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New benzimidazole derivatives as inhibitors of Pteridine reductase 1: Design, molecular docking study and ADMET prediction

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ABSTRACT

Pteridine reductase 1 (PTR1) is a unique enzyme required for survival of *Leishmania* species, a causative organism for the disease leishmaniasis. We herein report the design, docking, and Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) prediction studies of 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl)methyl]azole derivatives (**B1–B14**) as PTR1 inhibitors. Molecular docking studies showed good binding interaction of the compounds with the active site of pteridine reductase from *Leishmania Major*, with compounds **B5** and **B12** showing docking scores of −61.5232 and −62.5897, respectively, which were comparable with the original ligand, dihydrobiopterin. Large substituents on the azole ring, as well as substitutions on sixth position of the benzimidazole ring, were found to be favorable for interaction with PTR1 active site. Physicochemical properties, bioactivity prediction, and toxicity profiles of the compounds were studied using the Molinspiration and admetSAR web servers. All compounds followed Lipinski's rule of five and can be considered as good oral candidates. Bioactivity prediction indicated that the compounds were enzyme inhibitor, thus the rationale for designing PTR1 inhibitors was met. Most of the compounds were predicted to have good ADMET properties in terms of Gastrointestinal (GI) absorption, absence of P-glycoprotein interaction, and LD$_{50}$ values in rats. The designed molecules can be further explored for their antileishmanial activity.

INTRODUCTION

The 17 neglected tropical diseases (NTDs) prioritized by the World Health Organization affect more than 1 billion people worldwide and are endemic in 149 countries (Hotez *et al*., 2020). Of these NTDs, leishmaniases are a group of diseases caused by the protozoan parasite *Leishmania*. There are around 20 *Leishmania* species that are transmitted to humans during the blood meal by infected female phlebotomine sandflies. Leishmaniasis is known to affect over 98 countries, with more than 350 million people at risk. It is estimated that 700,000–1.2 million new cases of leishmaniasis are reported per year. Visceral leishmaniasis (VL), caused by the species *Leishmania Donovani*, is the most fatal of all infections caused by different leishmanial species which is

manifested as a chronic disease in the liver and spleen. It occurs mainly in the Indian subcontinent and East Africa (Tedla *et al*., 2018). Treatment options for VL are limited. Current treatments mostly rely on drugs that date back to more than 50 years and have certain limitations, such as toxicity (sodium stibogluconate, paromomycin, and miltefosine), increased resistance (pentavalent antimonial chemotherapy), and expensive long-term treatment (Amphotericin B). Thus, the need for new agents that are safe, inexpensive, selective, and effective against resistance is both considerable and urgent. Currently, research on leishmaniasis focuses on the design of specific inhibitors directed toward particular metabolic activities which will damage parasites without affecting the host (Kaur *et al*., 2010).

For developing specific inhibitors, knowledge of the differences between infective organisms and their respective host's biochemical pathways, metabolism, and macromolecular structure, as well as detailed characterization of target proteins and macromolecules, is of primary importance (Datta *et al*., 2008). *Trypanosomatid* species have been widely studied. Their

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molecular biology, enzymology, and genome sequencing are well understood. This can help in exploring new targets for combating *trypanosomatid* infections (Schuttelkopf *et al.*, 2005).

Leishmania species are auxotrophic for folates and pterins. They depend on an exogenous source for folates and biopterins. Inhibition of the enzymes involved in the biochemical cascade can prove to be suitable targets for the treatment of leishmaniasis (Cavazzuti *et al.*, 2008). Dihydrofolate reductase (DHFR) and thymidylate synthase (TS) are the two important enzymes that reduce folates obtained by the organism from an exogenous source, which are utilized by the *leishmania* species. Along with these enzymes, Pteridine reductase 1 (PTR1), which belongs to the reductase family, catalyzes the Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH)-dependent reduction of folates and pterins. PTR1 reduces oxidized pterins to dihydrobiopterins (DHB) and further to tetrahydrobiopterin and also reduces folates to dihydrofolates and tetrahydrofolates, which are required for the growth of *Leishmania*. PTR1, thus, acts as a bypass for folate and pterine metabolism when the DHFR-TS system is inhibited. Failure of the DHFR targeting drug therapies against *trypanosomatids* is due to this enzyme (Chandrasekaran *et al.*, 2016; Kaur *et al.*, 2012). Thus, PTR1 that is responsible for providing essential elements to *leishmania* for its survival can act as an effective drug target against leishmaniasis.

This study aims at designing PTR1 inhibitors with potential antileishmanial activity. The enzyme PTR1 is a tetramer (subunits A, B, C, and D) (Gourley *et al.*, 2001). Figure 1 shows the 3D structure of the receptor bound to DHB. A series of crystallographic analysis of PTR1 from different *leishmania* species has been studied. 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 (Ferrari *et al.*, 2011; Kaur *et al.*, 2012; Kumar *et al.*, 2008; Mpamhanga *et al.*, 2009). 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. Thus, a structure containing an aromatic ring system, a hydrophobic group, and H-bond acceptors would act as a good substrate for the receptor. These interactions have been made use of to develop potential enzyme inhibitors (Dube *et al.*, 2012; Mpamhanga *et al.*, 2009; Tulloch *et al.*, 2010). Also, heterocyclic compounds, like aminobenzimidazole (Mpamhanga *et al.*, 2009; Spinks *et al.*, 2011), aminobenzothiazole (Mpamhanga *et al.*, 2009), 2,4-diaminopteridine and 2,4-diaminopyrimidine (Tulloch *et al.*, 2010), 1,3,4-thiadiazole (Ferrari *et al.*, 2011), oxadiazoles and triazoles (Cottrell *et al.*, 2004; Ferreira *et al.*, 2007; Rastogi *et al.*, 2006), pyrrolopyrimidine (Khalaf *et al.*, 2014) and pyrimido[1,2-b]pyrimidinone (Kaur *et al.*, 2011), have been studied for PTR1 inhibitory antileishmanial activity.

Blending the data obtained from literature, a series of 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl)methyl] azole derivatives (**B1–B14**) have been designed which can act as PTR1 inhibitors. Figure 2 shows the general structure of the series designed. Molecular docking studies of 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl)methyl] azole derivatives (**B1–B14**) were carried out against PTR1. The results of the docking studies gave some valuable information about the interaction of the designed compounds with the receptor. Druglikeness and Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) prediction studies were undertaken.
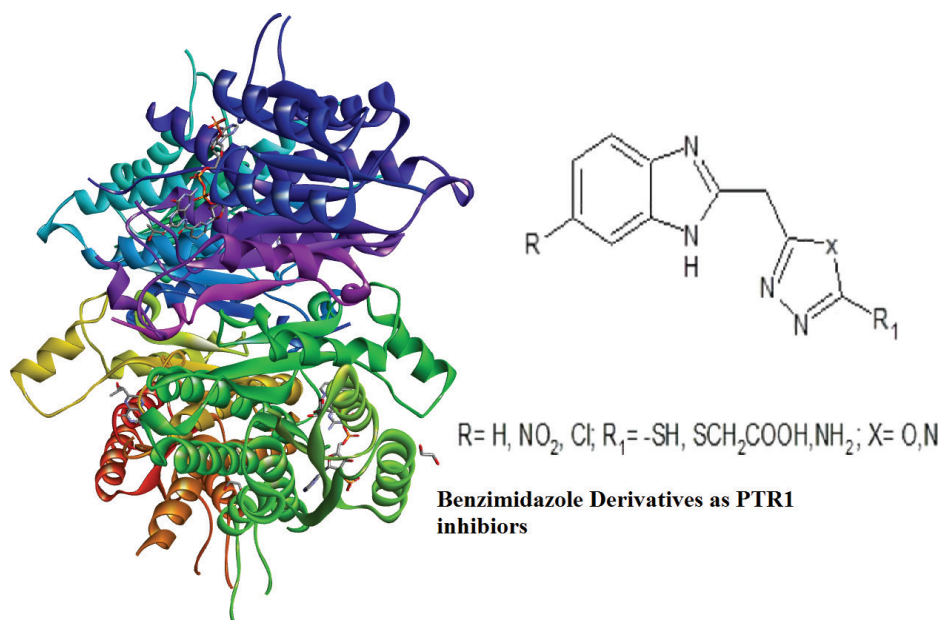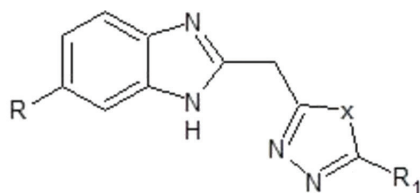

R= H, NO$_2$, Cl; R$_1$= -SH, SCH$_2$COOH, NH$_2$; X= O,N

Benzimidazole Derivatives as PTR1 inhibiors

Figure 1. 3D structure of PTR1 (PDB ID 1E92) complexed with the ligand DHB.

R= H, NO$_2$, Cl; R$_1$= -SH, SCH$_2$COOH, NH$_2$; X= O, N

Figure 2. General structure of 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl)methyl] azole derivatives.

MATERIALS AND METHODS

Molecular docking studies

Computational resources

Molecular docking simulations were used to predict the correct binding conformation of the ligand in the enzyme-binding pocket. The scoring function (dock score) comprised steric and electrostatic components of binding parameterized universal force fields. This utility allowed screening a set of compounds for lead optimization. VLifeMDS (Molecular design suite) uses genetic algorithms (GA), piecewise linear pairwise potential function, and grid algorithms to minimize the interaction energy between ligand and receptor.

All molecular modeling studies were carried out using the Molecular Design Suite (VLifeMDS software package, version 4.4; from VLife Sciences, Pune, India), molecular docking carried out by using a dell Personal Computer (PC) with a Pentium IV processor and Windows 7 operating system. Docking studies were carried out using the Ligand docking methology (GRIP) batch docking method implemented in VLifeMDS 4.4 software package.

X-ray crystal structure

The X-ray crystal structure of *L. major* PTR1 (PDB ID: 1E92) was imported from Protein Data Bank (available at http://www.rcsb.org/). The X-ray crystal structure of the PTR1 domain had a resolution of 2.2Å.

Protein preparation

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

Ligand preparation

The 2D structures of the designed molecules and the reference ligand, methotrexate, were sketched using MarvinSketch 5.11.5 and then converted to 3D structures using the VLifeMDS 4.4 software. The 3D structures were then energy-minimized to an Root mean square (RMS) gradient of 0.01 kcal/mol Å using Universal Force Field. Conformers of all the designed ligands were selected and the number of seeds used for searching the

conformational space was set as 5. All conformers were then energy-minimized to an RMS gradient of 0.01 kcal/mol Å, and then saved in separate folder.

Docking

Flexible docking algorithm was used which not only predicted the binding mode of a molecule more accurately than rigid body algorithms, but also its binding affinity relative to other compounds (Verkhivker *et al.*, 2000). All conformers were docked using an exhaustive method. The number of placements was fixed to a value of 30 and the rotation angle to a value of 15°. The docking score was used as a scoring function. By rotation angle, the ligand was rotated to obtain different poses. By placements, the method checked for all the 30 possible placements into the active site pocket and picked out few best placements out of 30. For each ligand, all the conformers with their best placements and their docking scores were saved to the output folder. The ligand forming the most stable drug receptor complex was the one which had the 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 involved hydrogen bonding, van der Waal's interaction, aromatic/π stacking, hydrophobic and other charge interactions.

Druglikeness, bioactivity prediction, and ADMET properties

The *in-silico* studies helped to determine the activity of the compound when inside the body and served as an important tool for drug discovery and lead optimization.

Molecular descriptors and druglikeliness properties of 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl)methyl] azole derivatives, DHB, and methotrexate (MTX) were analyzed using the Molinspiration tool server (http://www.molinspiration.com, accessed on 21 September 2019), which is based on Lipinski's rules of five (RO5). The pharmacokinetic properties, such as absorption, distribution, metabolism, excretion, and toxicity, of the compounds were checked using the admetSAR online database (http://lmmd.ecust.edu.cn/admetsar2/, accessed on 05 December 2019) (Cheng *et al.*, 2012). The structures were drawn using Advanced Chemistry Department (ACD)/ChemSketch version 12.0, and Simplified molecular-input line-entry system (SMILES) notation data were generated and fed into these softwares to calculate the parameters.

RESULTS AND DISCUSSION

Designing

The series was designed based on the findings reported in the literature related to 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. The striking features learnt from the literature were that H-bonding interactions and π-stacking interactions are important 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 some important points related to the active site. It was learnt that the hydrophobic region 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 compare to the thiadiazole ring system. Scaffolds, such as aminobenzimidazole and aminobenzothiazole, show selective binding to *Lm*PTR1. Taking all these studies as the basis and using the principle of bioisosterism, benzimidazole derivatives bearing a substituted azoles ring fulfilling the hydrophobic, aromatic, and hydrophilic requirements for binding in the active site of PTR1 were designed. Substitutions on the azole ring consisted of functional groups that can act as H-bond acceptors. Moreover, substitutions on the benzimidazole ring were incorporated to study the effect of these substituents on binding. 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl)   methyl] azole derivatives (**B1–B14**) are enlisted in Table 1.

Molecular docking

The elucidation of interactions between PTR1 and the designed series is crucial to check whether the compounds are able to mimic the binding mode of the substrate. Molecular docking was carried out to evaluate the interactions of designed compounds against the *L. Major* PTR1 crystal structure (PDB Code: 1E92) by using VLifeMDS software package, version 4.4. *L. major* and *L. donovani* enzymes share 91% sequence identity and homology modeling and later suggest a structural relationship in and around the active site. Docking studies showed that the designed molecules fit well in the active site pocket made up of the following 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 to the original ligand, DHB, and reference molecule, methotrexate (MTX). As per the available crystallographic data, the substrate DHB binds to *Lm*PTR1 by forming an extended network of H-bonds and aromatic interaction

with a 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 (Kaur *et al.*, 2011). The designed molecules mimic the key interactions, which include hydrogen bonding, hydrophobic, aromatic, and van der Waal's interactions. It was observed that the benzimidazole ring, the azole ring and the spacer methylene group were involved in hydrophobic interactions and aromatic π-stacking interaction majorly with amino acid residues Leu 229, Val 230, Phe 113, respectively. The hydrophilic substituents on the azole ring (−SH, −SCH$_2$COOH, −NH$_2$) formed an H-bond with the active site with residues Arg17 and Ser111 in most of the structures, which is similar to the interaction of DHB with the active site. The 2D and 3D interaction images were developed using Discovery studio visualizer v20 (Biswal *et al.*, 2019). Figures 3–6 show the 2D and 3D interactions of the original ligand (DHB), the reference ligand (MTX), compounds **B5** and compound **B12**, respectively, with the active site of PTR1.

The docking scores of the molecules (**B1–B14**) are presented in Table 2 and their interactions with the amino acids in the active site of PTR1 are listed in Table 3. Compounds **B5** and **B12** showed the lowest interaction energy of −61.5232 and −62.5897, respectively, which is comparable with the docking scores of the original ligand, DHB, which is −68.4502. It was observed that the length of substituents and H-bond acceptor functionalities on the azole ring played a crucial role in the interaction, which is reflected in the docking scores. Derivatives with a larger group, like SCH$_2$COOH, displayed good affinity when compared to −SH and −NH$_2$. The substituents on the benzimidazole ring (−Cl and −NO$_2$) were more favorable for hydrophobic interactions when compared to the unsubstituted benzimidazole derivatives.

Druglikeness, bioactivity prediction, and ADMET Studies

Absorption, distribution, metabolism, and elimination (ADME) properties play a major role in the success or failure of candidate molecules in drug development. Poor properties can limit the exposure of the compound to the target protein. Toxicity is another very important factor which often overshadows the ADME behavior. Lipinski's RO5 is useful in assessing the bioavailability of the orally administered compound. According to the rule, a molecule bearing hydrogen bond donors <5 (OH and NH groups), hydrogen bond acceptors <10 (N and O atoms), molecular weight <500, and calculated logP <5 have a great potential for oral bioavailability (Lipinski *et al.*, 2001). Other parameters that give the measure of absorption include water solubility (Log S), topographical polar surface areas (TPSA), human intestinal absorption (HIA), Caco-2 permeability, and blood–brain barrier (BBB) penetration (Ouassaf *et al.*, 2018). For distribution and transport of drugs, generally, only the unbound drug molecule is available for diffusion or transport across cell membranes and for interaction with a pharmacological target. P-glycoprotein (P-gp), an efflux membrane transporter, is widely distributed throughout the body and is responsible for limiting cellular uptake and the distribution of xenobiotic and toxic substances. Many drugs are substrates for this transporter. This transporter can impede the absorption, permeability, and

Table 1. Designed series (**B1–B14**) with the substituents.

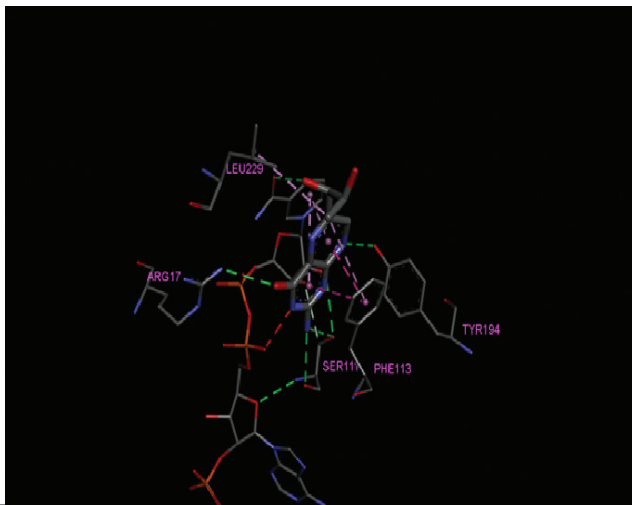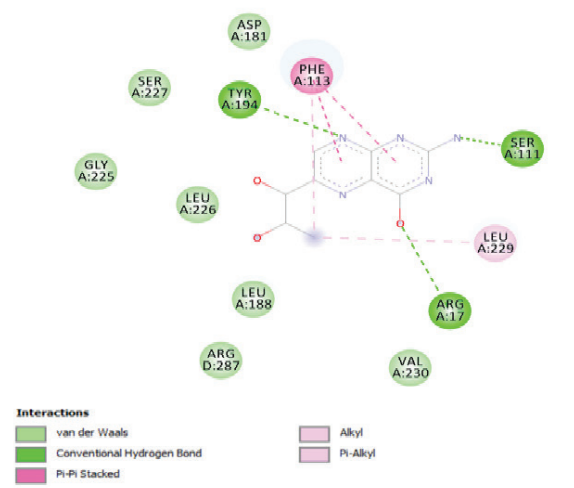| Compound	X	R	R$_1$
B1	–O	–H	–SH
B2	–O	–H	–SCH$_2$COOH
B3	–O	–H	–NH$_2$
B4	–O	–NO$_2$	–SH
B5	–O	–NO$_2$	–SCH$_2$COOH
B6	–O	–NO$_2$	–NH$_2$
B7	–O	–Cl	–SH
B8	–NH	–H	–SH
B9	–NH	–H	–SCH$_2$COOH
B10	–NH	–H	–NH$_2$
B11	–NH	–NO$_2$	–SH
B12	–NH	–NO$_2$	–SCH$_2$COOH
B13	–NH	–NO$_2$	–NH$_2$
B14	–NH	–Cl	–SH

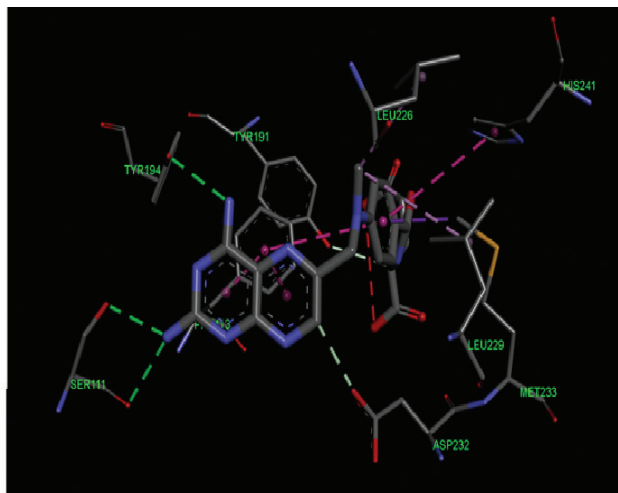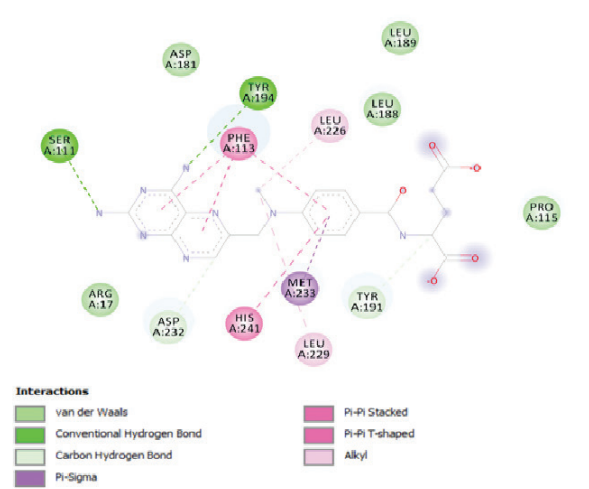**Figure 3.** 2D and 3D interactions of the original ligand DHB with active site residues of PTR1.

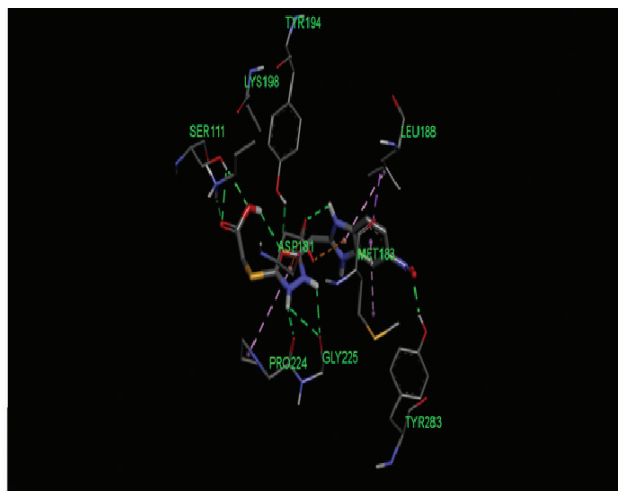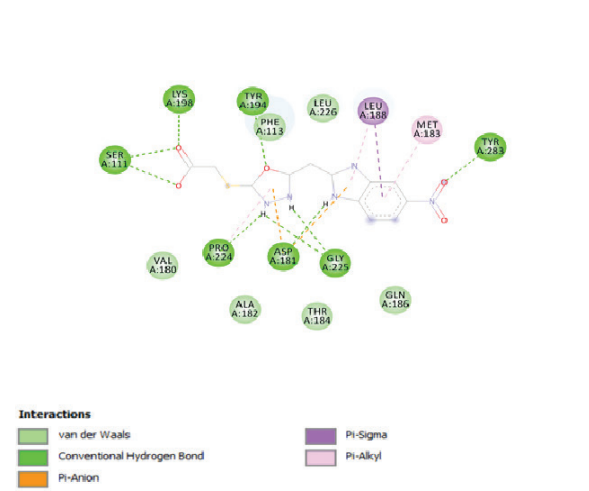**Figure 4.** 2D and 3D interactions of the reference ligand MTX with active site residues of PTR1.

Figure 5. 2D and 3D interactions of compound **B5** with active site residues of PTR1.
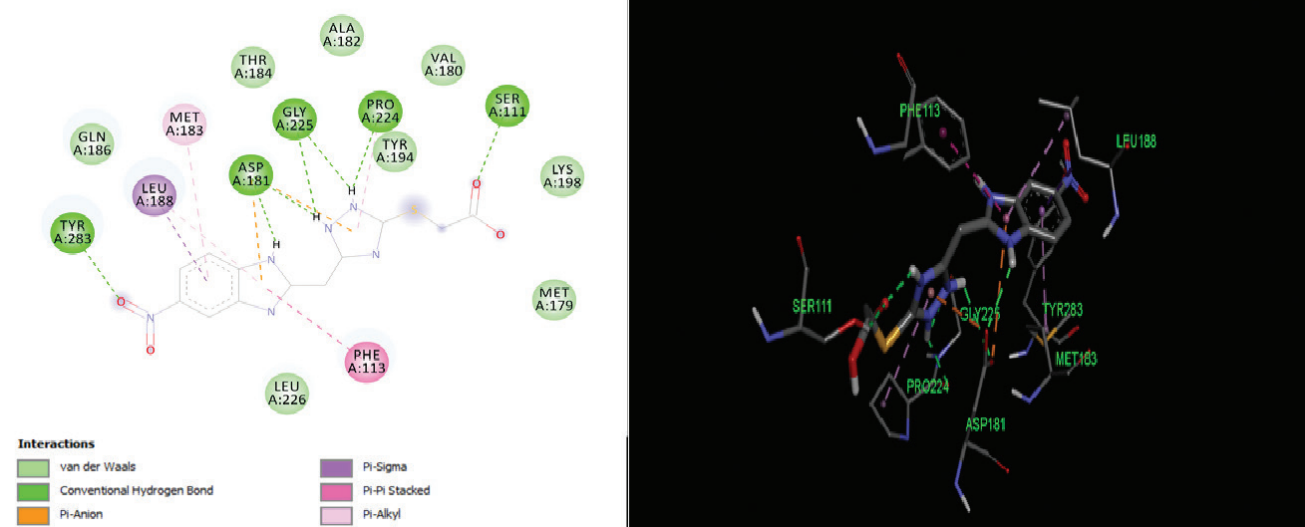
Figure 6. 2D and 3D interactions of compound **B12** with active site residues of PTR1.

Table 2. Docking scores of the designed compounds.

Compound	Dock score	Compound code	Dock score
B1	−38.1033	B9	−44.8633
B2	−46.6293	B10	−48.8593
B3	−46.9540	B11	−40.6646
B4	−44.6481	B12	−62.5897
B5	−61.5232	B13	−41.4732
B6	−41.9778	B14	−50.1494
B7	−49.3699	DHB	−68.4502
B8	−37.7739	Methotrexate	−75.6695

retention of the drugs by extruding them out of the cells (Amin, 2013). When it comes to drug metabolism, most of the drugs are metabolized by the cytochrome P450 class of enzymes. Majority of drug molecules are metabolized by the two isoforms: 2D6 and 3A4. Thus, it is necessary to know whether the designed compounds are substrates for these enzymes. At the same time, the molecules should not inhibit these enzymes as it may hinder the metabolic fate of other drugs. Toxicity profiles of the compounds can be understood by studying the mutagenicity, carcinogenicity, acute oral toxicity, and LD_{50} in rats (Belal, 2018).

The designed molecules were checked for the druglikeness (molecular properties) and bioactivity using an online server database, Molinspiration Cheminformatics software. admetSAR, a free online server, was used to predict ADMET properties. All the molecules (**B1–B14**) showed no violation from Lipinski's RO5. All compounds followed Veber's rule as they have rotatable bonds ≤10 and TPSA ≤140º (except for compounds **B5** and **B12**). It indicates that most compounds may have good oral absorption (Veber *et al.*, 2002). While the substrate (DHB) and reference standard MTX show violation from the rules, they both have negative logP values, indicating high affinity to the aqueous phase and high hydrophilicity quotient. Table 4 presents the details

of druglikeness studies by using the Molinspiration software. HIA values were found to be 0.9 and above, indicating good intestinal absorption. The Caco-2 cell permeability was found to be moderate, with some molecules having poor permeability values. 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. Log S value indicates water solubility. The lesser the log S value the greater the solubility (Nisha *et al.*, 2016). All compounds displayed log S values in the range of −3.2 to −1.8, indicating good water solubility. Overall, the compounds showed good absorption, distribution, and permeability through biological membranes.

Compounds were found to be non-substrates and non-inhibitors of the P-glycoprotein efflux transporter, and thus will not be extruded out of the cell, and the absorption and permeability of the compounds will not be impeded. Table 5 presents the absorption and distribution profiles of the series. With regard to metabolism, none of the compounds were found to be substrate or inhibitor of CYT2D6 isoform, while for CYT3A4, mixed data was obtained. Some compounds seemed to be both a substrate as well as an inhibitor of this isoform, while some were neither substrate nor inhibitor and some were either substrates or inhibitors. AMES toxicity test is employed to know whether a compound is mutagenic or not. Compounds **B1–B3**, **B8–B10**, and **B14** displayed negative AMES values, which mean they are non-mutagenic. Most of the compounds were found to be non-carcinogenic, except compounds **B4–B6** and **B13**. Mutagenicity and carcinogenicity can be attributed to the nitro substituents present in the above-mentioned compounds. All compounds showed lower oral acute toxicity than the reference MTX. LD_{50} is a dose that causes death of 50% of the test population. LD_{50} of the compounds was found to be relatively higher (ranging from 1.693 to 2.284 mol/kg) and can be considered to be safe. Table 6 presents the metabolism and toxicity data for the series obtained by the admetSAR tool.

Table 3. Interactions of the designed compounds, original substrates, and reference ligands with PTR1.

Compound	Hydrogen bond interaction	Charge interaction	Aromatic interaction	Hydrophobic interaction
Dihydrobiopterin (DHB)	Arg 17, Ser 111, Tyr 194	Nil	Phe 113	Leu 229
Methotrexate	Ser 111,Try 191, Try 194, Asp 232	Nil	Phe 113 His 241	Leu 226, Leu 229
B1	Val 230	Nil	Phe 113	Val 230
B2	Arg 17, Tyr 191	Nil	Phe 113	Leu 226, Ser 227
B3	Arg 17, Asp 232, NDP 268	Nil	Phe 113, Tyr 191	Leu 188, Leu226, Leu 229, Met 233,
B4	Arg17	Nil	Phe 113	Leu 226, Ser 227
B5	Ser 111, Asp 181, Tyr 194, Lys 198, Pro 224, Gly 225, Tyr 283	Nil	Nil	Met 183, Leu 188
B6	Arg 17, Val 230	Nil	Phe 113	Ser112, Phe 113
B7	Nil	Nil	Phe 113	Leu 229, Asp 232, Met 233
B8	Arg 17	Nil	Phe 113	Leu 229, Val230
B9	Ser 227	Nil	Phe 113	Leu 229, Val230
B10	Nil	Nil	Phe 113	Asp 232
B11	Arg 17, Phe 113 Val230	Nil	Phe 113	Ser112, Phe 113
B12	Ser111, Asp 181, Pro 224, Gly 225, Tyr 283	Nil	Phe 113	Leu 188, Leu226, Leu 229, Asp 232, Met 233,NDP 268
B13	Arg17	Nil	Phe 113	Nil
B14	Nil	Nil	Phe 113	Leu 229, Asp 232,Met 233

Table 4. Druglikeness properties of the series using the Molinspiration software.

Compound	Molecular weight	Log P	H-bond acceptor n ON	H-bond donor n OHNH	No. of rotating bond	TPSA	N violation
B1	232.27	1.52	5	1	2	67.61	0
B2	290.30	0.97	7	2	5	104.91	0
B3	215.22	0.61	6	3	2	93.63	0
B4	277.26	1.45	8	1	3	113.43	0
B5	335.30	0.90	10	2	6	150.73	0
B6	260.21	0.54	9	3	3	139.45	0
B7	266.71	2.17	5	1	2	67.61	0
B8	231.28	1.77	5	2	2	70.26	0
B9	289.32	1.22	7	3	5	107.56	0
B10	214.23	0.86	6	4	2	96.28	0
B11	276.28	1.70	8	2	3	116.08	0
B12	334.32	1.15	10	3	6	153.38	0
B13	259.23	0.79	9	4	3	142.10	0
B14	265.73	2.42	5	2	2	70.26	0
DHB	239.24	−1.65	8	6	1	136.62	1
MTX	454.45	−1.97	12	7	9	210.55	2

The bioactivity scores of the designed compounds as G-protein coupled receptor (GPCR) ligand, ion channel modulator, nuclear receptor ligand, a kinase inhibitor, protease inhibitor, and enzyme inhibitor were studied and are 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 −0.50–0.00 are expected to be moderately active and if the score is less than −0.50, it is presumed to be inactive (Ertl *et al.*, 2000). Bioactivity scores are more towards 0.0 for enzyme inhibition as compared to other mechanisms.

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

Compound	Log S	HIA	Caco-2 permeability	BBB	Human oral bioavailability	P-glycoprotein
B1	−2.808	0.9776	+0.7188	0.9757	0.6571	NS/NI
B2	−3.022	0.8992	−0.6575	0.9716	0.5143	NS/NI
B3	−2.601	0.9829	+0.6637	0.9790	0.6714	NS/NI
B4	−3.132	0.9604	+0.513	0.9742	0.6857	NS/NI
B5	−3.137	0.8995	−0.7773	0.9706	0.6000	NS/NI
B6	−2.964	0.9668	−0.5613	0.9768	0.7143	NS/NI
B7	−3.243	0.9788	−0.6198	0.9750	0.6000	NS/NI
B8	−2.494	0.9836	+0.6303	0.9745	0.5286	NS/NI
B9	−2.721	0.9174	−0.7530	0.9739	0.5429	NS/NI
B10	−1.873	0.9879	+0.6467	0.9571	0.5714	NS/NI
B11	−3.033	0.9700	+0.4034	0.9748	0.6286	NS/NI
B12	−3.072	0.9170	−0.8148	0.9730	0.6143	NS/NI
B13	−2.624	0.9751	+0.4897	0.9747	0.7000	NS/NI
B14	−3.022	0.9844	−0.5173	0.9762	0.5714	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 by using the admetSAR tool.

Compound	CYP2D6[a]	CYP3A4[a]	AMES toxicity	Carcinogenicity[a]	Acute oral toxicity	LD$_{50}$ in rats
B1	NS/NI	NS/NI	Nontoxic	NC	0.5084	2.007
B2	NS/NI	NS/NI	Nontoxic	NC	0.5564	1.875
B3	NS/NI	NS/NI	Nontoxic	NC	0.6108	1.798
B4	NS/NI	S/I	Toxic	C	0.5406	2.1
B5	NS/NI	S/NI	Toxic	C	0.5794	2.07
B6	NS/NI	S/I	Toxic	C	0.6071	1.693
B7	NS/NI	S/I	Toxic	NC	0.5950	1.935
B8	NS/NI	NS/I	Nontoxic	NC	0.4399	1.937
B9	NS/NI	NS/NI	Nontoxic	NC	0.5368	2.05
B10	NS/NI	NS/NI	Nontoxic	NC	0.6477	2.011
B11	NS/NI	S/I	Toxic	NC	0.5032	2.27
B12	NS/NI	S/NI	Toxic	NC	0.5593	2.284
B13	NS/NI	NS/I	Toxic	C	0.5464	2.148
B14	NS/NI	NS/I	Nontoxic	NC	0.4827	1.921
DHB	NS/NI	NS/NI	Nontoxic	NC	0.6048	2.051
MTX	NS/NI	S/NI	Nontoxic	NC	0.7310	3.077

[a]NS = Non-substrate; NI = Non-inhibitor; S = Substrate; I = Inhibitor; NC = Non-carcinogenic; C = Carcinogenic.

Compounds **B3**, **B6**, and **B10** exhibited bioactivity scores more than 0.00 for enzyme inhibition, thus they can be considered to exhibit significant biological activity by the above-mentioned mechanism. DHB displayed a bioactivity score of 0.34 under enzyme inhibition mechanism. The remaining compounds gave bioactivity score between −0.28 and −0.01. These scores justify the rationale behind designing the series as PTR1 inhibitor. MTX displayed positive scores in most of the heads, indicating that it exerts physiological action by different mechanisms. From the above set of studies, it was observed that compounds **B5** and **B12** exhibited the best docking scores, but are associated with potential mutagenicity and carcinogenicity. Compounds **B2**, **B3**, and **B10** have comparable docking scores and satisfactory druglikeness, bioactivity scores, and ADMET properties. It can be summarized that the substituent on the benzimidazole ring and substitution on the azole ring have an important role in affinity to the receptor, as well the ADMET properties which can be explored in the future.

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

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
B1	−0.87	−0.76	−0.41	−1.52	−0.56	−0.13
B2	−0.63	−1.01	−0.42	−1.02	−0.27	−0.18
B3	−0.25	−0.27	0.01	−0.94	−0.48	0.18
B4	−0.79	−0.67	−0.34	−1.21	−0.53	−0.18
B5	−0.66	−0.92	−0.44	−0.90	−0.32	−0.28
B6	−0.27	−0.25	0.01	−0.73	−0.47	0.08
B7	−0.73	−0.64	−0.34	−1.39	−0.53	−0.11
B8	−0.69	−0.38	−0.57	−138	−0.78	−0.22
B9	−0.40	−0.54	−0.51	−0.86	−0.28	−0.14
B10	−0.23	−0.03	−0.20	−1.32	−0.46	0.07
B11	−0.64	−0.35	−0.48	−1.09	−0.71	−0.26
B12	−0.45	−0.51	−0.51	−0.76	−0.33	−0.25
B13	−0.25	−0.05	−0.17	−1.04	−0.45	−0.01
B14	−0.56	−0.28	−0.49	−1.26	−0.73	−0.19
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

CONCLUSION

With a view to develop PTR1 inhibitors, a series of 2-substituted-5-[(6-substituted-1H-benzimidazol-2yl)methyl] azole derivatives (**B1**–**B14**) was designed. The bioactivity prediction studies substantiated the designed series as enzyme inhibitors. These compounds were subjected to docking studies which showed significant binding of the compounds with PTR1. It was observed that substituents on the benzimidazole ring (−Cl, −NO$_2$) were favorable for binding in the active site of PTR1, as seen in compounds **B5** and **B12**, but may be responsible for mutagenicity and carcinogenicity. Thus, other substituents that are devoid of the toxicity can be explored. Compounds **B2**, **B3**, and **B10** were found to possess comparable docking scores and satisfactory druglikeness, bioactivity scores, and ADMET properties, and can be considered as good oral drug candidates. In the future, based on the findings of docking studies and *in-silico* studies, the new series will be designed with structural modifications that would further improve receptor binding and toxicity profiles. The series will be synthesized and tested for antileishmanial activity.

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AUTHORS' CONTRIBUTION

Dr. Rakesh Somani, Dr. Devender Pathak, and Mrs. Shraddha Phadke contributed equally to the conception and design, critical review, statistical analysis, administrative and technical support, as well as final approval of the article. Dr. Rakesh Somani and Dr. Devender Pathak also supervised the process of article preparation. Drafting the article, data acquisition, interpretation, communication, and correspondence were conducted by Mrs. Shraddha Phadke.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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