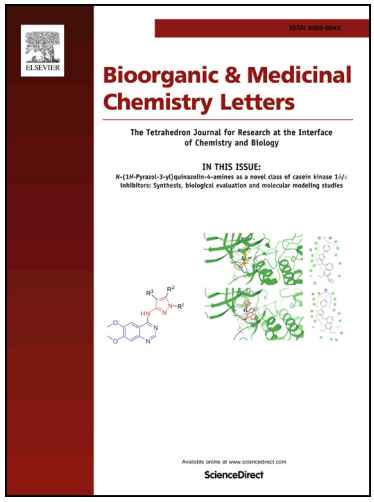
Accepted Manuscript



Antileishmanial potential of fused 5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiols: Synthesis, biological evaluations and computational studies

Sanjeev R. Patil, Ashish Asrondkar, Vrushali Patil, Jaiprakash N. Sangshetti, Firoz A. Kalam Khan, Manoj G. Damale, Rajendra H. Patil, Anil S. Bobade, Devanand B. Shinde

PII: S0960-894X(17)30655-8
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.06.053
Reference: BMCL 25087

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 17 March 2017
Revised Date: 6 April 2017
Accepted Date: 21 June 2017

Please cite this article as: Patil, S.R., Asrondkar, A., Patil, V., Sangshetti, J.N., Kalam Khan, F.A., Damale, M.G., Patil, R.H., Bobade, A.S., Shinde, D.B., Antileishmanial potential of fused 5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiols: Synthesis, biological evaluations and computational studies, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.06.053

Antileishmanial potential of fused 5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiols: Synthesis, biological evaluations and computational studies

Sanjeev R. Patil[a,b], Ashish Asrondkar[b], Vrushali Patil[b], Jaiprakash N. Sangshetti[c], Firoz A. Kalam Khan[c], Manoj G. Damale[d], Rajendra H. Patil[e], Anil S.Bobade[b], Devanand B. Shinde[f]*

[a]*Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431 004, MS, India*

[b]*Haffkine Institute for Training, Research and Testing, Parel, Mumbai-400 012, MS, India*

[c]*Y.B. Chavan College of Pharmacy, Aurangabad 431 001, MS, India.*

[d] *Shri Bhagwan College of Pharmacy, Aurangabad 431 003, MS, India.*

[e]*Department of Biotechnology, Savitribai Phule Pune University, Pune MS India*

[f] *Shivaji University, Vidyanagar, Kolhapur*-416004, MS, India

**Corresponding author E-mail: dbssanjeev2015@rediffmail.com*

Tel No.: 91-231-2609060 , Fax No.: 91-231-2692333

A series of newer 1,2,4-triazole-3-thiol derivatives **5(a-m)** and **6(a-i)** containing a triazole fused with pyrazine moiety of pharmacological significance have been synthesized. All the synthesized compounds were screened for their *in vitro* antileishmanial and antioxidant activities. Compounds **5f** (IC_{50}= 79.0 µM) and **6f** (IC_{50}= 79.0 µM) were shown significant antileishmanial activity when compared with standard sodium stilbogluconate (IC_{50}= 490.0 µM). Compounds **5b** (IC_{50}= 13.96 µM) and **6b** (IC_{50}= 13.96 µM) showed significant antioxidant activity. After performing molecular docking study and analyzing overall binding modes it was found that the synthesized compounds had potential to inhibit *L. donovani pteridine reductase 1* enzyme. In silico ADME and metabolic site prediction studies were also held out to set an effective lead candidate for the future antileishmanial and antibacterial drug discovery initiatives.

***Keywords**:* Pyrazine; 1,2,4-Triazole; Antioxidant activity; Antileishmanial activity; Cytotoxicity activity; Molecular docking, ADMET analysis.

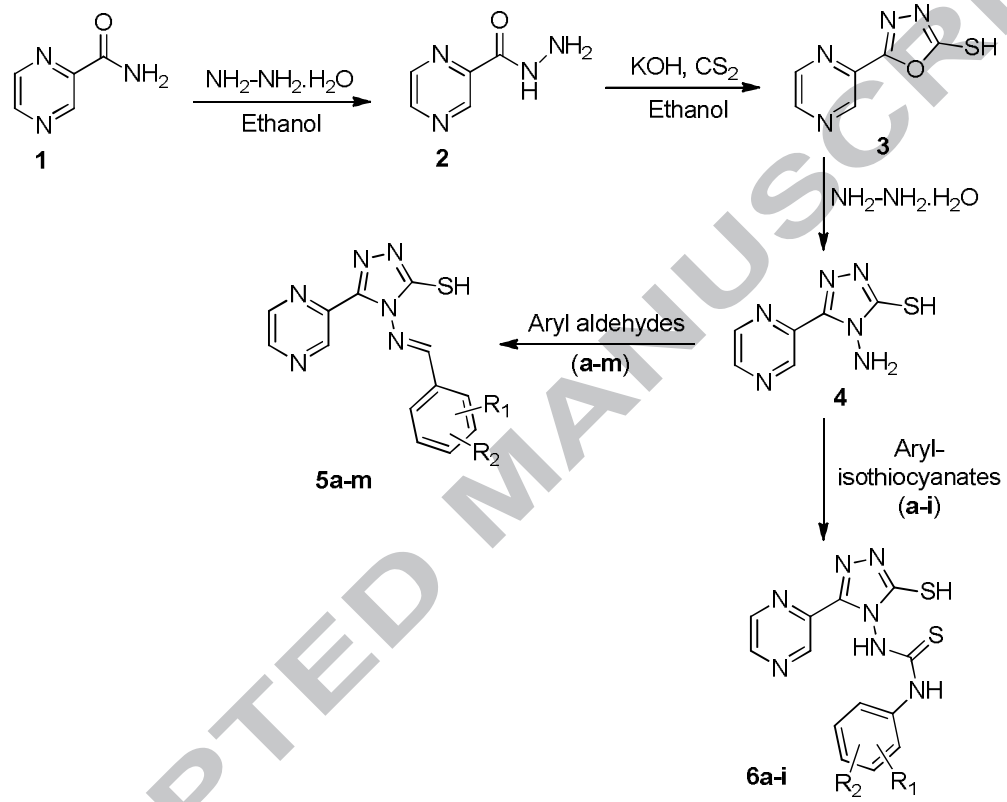
Leishmaniasis is a group of diseases caused by infection with intracellular species of the parasitic protozoan of the genus *Leishmania* with different clinical forms ranging from cutaneous leishmaniasis (CL) with skin lesions to visceral leishmaniasis (VL) with enlargement of liver, spleen, and bone marrow dysfunctions. According to the World Health Organization, leishmaniasis is an uncontrolled tropical disease with high morbidity and mortality rates in africa, asia, and the america.[1] The drugs currently in use are expensive, require long term treatment,[2] display high liver and heart toxicities, develop clinical resistance after few weeks of treatment and currently contribute to increase leishmaniasis-AIDS co-infections in some countries[3] and hence there is a need for new antileishmanials with improved efficacy and less side effects. Oxidative and free-radical-mediated processes reactions seem to play an important role in the progression of various diseases, including cancer and coronary heart disease. Radical damage has also been implicated in neurodegeneration occurring both through normal aging processes and in diseases such as Alzheimer's disease.[4] Thus, there is also a need for development of safe and effective antioxidants to prevent radical-mediated damage.

The therapeutic effects of 1,2,4-triazole containing compounds have been well studied for a number of pathological conditions including inflammation, cancer, pain, tuberculosis or hypertension. Moreover, synthesis of triazoles fused to another heterocyclic ring has attracted wide spread attention due to their diverse applications as antimicrobial,[5] molluscicidal agents,[6] antidepressant,[7] pesticides, herbicides,[8] antiviral,[9] antitumorial,[10] analgesic,[11] anti-inflammatory agents.[12] Among these, the commonly known systems are generally triazoles fused to thiadiazines, pyridines, pyridazines, pyrimidines, pyrazines and triazines. Although there are not many triazoles fused to thiadiazines, a number of them are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities such as anticancer,[13] antibacterial,[14] anti-fungal,[15] anti-inflammatory,[16] diuretic,[17] analgesic,[18] hypoglycemic[19] agents. Schiff bases are important organic compounds and well known intermediate for the preparation of azetidinone, thiazolidinone, formazone, aryl acetamide and many other derivatives. These are the compounds contain characteristic CH=N group. They are used as pigments and dyes, catalysts, intermediates in organic synthesis and as polymer stabilisers.[20] Schiff bases have also been shown to exhibit a broad range of biological activities.

In view of these marked activities of fused [1,2,4] triazole [3,4-*b*] [1,3,4] thiadiazine derivatives[21] and in continuation of our previously reported studies on heterocyclic derivatives

of pyrazinamide[22], it was contemplated to synthesize the title compounds and evaluate their biological potency.

As part of our efforts on the development of a new route for the preparation of biologically active molecules [23] and, considering the importance of biological properties of schiff base herein, we described an efficient and simple method for the synthesis of 1,2,4-triazole-3-thiol derivatives **5(a-m)** and **6(a-i)** containing triazole fused with pyrazine moiety (**Scheme 1**).



Scheme 1. Synthesis of titled compounds **5(a-m)** and **6(a-i)**

In the present study, a series of schiff bases of 1,2,4-triazole 3-thiol possessing pyrazine nucleus **5(a-m)** have been synthesized by the reaction of 4-amino-5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiol and substituted benzaldehydes.[24] The condensation between pyrazinamide (**1**) and hydrazine hydrate gave pyrazine-2-carbohydrazide (**2**) which on further cyclization with carbon disulfide in presence of base potasium hydroxide in ethanol as a solvent gave intermediate 5-(pyrazin-2-yl)-1,3,4-oxadiazole-2-thiol (**3**). The intermediate (**3**) was refluxed with hydrazine hydrate in ethanol produced key intermediate 4-amino-5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiol (**4**) which on further reaction with substituted aromatic aldehydes and

isothiocyanates and resulted in 4-(benzylideneamino)-5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiols **5(a-m)** and 1-(3-mercapto-5-(pyrazin-2-yl)-4*H*-1,2,4-triazol-4-yl)-3-phenylthiourea **6(a-i)**, respectively. The structures of all newely synthesized compounds were established by ^{1}H NMR, ^{13}C NMR, FT-IR and mass spectroscopy (Supporting information). The mass spectrum of **5f** displayed the molecular ion peak (M^{+}) peak at m/z 299, which was consistent with the product structure. The ^{1}H NMR spectrum of **5f** exhibited singlet of proton at δ 14.30 which indicates the presence of -SH group. Another singlet of one proton at δ 10.34 confirmed the formation of Schiff base (-N=C-H) proton. The peak observed at δ= 9.43 ppm confirmed the presence of the hydroxyl group. In the ^{13}C NMR spectrum of compound **5f**, characteristic peaks for C-SH and N=C-H were observed at δ 162.35 and 163.60 ppm, respectively. C-OH peak at δ 166.48 ppm indicated the presence of the hydroxyl group. The IR spectrum of **5f** exhibited characteristic absorption band at 1575.91 cm^{-1} for (C=N linkage) and all other general frequency bands were in good agreement with the structure. The physicochemical properties are as shown in **Table 1**. The isolated yields of synthesized compounds were in the range of 85.0–92.0%. Melting points were determined in open capillary tubes and are uncorrected.

Table 1. The physicochemical properties of compounds **5(a-m)** and **6(a-i)**

Entry	R_1	R_2	Mol. Formula	Mol. Wt.	Melting range (°C)	Rf value	Yield (%)
5a	*p*-OC_2H_5	*m*-OCH_3	$C_{16}H_{16}N_6O_2S$	356.4	207-210	0.62	86.5
5b	*p*-$(N(CH)_3)_2$	H	$C_{15}H_{15}N_7S$	325.39	192-194	0.74	87.0
5c	C_4H_4	*o*-OH	$C_{17}H_{12}N_6S$	332.38	220-222	0.59	91.0
5d	*o*-OH	H	$C_{13}H_{10}N_6OS$	298.32	189-192	0.64	90.5
5e	*o*-OH	*p*-OH	$C_{13}H_{10}N_6O_2S$	314.32	266-269	0.44	86.0
5f	*p*-OH	H	$C_{13}H_{10}N_6OS$	298.32	205-208	0.49	85.0
5g	*p*-F	H	$C_{13}H_9FN_6S$	300.32	178-180	0.68	89.5
5h	*p*-CN	H	$C_{14}H_9N_7S$	307.33	189-192	0.56	87.5
5i	*m*-NO_2	H	$C_{13}H_9N_7O_2S$	327.32	180-183	0.65	89.0
5j	*o*-Cl	*p*-Cl	$C_{13}H_8Cl_2N_6S$	351.21	266-269	0.73	88.0
5k	*p*-C_2H_5	H	$C_{14}H_{15}N_6S$	310.38	179-181	0.59	90.0

5l	CHO	H	$C_{13}H_{10}N_6S$	282.32	280-282	0.64	92.0
5m	*p*-Cl	H	$C_{13}H_9ClN_6S$	316.77	224-227	0.59	90.5
6a	H	H	$C_{13}H_{11}N_7S$	329.4	186-188	0.42	86.5
6b	*m*-F	H	$C_{13}H_{10}FN_7S_2$	347.39	173-176	0.52	87.0
6c	*p*-F	H	$C_{13}H_{10}FN_7OS$	331.33	228-232	0.82	91.0
6d	C_4H_4	H	$C_{17}H_{13}N_7S_2$	379.46	181-183	0.74	90.5
6e	*p*-Cl	H	$C_{13}H_{10}ClN_7S_2$	363.85	186-189	0.62	86.0
6f	*o*-Cl	*p*-Cl	$C_{13}H_9Cl_2N_7S_2$	298.32	238-242	0.54	85.0
6g	*o*-F	*p*-F	$C_{13}H_9F_2N_7S_2$	365.38	221-223	0.64	89.5
6h	*p*-CF3	H	$C_{14}H_{10}F_3N_7S_2$	397.40	198-201	0.68	87.5
6i	*m*-Cl	*p*-Cl	$C_{13}H_9Cl_2N_7OS$	382.23	222-226	0.52	89.0

Solvent of re-crystallization was ethanol; Eluents used in TLC were Ethyl acetate: n-hexane (8:2) for all compounds.

All the synthesized compounds **5(a-m)** and **6(a-i)** were tested for their *in vitro* antileishmanial activity against a culture of *Leishmania donovani promastigotes* (NHOM/IN/80/DD8) using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and sodium stibogluconate was used as standard drug.[25] Compounds **5(a-m)** and **6(a-i)** showed varying degrees of antileishmanial activities with IC_{50} ranging between 79.0 to 382.4 µM (**Table 2**). Compounds **5f**, **6f** and **5l** were found to be most promising compounds exhibiting IC_{50} value of 79.0, 79.0 and 95.2 µM, respectively when compared with sodium stilbogluconate. All the synthesized compounds showed better activity than standard sodium stibogluconate (IC_{50} = 490.0 µM) against *L. donovani promastigotes*. Structure activity relationship revealed that the activity mainly depends upon the presence of substituent on phenyl ring. In the first series **5(a-m)**, in the compound **5f** (IC_{50}= 79.0 µM), introduction of the *hydroxyl* group at the *para* position of phenyl gives most active compound, however, *fluoro* group at *para* position in compound **5g** gives least active compound with IC_{50} value 382.4 µM. From the series **6(a-i)**, the compound **6f** having the *chloro* substitution at *ortho* and *para* position exhibited excellent activity with IC_{50} value 79.0 µM, however, in compound **6e** *chloro* group at *para* position of phenyl ring displayed the least activity among all the synthesized compounds. All

other compounds from the series displays good to moderate antileishmanial activity compared to the standard.

Cytotoxic study was performed on HeLa cell line and evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[26] The cells were seen under the microscope (Ziess, Germany) at 10x magnification. The cytotoxic study against HeLa cell line of compound **5f** was carried out at concentration of 500 µg/mL. The compound **5f** had shown no cytotoxicity effect against HeLa cell lines to its tested concentration (**Figure 1**).

Table 2. Antileishmanial and antioxidant activity of compounds **5(a-m)** and **6(a-i)**

Entry	Antileishmanial IC_{50} µM	Antioxidant IC_{50} µM	Entry	Antileishmanial IC_{50} µM	Antioxidant IC_{50} µM
5a	190.0	58.80	**5l**	95.2	52.40
5b	113.0	13.96	**5m**	198.2	39.80
5c	178.0	69.18	**6a**	*	58.80
5d	102.0	75.86	**6b**	287.3	13.96
5e	106.4	75.86	**6c**	322.1	69.18
5f	79.0	58.90	**6d**	336.3	75.86
5g	382.4	58.80	**6e**	*	75.86
5h	99.0	47.80	**6f**	79.0	58.90
5i	347.1	72.40	**6g**	176.3	58.90
5j	241.3	81.20	**6h**	288.9	58.80
5k	201.1	47.70	**6i**	229.1	81.20
SS	490.0	-	-	490.0	-
AA	-	14.0	-	-	14.0

* No activity upto 500 µM; IC_{50} represents the mean values of three replicates; standard errors were all within 10% of the mean; SS: Sodium stibogluconate; AA: Ascorbic acid.

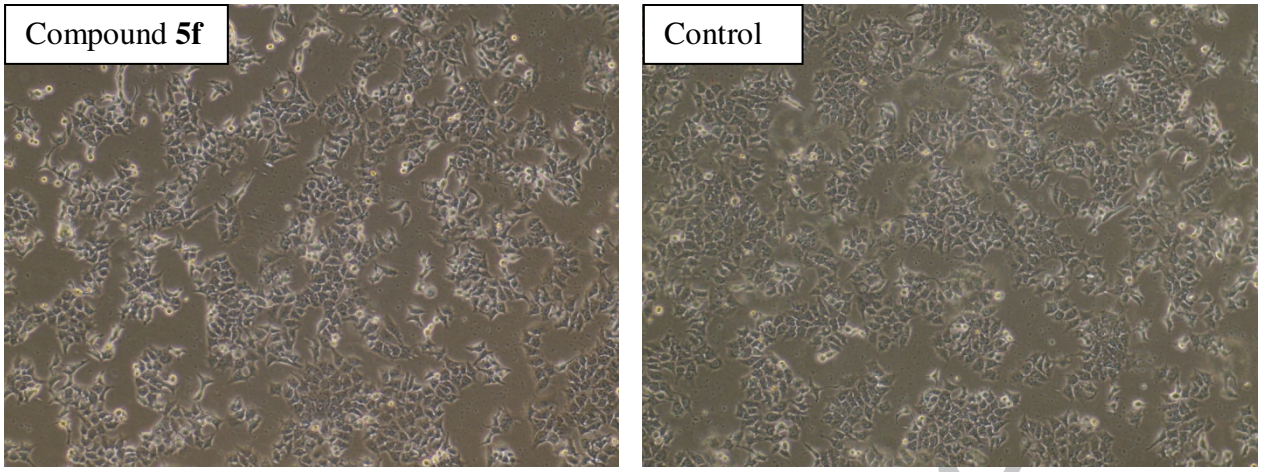


Figure 1. Cytotoxic study on HeLa cell line of compound **5f**.

All the compounds **5(a-m)** and **6(a-i)** were evaluated for antioxidant activity using free radical scavenging activity DPPH (1,1-diphenyl-2-picryl-hydrazil) method[27] and ascorbic acid was used as standard (**Table 2**). All synthesized compounds has shown high to moderate percentage of inhibition as compared to ascorbic acid. From the series **5(a-m)**, compound **5b** $N(CH_3)_2$ group at *para* position with inhibition concentration (IC_{50}) 13.96 µM found to equipotent with respect to standard ascorbic acid (IC_{50}= 14.0 µM). Compounds **5m** *chloro* group at *para* position, **5k** *ethyl* group at *para* position and **5h** *cyano* group at *para* position with IC_{50} 39.8, 47.7 and 47.8 µM, respectively, showed comparatively significant antioxidant activity with respect to standard ascorbic acid. The remaining compounds from the synthesized series **5(a-m)** showed less activity compared to the standard. Similarly, from the series **6(a-i)**, compound **6b** having *fluoro* group at *meta* position of phenyl ring with EC_{50} 13.96 µM was found to most active compound compared with standard drug ascorbic acid. Remaining all the compounds from this series was found to be less active.

Molecular docking study of synthesized compounds **5(a-m)** and **6(a-i)** were performed to understand binding interactions with using *Pteridine reductase 1* (PTR1) enzyme of *L. donovani* (PDB ID: 2XOX)[30] was performed Surflex-Dock module of Sybyl 2.1.1 package following standard procedure. Pyrazine derivatives have been reported to act as antileishmanial by inhibiting *Pteridine reductase 1* enzyme.[29] PTR1 is an essential enzyme for pterin salvage in the *Leishmania* parasite, and can potentially be used as a target in the development of improved therapies. PTR1 is a homotetramer with a subunit molecular mass of approximately 30 kDa. PTR1 is able to catalyze the two-step reduction of folate to H_2 folate and subsequently to H_4

folate. This ability to reduce folate is the same reaction catalyzed by the dihydrofolate reductase (DHFR) component of the DHFR-thymidylate synthase bifunctional polypeptide (DHFR-TS) which is the presumptive primary cell target for antiprotozoal activity. [30-33]

The synthesized compounds had shown good binding affinity as predicated by non covalent interactions such as hydrogen bond interaction, van der Waal's interaction, Pi-anion interaction, Pi-Pi shaped interaction, and alkyl interaction. To represent the details of docking score, terms like total score (total docking score), crash score (degree of inappropriate penetration by the ligand into the protein and of interpenetration between ligand atoms that are separated by rotatable bonds of compounds) and polar score gives (the contribution of the polar non-hydrogen bonding interactions to the total score) are used and presented in **Table 3**. The compounds **5(a-m)** had shown shown overall very efficient binding mode and penetrating active site cavity by forming the various interactions with active site residues such as GLU29, ASP259, VAL260, ILE262, PHE263, CYS265, SER266, SER267, LYS268. In case of most active compound **5f**, active site residue ASP259 had formed the hydrogen bond interactions with OH and SH The most active compound **5f** had shown good docking score that is 4.31. groups of aryl and triazole ring with a distance of 1.60 to 1.75Å respectively, whereas SER266 had formed the hydrogen bond interactions with heterocyclic pyrazine moiety with a distance of 1.65Å (**Figure 2**). The synthesized series **6(a-i)** had also shown good docking intections with *Pteridine reductase 1* enzyme. The most active compound **6f** had shown good docking score that is 4.29. The compounds **6(a-i)** were penetrated into active site cavity by forming the various interactions with active site residues such as GLU29, ASP259, ILE262, PHE263, SER266, SER267, and LYS268. In case of most active compound **6f**, amino acid residue ASP259 had formed the hydrogen bond interactions with SH groups of triazole ring with a distance of 1.60 Å. The aryl and pyrazine ring system had shown strong non covalent interactions such as Pi-Sigma, Pi-Pi-T-Shaped and amide Pi-stacked interactions with active site amino acids such as ILE262 and PHE263 (**Figure 2**). On the basis of activity data and molecular docking analysis, it was found that synthesized compounds had potential to inhibit *pteridine reductase 1* enzyme.

Table 3. Molecular docking stastics of synthesized compounds **5(a-m)** and **6(a-i)**

Entry	Total Score (Logki)	Crash	Polar	Entry	Total Score (Logki)	Crash	Polar
5a	3.12	-0.7790	3.19	**5l**	4.11	-4.2131	2.25
5b	3.90	-1.1928	2.60	**5m**	3.01	-4.0137	2.94
5c	3.95	-0.7190	2.65	**6a**	2.65	-2.6578	2.31
5d	3.83	-0.7051	2.70	**6b**	2.86	-2.8695	2.10
5e	3.19	-0.9636	3.35	**6c**	3.53	-4.5349	4.21
5f	4.31	-1.3329	2.97	**6d**	2.79	-2.7988	4.23
5g	3.85	-0.7980	1.94	**6e**	2.81	-2.8185	3.85
5h	3.83	-0.5621	2.27	**6f**	4.29	-2.1991	2.19
5i	3.62	-0.5467	3.41	**6g**	3.40	-3.4021	2.30
5j	4.05	-0.6038	3.27	**6h**	2.54	-2.5470	1.74
5k	3.84	-0.6622	1.84	**6i**	3.46	-3.4660	2.99

Total score: Total docking score; Crash: Degree of inappropriate penetration by the ligand into the protein and of interpenetration between ligand atoms that are separated by rotatable bonds; Polar: Contribution of the polar non-hydrogen bonding interactions to the total score.

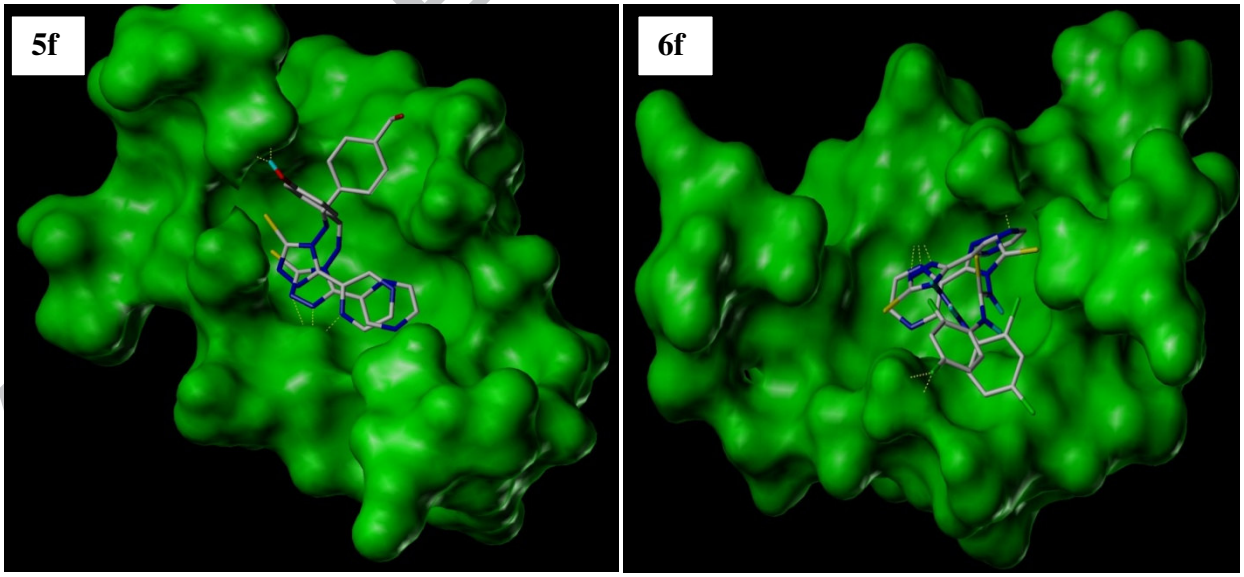


Figure 2: Binding modes of most active compounds **5f** and **6f** in the putative active site of PRT1 enzyme.

Early prediction of drug like properties of lead compounds is an important task as it decides the time and cost of drug discovery and development. The many of the active agents who has shown significant biological activity fails in clinical trials because of inadequate drug like properties. [34] The drug like properties has been predicted by analyzing absorption, distribution, metabolism, and elimination (ADME) parameters based on Lipinski's rule of five and its variants. [35,36] We had calculated and analyzed various physical descriptors and pharmaceutical relevant properties for ADMET prediction by using FAFDrugs2 and data is summarized in **Table 4**. All the compounds showed significant values for the various parameters analyzed and showed good drug-like characteristics based on Lipinski's rule of five and its variants. None of the synthesized compounds **5(a-m)** and **6(a-i)** had violated the Lipinski's rule of five its variants. The value of polar surface area (PSA), log *P* and H/C ratio of synthesized compounds had indicated for good oral bioavailability. The parameters, like number of rotatable bonds and number of rigid bonds are linked with the intestinal absorption result and showed that all synthesized compounds **5(a–m)** and **6(a-i)** had good absorption. Also, all the synthesized compounds was found to be nontoxic.

Table 4: Pharmacokinetic parameters important for agents to have good oral bioavailability of synthesized compounds **5(a-m)** and **6(a-i)**.

Entry	MW	Log *P*	PSA	n-Rot Bond	n-Rig Bond	HBD	HBA	Rings	Ratio H/C	Toxicity
5a	356.40	2.31	100.10	6	18	1	8	3	0.56	NT
5b	325.39	1.97	84.89	4	18	1	6	3	0.53	NT
5c	348.38	2.76	101.90	3	23	2	7	3	0.47	NT
5d	298.32	1.61	101.90	3	18	2	7	3	0.61	NT
5e	314.32	1.31	122.10	3	18	3	8	3	0.69	NT
5f	298.32	1.61	101.90	3	18	2	7	3	0.61	NT
5g	300.31	2.04	81.65	3	18	1	6	3	0.61	NT
5h	307.33	1.77	105.40	3	19	1	6	3	0.57	NT
5i	327.32	2.33	121.60	4	19	1	8	3	0.76	NT
5j	351.21	3.21	81.65	3	18	1	6	3	0.69	NT
5k	310.37	2.46	81.65	4	18	1	6	3	0.46	NT

5l	310.33	1.71	98.72	4	19	1	7	3	0.57	NT
5m	316.76	2.55	81.65	3	18	1	6	3	0.61	NT
6a	329.40	2.11	125.44	5	18	3	6	3	0.69	NT
6b	347.39	2.24	125.44	5	18	3	6	3	0.76	NT
6c	331.32	2.08	110.42	3	20	3	6	3	0.76	NT
6d	379.46	3.26	125.44	5	23	3	6	3	0.52	NT
6e	363.84	2.76	125.44	5	18	3	6	3	0.76	NT
6f	398.29	3.41	125.44	5	18	3	6	3	0.84	NT
6g	365.38	2.38	125.44	5	18	3	6	3	0.84	NT
6h	397.40	3.12	125.44	6	18	3	6	3	0.85	NT
6i	382.22	3.25	110.42	3	20	3	6	3	0.84	NT

MW: molecular weight; Log *P*: logarithm of partition coefficient of compound between n-octanol and water; PSA: Polar surface area; n-Rot Bond: number of rotatable bonds; n-Rig Bond: number of rigid bonds; HBA: hydrogen bond acceptors; HBD: hydrogen bond donor; and NT: Non toxic.

MetaPrint2D-React, a metabolic product predictor server developed by Unilever Cambridge, Centre for Molecular Science Informatics, University of Cambridge, UK. It is online tool that predicts fate of Xenobiotics metabolism through data-mining and statistical analysis of known metabolic transformations reported in scientific literature[37, 38]. The MetaPrint2D data is generated through processing of the transformations found in the Symyx Metabolite database previously known as MDL, for each transformation, the differences between the structures of the reactant. In order to make predictions of on the drug molecule the each atom environment is calculated and Symyx Metabolite database mining is done to search similar environment. As based on the interest of the study, only Phase I additions (defined as the addition of a single oxygen atom; covering hydroxylation, oxidation and epoxidation), and eliminations (e.g., dealkylation, ester and amide hydrolysis) are engaged [39]. For an addition, the atom neighboring the added oxygen highlighted as a reaction center. One of the most active compound **5f** (**Figure**

3) was analyzed for metabolic product prediction through an online web server of MetaPrint2D-React for prediction of possible metabolic pathways and the predicted site of metabolism prediction done for Human only. The plot of MetaPrint2D shown possible Site of metabolism of individual atom neither indicated by NOR values (Normalized Occurrence Ratio). The higher the NOR value indicates site frequently undergoes through particular metabolism phase I metabolic reactions. [40, 41] The NOR ratio for **5f** was observed as Red 0.66 ≤ NOR ≤ 1.00, Orange 0.33 ≤ NOR < 0.66, Green 0.15≤ NOR < 0.33, White (No color) 0.00 ≤ NOR < 0.15, and Grey Little/no data. **Figure 3** indicates that most of the atoms in skeleton of compound **5f** are less prone to metabolic deactivation marked by grey color.

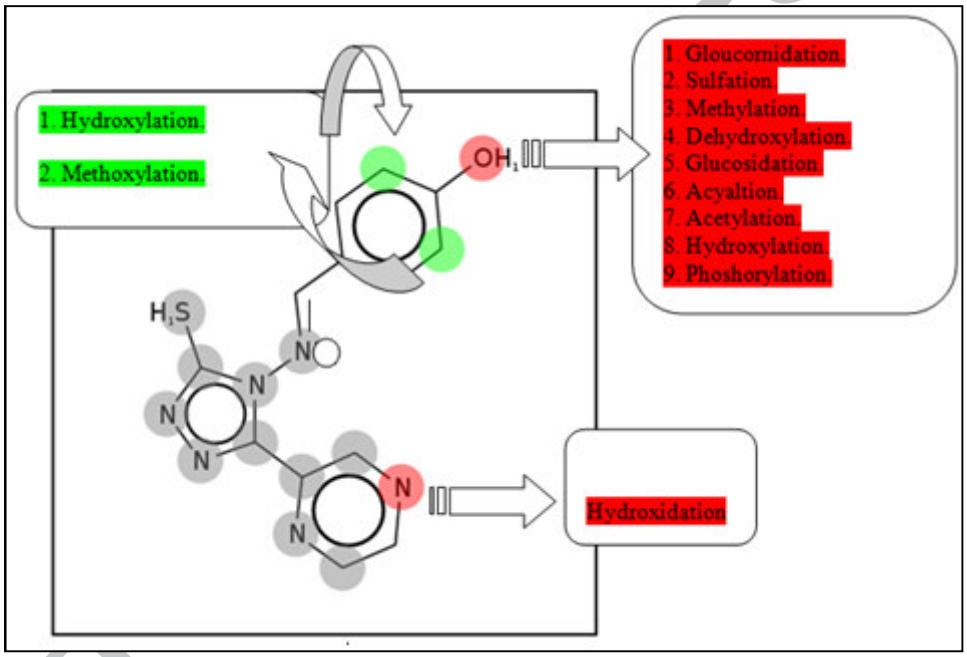


Figure 3: Metabolic/Biotransformation pathways for **5f** predicted by MetaPrint-2D React server.

In Summary, we have carried out the efficient synthesis of 5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiols **5(a-m)** and **6(a-i)**. All the synthesized compounds have been investigated for their *in vitro* antileishmanial and antioxidant activities. Among them compound **5f** and **6f** with IC_{50} = 79.0 µM exhibited excellent antileishmanial activity, whereas compound **5b** and **6b** with IC_{50} = 13.96 µM displayed excellent antioxidant activity. Compound **5f** showed no cytotoxicity to HeLa cell lines upto its highest tested concentrations 500 µg/mL. A molecular docking study suggested good binding interactions of these compounds with *Pteridine reductase 1* enzyme of *L. donovani*. In silico ADME and metabolic sites prediction studies of synthesized compounds indicated that compounds had potential to develop as good oral drug like candidate. These data

suggest that 5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiols chemical scaffold can be used as a template for further structure optimization for generating compounds with higher antileishmanial and antioxidant activity.

Acknowledgements

The authors are grateful to the Dept. Of Chemical technology, Aurangabad And Haffkine Institute, Mumbai, India for providing laboratory facilities. The authors are also thankful to the Department of Biotechnology, Savitribai Phule Pune University, Pune and Dr. Rafiq Zakaria Campus, Y.B. Chavan College of Pharmacy, Aurangabad, India for providing biological activities.

References and notes

1. World Health Organization, Leishmaniases Fact Sheet No. 116, **2000**, http://www.who.int/mediacentre/factsheets/fs116/en/.
2. Murray, H. W. *Am. J. Trop. Med. Hyg.* **2004**, *71*, 787.
3. (a) Croft, S. L.; *Trends Pharmacol. Sci.* **1988**, *9*, 376; (b) Berman, J. D. *Rev. Infect. Dis.* **1988**, *10*, 560.
4. (a) Gey, K. F. *Biochem. Soc. Trans.* **1990**, *18*, 1041; (b) Smith, M. A.; Perry, G.; Richey, P. L. L.; Sayre, M.; Anderson, V. E.; Beal, M. F.; Kowal, N. *Nature* **1996**, *382*, 120; (c) Diaz, M. N.; Frei, B.; Vita, J. A.; Keaney, J. F. *N. Engl. J. Med*. **1997**, *337*, 408.
5. Griffin, D. A.; Mannion, S. K. *Chem. Abstr*. **1987**, *106*, 98120u.
6. Shehry, M. F.; El Abu-Hashem, A. A.; El-Telbani, E. M. *Eur. J. Med. Chem*. **2010**, *45*, 1906.
7. Chiu, S. H. L.; Huskey, S. E. W. *Drug MetabDispos*. **1998**, *26*, 838.
8. Chiara, B. V.; Maurizio, M.; Augusto, C. V.; Mario, G. *J. Heterocyclic. Chem*. **1998**, *35*, 29.
9. Abdel-Rahman, F.; Clercq, E. D.; El-Kashefa, H. *ARKIVOC* **2006**, *X*, 137.
10. Demirbas, N.; Demirbas, U. A.; *Bioorg. Med. Chem*. **2002**, *10*, 3717.
11. Turan-Zitouni, G.; Kaplancikli, Z. A.; Erol, K.; Kilic, F. S. *Farmaco*. **1999**, *54*, 218.
12. Holla, B. S.; Rao, B. S.; Sarojini, B. K.; Akberali, P. M.; Kumari, N. S. *Eur. J. Med. Chem*. **2006**, *41*, 657.
13. Maxwell, J. R.; Wasdahl, D. A.; Wolfson, A. C.; Stenberg, V. *J. Med. Chem*. **1984**, *27*, 1565.
14. Sahar, M. I.; Badr, Rasha, M.; Barwa, *Bioorg. Med. Chem*. **2011**, *19*, 4506.
15. Hui, X. P.; Zhang, L. M.; Zhang, Wangb, Z. Y. J. Q.; Wang, F. *Chin. Chem. Soc*. **2000**, *47*, 535.
16. Ray, W. A.; Stein, C. M.; Hall, K.; Daugherty, J. R.; Griffin, M. R. *Lancet* **2002**, *359*, 118.
17. Hester, J. B.; Lu dens, J. H.; Emmert, D. E.; West, B. E. *J. Med. Chem*. **1989**, *32*, 1157.
18. Turan, Z. G.; Kaplancikli, Z. A.; Erol, K. *Farmaco*. **1999**, *54*, 218.
19. Holla, B. S.; Veerndra, B.; Shivananda, M. K.; Poojary, B. *Eur. J. Med. Chem*. **2003**, *38*, 759.
20. Dhar, D. N.; Taploo, C. L. *J. Sci. Ind. Res*, **1982**, *41*, 501.

21. Prasad, A. R.; Ramalingam, T.; Rao, A. B. *Eur. J. Med. Chem*. **1989**, *24*, 199.
22. (a) Vijaya Raj, K. K.; Narayana, B.; Ashalatha, B. V.; Suchetha, K. N. *Eur. J. Med. Chem*. **2007**, *42*, 425; (b) Vijaya Raj, K. K.; Narayana, B. *Phosphorus, Sulfur, Silicon Relat. Elem.* **2006**, *181*, 971; (c) Chidananda, N.; Poojary, B.; Sumangala, V.; Suchetha K. N.; Shetty, P.; Arulmoli, T. *Eur.J. Med. Chem*. **2012**, *51*, 124; (d) Chidananda, N.; Poojary, B.; Shettya, Sumangala, V.; Shenoy, S.; Shetty, P.; Arulmoli, T.; *Med.Chem. Res*. **2013**, *22*, 253.
23. (a) Sangshetti, J. N.; Shinde, D. B. *Eur. J. Med. Chem*. **2011**, *46*, 1040; (b) Sangshetti, J. N.; Khan, F. A. K.; Kulkarni, A. A.; Patil, R. H.; Pachpinde, A. M.; Lohar, K. S.; Shinde, D. B. *Bioorg. Med. Chem. Lett*. **2016**, *26*, 829 (c) Sangshetti, J. N.; Lokwani, D. K.; Chouthe, R. S.; Ganure, A.; Raval, B.; Khan, F. A. K.; Shinde, D. B. *Med. Chem. Res*. **2014**, *23*, 4893; (c)Kokare, N.D.; Sangshetti, J.N.;Shinde, D.B. *Chinese Chem. Lett.***2007**, *18*, 1309; (c) Sangshetti, J. N.;Lokwani, D.K.;Sarkate,A.P.; Shinde, D. B. *Chem. Biol. & Drug. Des.* **2011**, *78*, 800.
24. Kawade, D. P.; Khedekar, P. B. *Der. Pharma. Chemica*. **2012**, *5*, 1856.
25. Dutta, A.; Bandyopadhyay, S.; Mandal, C.; Chatterjee, M. *Parasitol. Int*. **2005**54, *54*, 119.
26. Denizlt, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271.
27. (a) Srivastava, B. K.; Solanki, M.; Mishra, B.; Soni, R.; Jayadev, S.; Valani, D.; Jain, M.; Patel, P. R. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 1924; (b) Sycheva, T. P.; Pavlova, T. N.; Shehukina, M. N. *Khimike-Farmatserti cheskii Journal*, **1972**, *6*, 6; (c) Lohray, B. B.; Srivastava, V. B.; Kapadnis, B. K.; P. B.; Pandya, P. *Bioorg. Med. Chem.* **2004**, *12*, 4557.
28. Barrack, K.L.; Tulloch, L.B.; Burke, L.A.; Fyfe, P.K.; Hunter, W.N. *Acta Crystallogr. Sect. F*, **2011**, *67*, 33.
29. (a) Gilbert, I. H. *Biochim. Biophys. Acta* **2002**, *1587*, 249; (b) Khan, S.; Khan, I.; Chauhan, P.M.S. *Chem. Biol. Interface* **2015**, *5*, 1.
30. Gao, J.; Liang, L.; Zhu, Y.; Qiu, S.; Wang, T.; Zhang, L. *Int. J. Mol. Sci*. **2016**, *17*, 1141.
31. Kazemi, B.; Tohidi, F.; Bandehpour, M.; Yarian, F. *Iran. Biomed. J.* **2010***, 14*, 97.
32. Gourley, D. G.; Luba, J.; Hardy, L. W.; Beverley S. M.; Hunter, W. N. *Acta Cryst*. **1999**, *D55*, 1608.
33. Sangshetti, J.N.; Khan, F.A.K.; Kulkarni, A.A.; Patil, R.H.; Pachpinde, A.M.; Lohar, K.S.; Shinde, D.B. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 829.

34. Sangshetti, J.N.; Khan, F.A.K.; Chouthe, R.S.; Damale, M.G.; Shinde, D.B. *Chinese Chem. Lett.* **2014,** *25*, 1033.
35. Lagorce, D.; Sperandio, O.; Galons, H.; Miteva, M.A.; Villoutreix, B.O. *BMC Bioinformatics* **2008**, *9*, 396.
36. Ertl, P.; Rohde, Bernhard, Selzer, P. *J. Med. Chem*. **2000**, *43*, 3714.
37. Boyer, S.; Zamora, I. *J. Comput. Aided Mol. Des.* **2002**, *16***,** 403.
38. Carlsson L.; Spjuth O.; Adams, S.; Glen, R.; Boyers, S. *BMC Bioinfo*rmatics **2010**,*11*, 362.
39. Boyer, S.; Arnby, C.H.; Carlsson L.; Smith, J.; Stein, V.; Glen,R.C. *J. Chem. Inf. Model.* **2007,** *47,* 583.
40. Braga, R.C.; Alves, V.M.; Fraga, C.M.; Barreiro, E.J.; de Oliveira, V.; Andrade, C.H. *J. Mol.Model*, **2012**, *18*, 2065.
41. Khan, F.A. K.; Zaheer, Z.; Sangshetti, J.N.; Patil, R.H.; Farooqui, M. *Bioorg Med. Chem. Lett.* **2017**, *27*, 567.

Captions

Scheme 1. Synthesis of titled compounds **5(a-m)** and **6(a-i)**

Table 1. The physicochemical properties of compounds **5(a-m)** and **6(a-i)**

Table 2. Antileishmanial and antioxidant activity of compounds **5(a-m)** and **6(a-i)**

Table 3. Molecular docking stastics of synthesized compounds **5(a-m)** and **6(a-i)**

Table 4. Pharmacokinetic parameters important for agents to have good oral bioavailability of synthesized compounds **5(a-m)** and **6(a-i)**

Figure 1. Cytotoxic study on HeLa cell line of compound **5f**

Figure 2. Binding modes of most active compounds **5f** and **6f** in the putative active site of PRT1 enzyme.

Figure 3. Metabolic/Biotransformation pathways for **5f** predicted by MetaPrint-2D React server

Graphical Abstract

Antileishmanial potential of fused 5-(pyrazin-2-yl)-4*H*-1,2,4-triazole-3-thiols: Synthesis, biological evaluations and computational studies

Sanjeev R. Patil[a,b], Ashish Asrondkar[b], Vrushali Patil[b], Jaiprakash N. Sangshetti[c], Firoz A. Kalam Khan[c], Manoj G. Damale[d], Rajendra H. Patil[e], Anil S.Bobade[b], Devanand B. Shinde[f]*

[a]*Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431 004, MS, India*

[b]*Haffkine Institute for Training, Research and Testing, Parel, Mumbai-400 012, MS, India*

[c]*Y.B. Chavan College of Pharmacy, Aurangabad 431 001, MS, India.*

[d] *Shri Bhagwan College of Pharmacy, Aurangabad 431 003, MS, India.*

[e]*Department of Biotechnology, Savitribai Phule Pune University, Pune MS India*

[f] *Shivaji University, Vidyanagar, Kolhapur*-416004, MS, India

**Corresponding author E-mail: dbssanjeev2015@rediffmail.com*

Tel No.: 91-231-2609060, Fax No.: 91-231-2692333