as well as studying the pharmacokinetic profile, drug likeness using *in silico* tools.

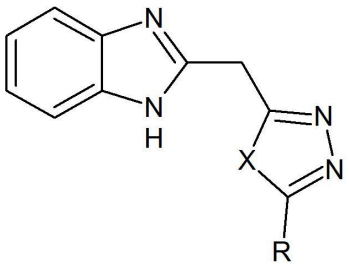


**Fig. 1. General structure of 2-substituted-5-[(1H-benzimidazol-2yl)methyl] azole derivatives**

## 2. MATERIALS AND METHODS

## 2.1 Molecular Docking Studies

### 2.1.1 Computational resources

All molecular modeling studies were performed using the Molecular Design Suite (V Life MDS software package, version 4.4; from V Life Sciences, Pune, India). Molecular Docking carried out using dell PC with a Pentium IV processor and Windows 7 operating system. Docking studies were performed using GRIP

batch docking method implemented in V Life MDS 4.4 software package.

#### 2.1.2 X-ray Crystal structure

The X-ray crystal structure of *Leishmania major* Pteridine reductase 1 (PDB ID: 1E92) was imported from Protein data bank (Available from http://www.rcsb.org/). The X-ray crystal structure of Pteridine reductase 1 domain had resolution of 2.2Å.

#### 2.1.3 Protein preparation

The crude PDB structure of the receptor was refined by completing the incomplete residues. Chloride ions and ADP were deleted. Water molecules were also removed, hydrogen atoms were added. The optimized receptor was saved as mol file and used for docking simulation.

#### 2.1.4 Ligand preparation

The 2D structures of the designed molecules and the reference ligand, Methotrexate were sketched using Marvin sketch 5.11.5 and then converted to 3D structures using V Life MDS 4.4 software. The 3D structures were then energy minimized to RMS gradient of 0.01kcal/mol Å using Universal Force Field (UFF). Conformers of all the designed ligands were selected and number of seeds used for searching the conformational space was set as 5. All conformers were then energy minimized to the RMS gradient of 0.01kcal/mol Å and then saved in separate folder.

#### 2.1.5 Docking

Flexible docking algorithm was used which not only predicts the binding mode of a molecule more accurately than rigid body algorithms but also its binding affinity relative to other compounds [23]. All conformers were docked using exhaustive method. Number of placements was fixed to a value of 30 and rotation angle to a value of $15^{o}$. The docking score was used as a scoring function. By rotation angle, the ligand is rotated to obtain different poses. By placements, the method checks for all the 30 possible placements into the active site pocket and results out few best placements out of 30. For each ligand, all the conformers with their best placements and their docking score were saved to the output folder. The ligand forming the most stable drug receptor complex is the one which has minimum docking score (interaction energy) and the scoring interaction energy of the standard drug ligand for comparison. The most stable drug receptor poses were studied for their interactions with the amino acid residues in the active site of the receptor. These interactions involve hydrogen bonding, Van der Waal's interaction, aromatic/π stacking, hydrophobic and other charge interactions.

### 2.2 Drug Likeness, Bioactivity Prediction and ADMET Properties

These *in silico* studies help to determine the activity of the compound when inside the body and can serve as an important tool for drug discovery and lead optimization.

Molecular descriptors and drug likeliness properties of 2-substituted-5-[(1H-benzimidazol-2yl)methyl] azole derivatives, DHB and MTX were analyzed using the tool Molinspiration software which is based on Lipinski's Rule of five (RO5). (Molinspiration server) (http://www.molinspiration.com), (accessed on 21 September 2019). The pharmacokinetic properties such as absorption, distribution, metabolism, excretion and the toxicity of the compounds were checked using admetSAR online software admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) (accessed on 05 December 2019). The structures were drawn using ACD labs Chemsketch v 12.0 and SMILES notation data was generated and fed into these softwares to calculate the parameters.

## 3. RESULTS AND DISCUSSION

### 3.1 Designing

The series was designed based on the available literature on the active binding site of PTR1, the structural features for effective interaction with the receptor, as well as various pharmacophores that have been explored in this context. According to the literature study, H-bonding interactions and π-stacking interactions are two most important interactions and necessary for the binding of inhibitors in the active site of PTR1. There are several hydrophilic and hydrophobic regions in the active site of *Lm*PTR1. The hydrophobic region is at the core of the active site surrounded by hydrophilic regions. Studies on interactions of 1, 3, 4-thiadizol-2-yl-amine derivatives with PTR1 revealed that the hydrophobic region of the active site had enough space to accommodate various substituted aromatic ring systems.

Increasing the hydrophobic quotient by adding a benzene ring to give benzothiazole showed better overlap with the hydrophobic region when compared to the thiadiazole ring system. Scaffolds such as aminobenzimidazole and amino benzothiazole, show selective binding to *Lm*PTR1.

Apart from the reported literature, docking and *in silico* study results from the previous designed series of 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl) methyl] azole derivatives were considered in designing the present series of PTR 1 inhibitors [21]. Following were the findings of the previous study. 1. An important structural feature that can improve ligand interaction was the length of substituents and H-bond acceptor functionalities on the azole ring. Within the series, derivatives with larger group like $SCH_2COOH$ displayed good affinity as compared to –SH and $–NH_2$. This conclusion was in agreement with the literature which states that the binding site has enough space to accommodate various substituents ranging from aliphatic to aromatic substituents with 0, 1 or 2 substituents. 2. The substituents on benzimidazole ring (–Cl and $–NO_2$) were more favorable for hydrophobic interactions as compared to unsubstituted benzimidazole derivatives but at the same time were thought to be contributing to mutagenicity and carcinogenicity. With these observations in mind, 2-substituted-5-[(1H-benzimidazol-2yl) methyl] azole derivatives were designed bearing the following structural modifications; a. Unsubstituted benzimidazole ring b. Larger substituents on triazole and thiadiazole ring that can act as H-bond acceptors.

The present series consists of 1,2,4-triazole and 1,3,4-thiadiazole derivatives. Substituents on these rings consists of benzimidazole ring which will contribute to the hydrophobic requirement along with the azole ring. While the H-bond acceptors selected were –SH and esters of thiol in case of 1,2,4-triazole ring and $–NH_2$, acetyl derivatives, Schiff bases and chalcone derivatives of 2-amino-1,3,4-thiadiazole ring respectively. 2-substituted-5-[(1H-benzimidazol-2yl) methyl] azole derivatives (A1-A12) are enlisted in Table 1.

## 3.2 Molecular Docking

Molecular docking was performed to evaluate the interactions of designed compounds against *Leishmania Major* PTR 1 (*Lm*PTR1) crystal structure (PDB Code: 1E92) using V Life MDS software package, version 4.4. Docking studies show that the designed molecules fit well in the active site pocket made up of key residues Arg 17, Leu 18, Ser 111, Phe 113, Tyr 191, Pro 224, Gly 225, Ser 227, Leu 229 and Val 230. The interactions were compared against the original ligand DHB and reference molecule Methotrexate (MTX). According to the literature the original substrate DHB bind to *Lm*PTR1 by forming H-bonds and aromatic interaction with co-factor and Phe 113. Similar interactions are observed in MTX with *Ld*PTR1 involving hydrogen bonding interaction of pteridine moiety with Ser 111, Tyr 194 and Arg 17 of *Ld*PTR [20]. The designed molecules were found to mimic the key interactions which include hydrogen bonding, hydrophobic, aromatic and Van der Waal's interactions. It was observed that benzimidazole ring, azole ring and the spacer methylene group are involved in hydrophobic interactions with amino acid residues Leu 226, Ser 227, Leu 229, Val 230 and azole ring is involved in aromatic π-stacking interaction with Phe 113.Compounds with phenyl substituent also showed aromatic π-stacking interaction with Phe 113. However, the compounds that had the best dock scores namely, A10 and A12 did not show aromatic π-stacking interaction. Rather hydrophobic interaction with the key residue( Phe 113) was observed in both compounds. The substituent present in both the compounds contain an nitrophenyl functionality which may be responsible for a different orientation of these compounds in the active site cavity. The azole ring nitrogen, substituents on azole ring and benzimidazole nitrogen are involved in H-bond with the active site. Amino acid residue Arg17 is involved in the interaction in most of the structures which is similar to the interaction of DHB with the active site. Thus if we compare the binding interactions of the designed molecules with the interactions of original substrate, it can be said that the molecules bind in a similar pattern with the enzyme active site. The three-dimensional interaction images were developed using Discovery studio visualizer v20 [24]. Figs. (2-6) show the 3D interactions of original ligand (DHB), reference ligand (MTX), compound A12 (hydrogen bonding, hydrophobic interactions and Van der Waal's interaction) with the active site of PTR1 respectively.

The docking score of the molecules (A1-A12) is presented in Table 2 and their interactions with the amino acids in the active site of PTR1 are listed in Table 3.

Dock scores of the designed series improved with increase in the size of the substituent. In 1,2,4-triazole derivatives the highest interaction was observed in the benzyl esters of thiols (compound A5) as compared to the thiol derivative, methyl and ethyl esters (compounds A1-A4). While amino-substituted-1,3,4-thiadiazole derivatives displayed better interactions than the triazole derivatives. Within the thiadiazole derivatives chalcone derivative of acetyl amino thiadiazole (Compound A10) and Schiff base derivative (Compound A12) showed the lowest interaction energy of -59.9765 and -60.4373 respectively which is comparable with the docking score of original ligand DHB which is -68.4502. Best docking scores of A10 and A12 can be due to the extended length of the substituents in these molecules as well as the presence of conjugated system and strong H-bond accepting functionality.

**Table 1. Designed Series (A1-A12) with the substituents**

| Compound | X | R | Compound | X | R |
|---|---|---|---|---|---|
| A1 | N | -SH | A7 | S | $-NH_2$ |
| A2 | N | $-SCH_2COOH$ | A8 | S | $-NHCOCH_3$ |
| A3 | N | $-SCH_2COOCH3$ | A9 | S | -NHCOCH=CHPh |
| A4 | N | $-SCH_2COOC2H5$ | A10 | S | $-NHCOCH=CHPhNO_2$ |
| A5 | N | $-SCH_2COOCH2Ph$ | A11 | S | -NH=CHPh |
| A6 | N | $-SCH_2C_6H_3Cl_2$ | A12 | S | $-NH=CHPhNO_2$ |

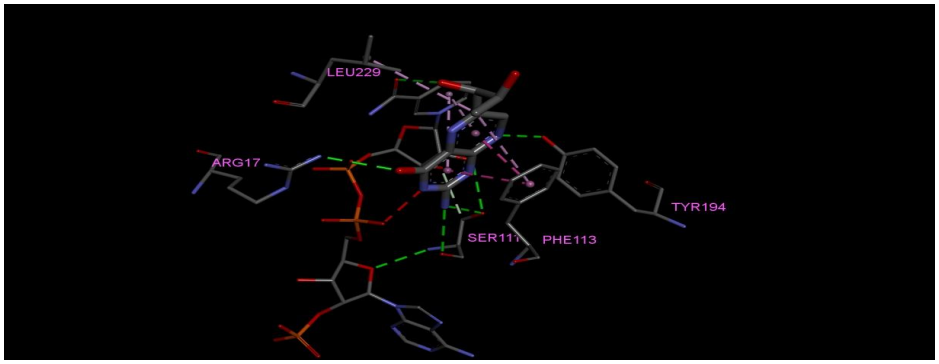


**Fig. 2. Three-dimensional interaction of original ligand DHB with active site residues of PTR1; green dotted lines represent hydrogen bond interaction, pink dotted lines represent the aromatic interactions and violet dotted lines represent hydrophobic interactions with the amino acid residues**

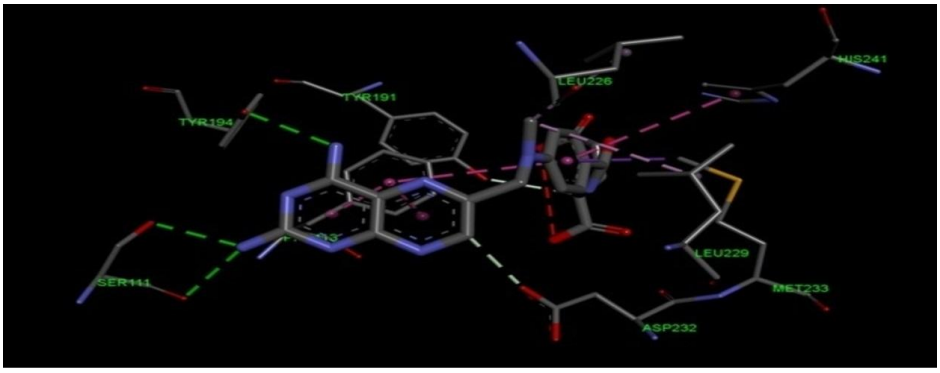


**Fig. 3. Three-dimensional interaction of reference ligand MTX with active site residues of PTR1; green dotted lines represent hydrogen bond interaction, pink dotted lines represent the aromatic interactions and violet dotted lines represent hydrophobic interactions with the amino acid residues**

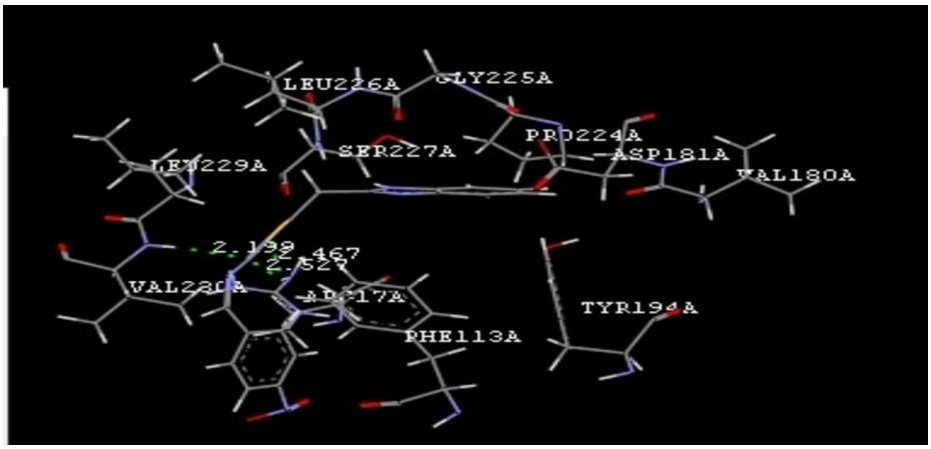


**Fig. 4. Three-dimensional interaction of compound A12 with active site residues of PTR1; green dotted lines represent hydrogen bond interaction with the amino acid residues**

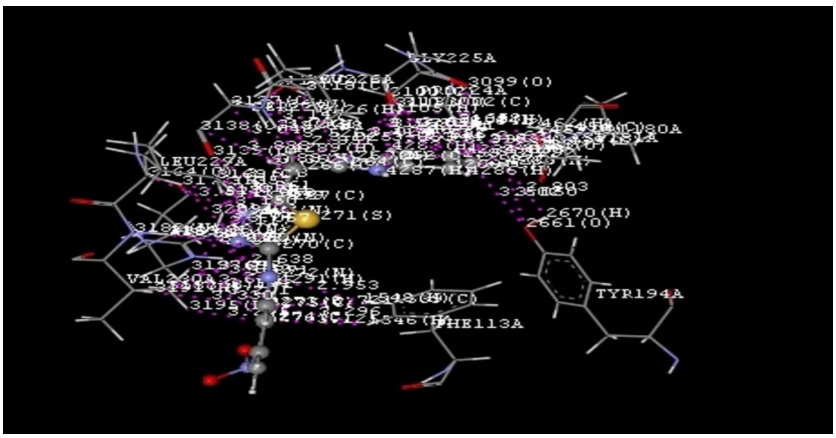


**Fig. 5. Three-dimensional interaction of compound A12 with active site residues of PTR1; pink dotted lines represent hydrophobic interactions with the amino acid residues**

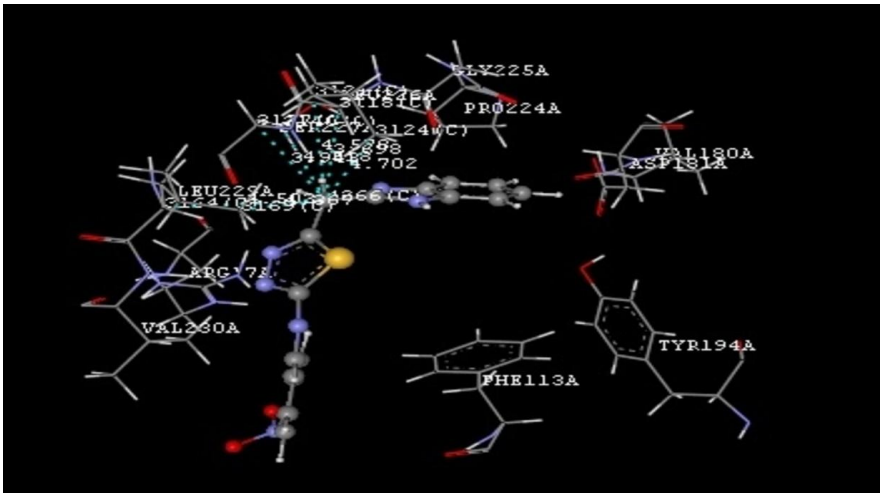


**Fig. 6. Three-dimensional interaction of compound A12 with active site residues of PTR1; blue dotted lines represent the hydrophobic interactions with the amino acid residues**

**Table 2. Dock score of designed compounds**

| Compound | Dock Score | Compound Code | Dock Score |
|---|---|---|---|
| A1 | -37.7739 | A8 | -39.1472 |
| A2 | -44.8633 | A9 | -56.8060 |
| A3 | -48.6035 | A10 | -59.9765 |
| A4 | -51.4926 | A11 | -56.1416 |
| A5 | -55.1623 | A12 | -60.4373 |
| A6 | -52.8165 | Dihydrobiopterin (DHB) | -68.4502 |
| A7 | -50.3321 | Methotrexate (MTX) | -75.6695 |

The hypothesis that the larger group substituents and H-bond acceptor functionalities on the azole ring display good affinity holds true.

### 3.3 Drug Likeness, Bioactivity Prediction and ADMET Studies

In the drug discovery process, out of the hundreds of molecules having pharmacological action very few possess appropriate pharmacokinetic properties. Oral bioavailability studies of the compounds (absorption, distribution, metabolism, and excretion) are essential to eliminate compounds with unacceptable pharmacokinetic properties and for successful drug discovery studies. *In silico* ADME and toxicological screening systems can provide an opportunity to predict performance *in vivo*. Structure of a molecule has a major role in absorption, distribution, metabolism, elimination and toxicity (ADMET) properties. [25,26].

The designed molecules were checked for the drug likeness (molecular properties) and bioactivity using an online server database, Molinspiration Chemoinformatics software. admetSAR, a free online server was used to predict ADMET properties. All the molecules (A1-A12) showed no violation from the Lipinski's Rule of five (molecular weight < 500, number of hydrogen bond donors < 5 number of hydrogen bond acceptors < 10, log P < 5). The compounds also comply with the Veber's rule (≤ 10 rotatable bonds and TPSA ≤ 140$^{o}$). This rule gives a measure of membrane permeability and bioavailability. It indicates that most compounds may have good oral absorption [27]. Violations were observed in original ligand and reference ligand. Table 4 gives the details of drug likeness studies using Molinspiration software.

Human Intestinal Absorption (HIA) and Caco-2 cell permeability model are a measure of human intestinal drug absorption. Greater HIA values and "+" Caco-2 cell permeability indicate good intestinal absorption. HIA values were found to be 0.87 and above indicating good absorption but in the Caco-2 cell permeability model most of the molecules show poor permeability. DHB and MTX were found to have poor Caco-2 cell permeability and may have poor intestinal absorption. All the compounds were found to have high BBB permeability as well as good oral bioavailability [28-30]. Log S value indicates water solubility. Lesser the log S value greater will be the solubility [31]. All compounds displayed log S in the range of -3.5 to -1.8 indicating good water solubility. Overall, the compounds show good absorption distribution and permeability through biological membranes.

P-glycoprotein efflux transporter parameter is used for studying the drug transport. Compounds were found to be non-substrate (NS) and non-inhibitor (NI) of P-glycoprotein efflux transporter except A5 which is inhibitor of the transporter. NS indicate that compounds will not be a substrate of P-glycoprotein transporter as a result will not be effluxed out of the cell also as they are not inhibitor of the transport system, they will not interfere with absorption, permeability of other drugs [32]. Table 5 gives the absorption and distribution profile of the series.

Most drugs are metabolized by the cytochrome P450 class of enzymes. Isoforms 2D6 and 3A4 are the major metabolizers of most of the drugs. It is essential to know whether the molecules are inhibitors of these enzymes as it may affect the metabolism of other drugs. None of the compounds were found to be substrate or inhibitor of CYT2D6 isoform while for CYT3A4 a mixed data was obtained. Some compounds seem to be both substrate as well as inhibitor of this isoform while some were neither substrate nor inhibitor and some were either substrates or inhibitors. Compounds A1-A8 displayed negative AMES values which mean they are non-mutagenic. The compounds were found to be non-carcinogenic except compounds A10 and A12. Mutagenicity and carcinogenicity can be attributed to the nitro substituent present in the above-mentioned compounds. All compounds

**Table 3. Interactions of the designed compounds, original substrate and reference ligand with PTR 1**

| Compound | Hydrogen bond interaction | Aromatic interaction | Hydrophobic interaction | Van der Waal interaction |
|---|---|---|---|---|
| Dihydrobiopterin(DHB) | Arg 17, Ser 111, Tyr 194 | Phe113 | Leu 229 | Asp 181, Leu 188, Gly 225, Leu226, Ser 227, Val 230, Arg 287 |
| Methotrexate(MTX) | Ser 111, Tyr 191, Tyr 194, Asp 232 | Phe113 His 241 | Leu 226, Leu 229 | Arg 17, Pro 115, Asp 181, Leu 188 |
| A1 | Arg 17 | Phe 113 | Leu 229, Val230 | Arg 17, Leu 18, Phe 113, Pro 224, Gly 225, Ser 227, Leu 229, Val 230 |
| A2 | Ser 227 | Phe 113 | Leu 229, Val230 | Arg 17, Leu 18, Phe 113, Pro 224, Gly 225, Ser 227, Leu 229, Val 230 |
| A3 | Gly225, Ser 227 | Phe 113 | Leu 226, Ser 227, Leu 229, Val 230 | Arg 17, Phe 113, Val 180, Asp 181, Pro 224, Gly 225, Leu 226, Ser 227, Leu 229, Val 230 |
| A4 | Gly 225, Ser 227, Val 230 | -- | Leu 226, Ser 227, Leu 229, Val 230 | Arg 17, Phe 113, Val 180, Asp 181, Pro 224, Gly 225, Leu 226, Ser 227, Leu 229, Val 230 |
| A5 | Arg 17 | Phe 113 | Leu 18, Ser227 | Arg 17, Leu 18, Gly 19, Asn 109, Phe 113, Tyr 191, Leu 226, Ser 227, Leu 229, Val 230 |
| A6 | -- | Phe 113, Try 194 | Ser 111, Phe 113, Leu 226, Leu 229 | Lys 16, Arg 17, Leu 18, Gly 19, Ser 111, Phe 113, Leu 188, Tyr 191, Leu 226, Ser 227, Leu 229, Val 230, Asp 232, Met 233 |
| A7 | Arg 17 | Phe 113 | Leu 229, Val 230 | Arg 17, Leu 18, Phe 113, Pro 224, Ser 227, Leu 229, Val 230 |
| A8 | Ser 111, Pro 224, Gly 225 | Phe 113 | Ser 111, Phe 113, Lys 198 | Ser 111, Ser112, Phe 113, Val 180, Asp 181, Tyr 194, Lys 198, Pro 224, Gly 225 |
| A9 | Arg 17 | Phe 113 | Leu 18, Ser227 | Arg 17, Leu 18, Gly 19, Asn 109, Phe 113, Tyr 191, Leu 226, Ser 227, Leu 229, Val 230 |
| A10 | Arg 17, Ser 111, Ser 227 | -- | Leu 229, Val 230 | Arg 17, Leu 18, Ser 111, Phe 113, Tyr 191, Pro 224, Gly 225, Leu 226, Ser 227, Leu 229, Val 230 |
| A11 | Arg 17 | Phe 113 | Leu 226, Leu 229 | Arg 17, Leu 18, Gly 19, Asn 109, Phe 113, Leu 229, Val 230 |
| A12 | Arg 17, Val 230 | -- | Leu 226, Ser 227, Leu 229 | Arg 17, Phe 113, Val 180, Asp 181, Tyr 194, Pro 224, Gly 225, Leu 226, Ser 227, Leu 229, Val 230 |

**Table 4. Drug likeness properties of the series using Molinspiration software**

| Compound | Molecular Weight | Log P | H bond acceptor n ON | H bond donor n OHNH | No. of rotating bond | TPSA | N violation |
|---|---|---|---|---|---|---|---|
| A1 | 374.23 | 3.66 | 6 | 2 | 5 | 79.63 | 0 |
| A2 | 231.28 | 1.77 | 5 | 2 | 2 | 70.26 | 0 |
| A3 | 289.32 | 1.22 | 7 | 3 | 5 | 107.56 | 0 |
| A4 | 303.35 | 1.83 | 7 | 2 | 6 | 96.56 | 0 |
| A5 | 317.37 | 2.21 | 7 | 2 | 7 | 96.56 | 0 |
| A6 | 379.44 | 3.43 | 7 | 2 | 8 | 96.56 | 0 |
| A7 | 231.28 | 1.43 | 5 | 3 | 2 | 80.49 | 0 |
| A8 | 273.32 | 1.16 | 6 | 2 | 3 | 83.56 | 0 |
| A9 | 361.43 | 3.47 | 6 | 2 | 5 | 83.56 | 0 |
| A10 | 406.43 | 3.43 | 9 | 2 | 6 | 129.39 | 0 |
| A11 | 319.39 | 3.48 | 5 | 1 | 4 | 66.83 | 0 |
| A12 | 364.39 | 3.44 | 8 | 1 | 5 | 112.66 | 0 |
| DHB | 239.24 | -1.65 | 8 | 6 | 1 | 136.62 | 1 |
| MTX | 454.45 | -1.97 | 12 | 7 | 9 | 210.55 | 2 |

**Table 5. Absorption and distribution profile of the series using admetSAR tool**

| Compound | Log S | HIA | Caco-2 permeability | BBB | Human Oral bioavailability | P-glycoprotein |
|---|---|---|---|---|---|---|
| A1 | -2.494 | 0.9836 | +0.6303 | 0.9745 | 0.5286 | NS/NI |
| A2 | -2.721 | 0.9174 | -0.7530 | 0.9739 | 0.5429 | NS/NI |
| A3 | -2.88 | 0.9693 | -0.7179 | 0.9742 | +0.5143 | NS/NI |
| A4 | -3.16 | 0.9801 | -0.6561 | 0.9740 | +0.5429 | NS/NI |
| A5 | -3.407 | 0.9693 | -0.7808 | 0.9739 | -0.6286 | NS/I |
| A6 | -3.861 | 0.9710 | -0.8080 | 0.9785 | -0.5429 | NS/NI |
| A7 | -1.847 | 0.9770 | +0.5293 | 0.9734 | +0.6286 | NS/NI |
| A8 | -2.854 | 0.9061 | -0.7474 | 0.9703 | -0.5413 | NS/NI |
| A9 | -3.485 | 0.8924 | -0.8422 | 0.9731 | -0.5571 | NS/NI |
| A10 | -3.514 | 0.8305 | -0.8607 | 0.9738 | +0.6429 | NS/NI |
| A11 | -3.056 | 0.9322 | -0.7924 | 0.9760 | +0.5143 | NS/NI |
| A12 | -3.319 | 0.8535 | -0.7211 | 0.9763 | +0.6714 | NS/NI |
| DHB | -3.091 | 0.9517 | -0.8841 | 0.9715 | +0.5714 | NS/NI |
| MTX | -3.065 | 0.9088 | -0.8662 | 0.9930 | -0.8286 | S/NI |

**NS: Non-substrate, NI: Non-inhibitor, S: Substrate, I: Inhibitor*

**Table 6. Metabolism and toxicity profile of the series using admetSAR tool**

| Compound | CYP2D6* | CYP3A4* | AMES toxicity | Carcino genicity* | Acute oral toxicity | $LD_{50}$ in rats |
|---|---|---|---|---|---|---|
| A1 | NS/NI | NS/I | Non-toxic | NC | 0.4399 | 1.937 |
| A2 | NS/NI | S/NI | Non-toxic | NC | 0.5368 | 2.05 |
| A3 | NS/NI | S/NI | Non-toxic | NC | 0.5098 | 2.66 |
| A4 | NS/NI | NS/NI | Non-toxic | NC | 0.5366 | 2.284 |
| A5 | NS/NI | S/I | Non-toxic | NC | 0.5448 | 2.18 |
| A6 | NS/NI | S/I | Non-toxic | NC | 0.5442 | 2.116 |
| A7 | NS/NI | NS/I | Non-toxic | NC | 0.5348 | 2.092 |
| A8 | NS/NI | S/I | Non-toxic | NC | 0.5454 | 1.895 |
| A9 | NS/NI | S/I | Toxic | NC | 0.6117 | 1.998 |
| A10 | NS/NI | S/I | Toxic | C | 0.6154 | 2.461 |
| A11 | NS/NI | NS/I | Toxic | NC | 0.5400 | 1.95 |
| A12 | NS/NI | S/I | Toxic | C | 0.5009 | 2.166 |
| DHB | NS/NI | NS/NI | Non-toxic | NC | 0.6048 | 2.051 |
| MTX | NS/NI | S/NI | Non-toxic | NC | 0.7310 | 3.077 |

**NS: Non-substrate, NI: Non-inhibitor, S: Substrate, I: Inhibitor, NC: Non-carcinogenic, C: Carcinogenic*

**Table 7. Bioactivity scores of the series using Molinspiration software**

| Compound | GPCR ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---|---|---|---|---|---|---|
| A1 | -0.69 | -0.38 | -0.57 | -138 | -0.78 | -0.22 |
| A2 | -0.40 | -0.54 | -0.51 | -0.86 | -0.28 | -0.14 |
| A3 | -0.48 | -0.63 | -0.51 | -1.06 | -0.36 | -0.32 |
| A4 | -0.49 | -0.62 | -0.52 | -0.99 | -0.34 | -0.35 |
| A5 | -0.28 | -0.43 | -0.36 | -0.77 | -0.10 | -0.18 |
| A6 | -0.26 | -0.47 | -0.25 | -0.74 | -0.20 | -0.17 |
| A7 | -0.23 | -0.03 | -0.20 | -1.32 | -0.46 | 0.07 |
| A8 | -0.22 | -0.45 | -0.12 | -0.67 | -0.49 | -0.09 |
| A9 | -0.32 | -0.47 | -0.06 | -0.46 | -0.30 | -0.11 |
| A10 | -0.42 | -0.48 | -0.18 | -0.51 | -0.41 | -0.20 |
| A11 | -0.64 | -0.81 | -0.23 | -0.83 | -0.65 | -0.31 |
| A12 | -0.72 | -0.78 | -0.34 | -0.83 | -0.69 | -0.39 |
| DHB | -0.59 | -0.57 | -0.53 | -1.30 | -0.81 | 0.34 |
| MTX | 0.51 | 0.23 | 0.38 | -0.38 | 0.27 | 0.72 |

showed lower oral acute toxicity than reference MTX. $LD_{50}$ of the compounds was found to be relatively higher (ranging from 1.895 - 2.461 mol/kg) and can be considered to be safe [33]. Table 6 illustrates the metabolism and toxicity data for the series obtained by admetSAR tool.

The bioactivity scores of the designed compounds as GPCR ligand, ion channel modulator, nuclear receptor ligand, a kinase inhibitor, protease inhibitor, enzyme inhibitor was studied and is summarized in Table 7.

A molecule having a bioactivity score of more than 0.00 is most likely to exhibit considerable biological activity, while values −0.50 to 0.00 are expected to be moderately active and if the score is less than -0.50, it is presumed to be inactive [34]. Bioactivity scores are more towards 0.0 for enzyme inhibition as compared to other mechanisms. Compounds A7 exhibits bioactivity score more than 0.00 for enzyme inhibition thus can be considered to exhibit significant biological activity by the above said mechanism. DHB displayed a bioactivity score of 0.34 under enzyme inhibition mechanism. The remaining compounds gave bioactivity score between -0.39 to -0.09 indicating moderate enzyme inhibition. These scores justify the rationale behind designing the series as PTR 1 inhibitor. While the bioactivity scores of MTX were positive in most of the heads.

From the above set of studies, it can be summarized that the designed series can prove to be a good scaffold as an inhibitor of the enzyme PTR1. Based on the docking studies compound A10 and A12 exhibit the best docking score. The series also gave favourable results for the *in-silico* drug likeness, bioactivity scores, ADMET studies. However, some molecules (A10 and A12) can be mutagenic and carcinogenic. This toxicity can be attributed to nitro group functionality present in these compounds. Thus, the nitro substituent was seen to be favourable for effective binding interaction but also may be responsible for the toxicity. Thus, replacement of this functionality with other substituents can be studied. Remaining compounds also have comparable docking scores and satisfactory drug likeness, bioactivity scores and ADMET properties.

## 4. CONCLUSION

With a view to develop PTR1 inhibitors, series of 2-substituted-5-[(1H-benzimidazol-2yl)methyl]azole derivatives (A1-A12) was designed. The series of 12 compounds consisted of 2,5-disubstituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives. *In silico* studies were carried out which included docking studies (using V Life software) to understand binding of the compounds with enzyme PTR1, ADMET studies, drug likeness studies for physicochemical properties and bioactivity studies to understand the possible mechanism of action of the compounds. It can be summarized that substitution on azole ring and its length have an important role in affinity to the receptor as well the ADMET properties. These results have given valuable information for further optimization of the series. A detailed study is required for further understanding relation between the nature of substituents and toxicity profile. The objective of structural modification of the previous series and studying the effect of these modifications on the binding and pharmacokinetic properties was thus accomplished. The above series will be taken

ahead for synthesis and anti-leishmanial testing. This study provides evidence for consideration of valuable ligands in 2,5-disubstituted 1,2,4-triazole and 1,3,4-thiadiazole derivatives as potential pteridine reductase 1 inhibitor and further in vitro and in vivo investigations may prove its therapeutic potential.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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