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Synthesis, antileishmanial activity and docking study of *N′*-substitutedbenzylidene-2-(6,7-dihydrothieno[3,2-*c*] pyridin-5(4*H*)-yl)acetohydrazides

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ABSTRACT

A series of *N′*-substitutedbenzylidene-2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetohydrazide derivatives is synthesized and evaluated for antileishmanial activity against *Leishmania donovani* promastigotes. Compounds **9a** and **9i** were shown significant antileishmanial when compared with standard sodium stilbogluconate. Antimicrobial study revealed that compound **9b** has potent as well as broad spectrum antibacterial activity when compared with ampicillin and compound **9e** showed promising antifungal activity when compared with miconazole. Also, none of the synthesized compounds showed cytotoxicity up to tested concentration. Further, docking study against pteridine reductase 1 enzyme of *L. donovani* showed good binding interactions. ADME properties of synthesized compounds were also analyzed and showed potential to develop as good oral drug candidates.

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[3,4] and hence there is a need for new antileishmanials with improved efficacy and less side effects. Also, there are several classes of antimicrobial agents are available and used for clinical treatment, their advances in medical care are threatened by a natural phenomenon known as 'drug resistance'.[5,6] This has created an urgent need to devote our continuous efforts for the discovery and development of new antimicrobials with broader spectrum of activity and lower toxicity.[7,8]

Thieno[3,2-*c*]pyridine compounds are known to possess diverse range of pharmacological activities such as antimicrobials,[9–13] antiarrhythmic activity,[14] antiplatelet agent[15] and antidiabetic activity.[16] Compounds containing acetohydrazide group are reported to posses various pharmacological activities like antimicrobial,[17–21] anticancer,[22,23] anticonvulsant[24,25] and antileishmanial.[26] To reduce the economical burden of developing new antileishmanial drugs from scratch and limited understanding of leishmanial biology, a current strategy is to study drugs known to possess anti-infective activity.[27,28] Many leishmanicidal drugs in distinct phases of development are derived from this approach, including some in current clinical use such as the amphotericin B[29] (antifungal) and paromomycin[30] and sitamaquine[31] (antibacterial). Structures of some anti-infective agents bearing thieno[3,2-*c*]pyridine ring and acetohydrazide chain reported in literature are presented in Figure 1.

Taking into account all of the aforementioned and due to the urgent need for innovative drugs based on new molecular scaffolds, and as an extension of our earlier work on thieno[3,2-*c*]pyridine derivatives[9,10] and the increased interest on new antileishmanial agents due to the lack of effective drugs, we decided to synthesize and test the efficiency of *′N′*-substitutedbenzylidene-2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetohydrazides **9**(**a**–**j**) for antimicrobial and antileishmanial activity. The computational parameters like docking study for

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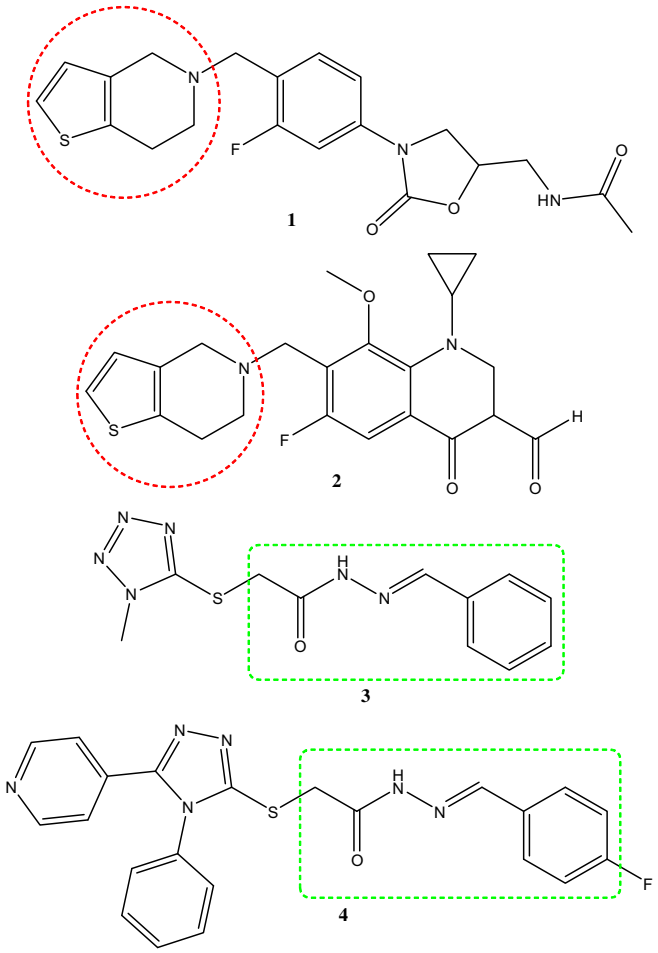


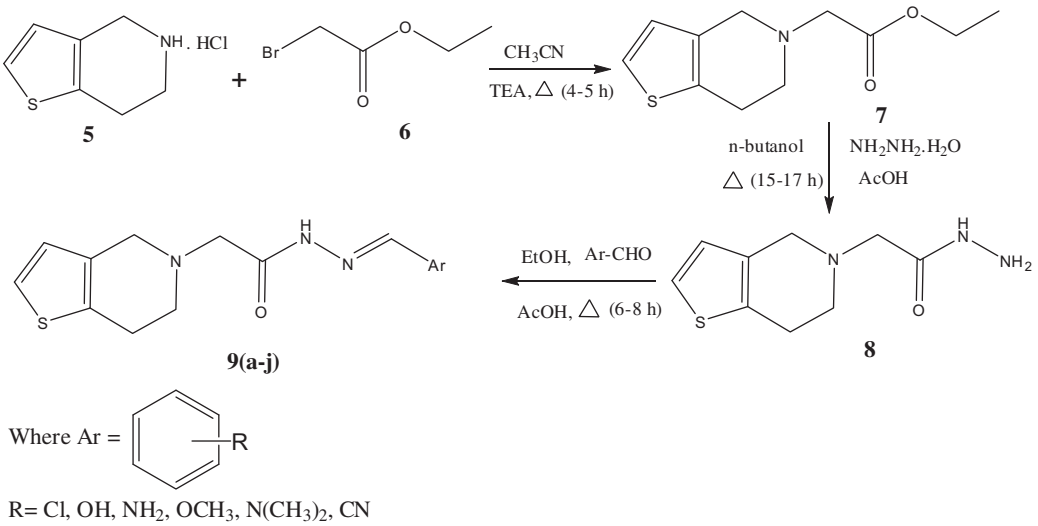
Figure 1. Structures of some anti-infective agents reported in literatures **1**–**4**.

antileishmanial and ADME prediction of synthesized compounds were also performed. The results suggest that the compound could be exploited as an antileishmanial drug.

The synthetic protocols employed for the synthesis of *N*′-substitutedbenzylidene-2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetohydrazides **9**(**a**–**j**) are presented in Scheme 1. The ethyl-2(6,7-dihydrothieno[3,2-*c*]pyridine-5(4*H*)-yl)acetate (**7**) was obtained via reaction of 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine hydrochloride (**5**) with ethyl-2-bromoacetate (**6**) using triethyl amine as catalyst. Ethyl-2(6,7-dihydrothieno[3,2-*c*]pyridine-5(4*H*)-yl)acetate (**7**) was reacted with hydrazine hydrate to give the compound 2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetohydrazide (**8**).[9]

Further, to expand the series, *N*′-substitutedbenzylidene-2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetohydrazides **9**(**a**–**j**) were prepared reacting the compound (**8**) with various substituted aromatic aldehydes in ethanol using glacial acetic acid as catalyst. The physical data of the synthesized compounds are presented in Table 1. All the reactions proceeded well in 6–8 h to give products in very good yields (74–90%). The purity of the synthesized compounds was checked by TLC and melting points were determined in open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. All synthesized derivatives **9**(**a**–**j**) were characterized using IR, ^{1}H NMR, ^{13}C NMR and Mass spectra.

The title compounds **9**(**a**–**j**) were tested for their in vitro antileishmanial activity against a culture of *Leishmania donovani* promastigotes (NHOM/IN/80/DD8). Parasite viability was evaluated using a modified 3-(4,5-dimethylthiazol-2 yl)-2,5-diphenyl tetrazolium bromide (MTT) assay wherein the amount of formazan produced is directly proportional to the number of metabolically active cells.[32] The concentration that decreased cell growth by 50% (IC_{50}) was determined by graphic interpolation and data obtained depicted in Table 2. Sodium stibogluconate and pentamidine were used as standard drugs. Compounds **9**(**a**–**j**) showed varying degrees of antileishmanial activities with IC_{50} ranging between 93.75 and 265 μg/mL. Amongst all tested compounds **9a** and **9i** were found to be most promising compounds showing IC_{50} value of 98.75 μg/mL and 93.75 μg/mL, respectively when compared with sodium stilbogluconate. All the synthesized compounds showed better activity than standard sodium stibogluconate (IC_{50} = 490 μg/mL) against *L. donovani* promastigotes. Structure activity relationship revealed that the activity mainly depends upon the presence of substituent on phenyl ring. If we compare the activity of most active member of the series, compounds with 2-chloro **9a** and 4-*N*,*N*-dimethylamino substituted phenyl ring **9i** showed significant antileishmanial activity against *L. donovani* promastigotes. Introduction of 4-chloro **9b** or 2,6-dichloro **9c** on phenyl ring showed decrease in activity. Substitution of methoxyl group on various position of phenyl ring contributed no significant



Scheme 1. Synthetic protocol for title compounds.

Table 1
Physical data for *N*′-substitutedbenzylidene-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)acetohydrazide derivatives **9**(**a**–**j**)

Entry	Ar	Molecular formula	Yield (%)	R_f value	Mp (°C)
9a	2-Chlorophenyl	$C_{16}H_{16}ClN_3OS$	88	0.62	138–140
9b	4-Chlorophenyl	$C_{16}H_{16}ClN_3OS$	80	0.74	158–160
9c	2,6-Dichlorophenyl	$C_{16}H_{15}Cl_2N_3OS$	76	0.59	220–222
9d	3,4-Dihydroxyphenyl	$C_{16}H_{17}N_3O_3S$	86	0.64	148–150
9e	2,4-Dimethoxyphenyl	$C_{18}H_{21}N_3O_3S$	82	0.44	200–202
9f	2,5-Dimethoxyphenyl	$C_{18}H_{21}N_3O_3S$	86	0.49	160–162
9g	3,4-Dimethoxyphenyl	$C_{18}H_{21}N_3O_3S$	88	0.68	208–210
9h	2-Amino-6-methoxy phenyl	$C_{17}H_{20}N_4O_2S$	90	0.56	146–148
9i	4-*N*,*N*-Dimethylaminophenyl	$C_{18}H_{22}N_4OS$	82	0.65	158–160
9j	4-Cyanophenyl	$C_{17}H_{16}N_4OS$	74	0.73	168–170

Solvent of recrystallization was ethanol; eluants used in TLC were petroleum ethyl acetate/*n*-hexane (8:2) for all compounds.

Table 2
In vitro antileishmanial evaluation and molecular docking statistics of synthesized compounds **9**(**a**–**j**)

Entry	*L. donovani* (IC_{50})		Docking result against *L. donovani* (Pteridine reductase 1)	
	μg/mL	μM	H-bond	Binding energy (kcal/mol)
9a	98.75	295.80	SER185-NH; SER185N=CH	−67.16
9b	246.25	737.62	SER185-NH	−53.75
9c	237.50	644.88	*	−50.58
9d	223.75	672.16	VAL180-OH; ASP181-OH	−52.13
9e	265.00	737.25	*	−53.32
9f	247.50	688.57	*	−54.11
9g	222.50	619.01	LYS16-OCH_3	−51.62
9h	248.75	722.20	*	−46.95
9i	93.75	273.75	LYS16-$N(CH_3)_2$; SER185O=C	−70.76
9j	225.00	693.58	LEU18-NH	−51.55
STD1	490.00	537.92	ND	ND
STD2	5.5	16.15	ND	ND

IC_{50} represents the mean values of three replicates; standard errors were all within 10% of the mean.
* Denotes no hydrogen bond interaction of ligands with protein; STD1: sodium stibogluconate; STD2: pentamidine; ND: not done.

activity. A representation of the effect of compounds on the *L. donovani* was as shown in Figure 2. In presence of compound, the organism loses its viability as seen by irregular shape morphology of the same. Cytotoxicity study on HeLa cell line was evaluated as per reported procedure[33] for synthesized compounds and none of the compounds showed cytotoxicity at concentration up to 300 μg/mL (Fig. 2).

The antibacterial activity was evaluated against two Gram-negative bacteria namely, *Escherichia coli* (NCIM-2256) and *Pseudomonas aeruginosa* (NCIM-2036) and two Gram-positive bacteria namely, *Staphylococcus aureus* (NCIM-2901) and *Bacillus subtilis* (NCIM-2063) using ampicillin and ciprofloxacin as standard drugs. The antifungal activity was evaluated against two fungal strains *Candida albicans* (NCIM-3471) and *Aspergillus niger* (NCIM-1196) using miconazole and fluconazole as standard drugs. Minimum inhibitory concentration (MIC) values for antibacterial and antifungal were determined using standard agar method.[34–36] Dimethyl sulfoxide was used as solvent control. MIC values of the tested compounds are presented in Table 3.

From the antibacterial activity data, comparison of antibacterial activity of synthesized compounds with that of ampicillin (MIC = 100 μg/mL), showed that compounds **9a**, **9b**, **9c**, **9d** and **9i** (MIC range = 25–50 μg/mL) against *E. coli* strain and compound **9b** (MIC = 50 μg/mL) against *P. aeruginosa* strain were most active. Compound **9j** (MIC = 100 μg/mL) was equipotent with ampicillin against *E. coli*. On comparison of compounds with ciprofloxacin (MIC = 25 μg/mL), compounds **9a** and **9b** (MIC = 25 μg/mL) showed equipotent activity against *E. coli*. The compounds **9a**, **9b**, **9e**, **9f** and **9g** (MIC range = 50–100 μg/mL) were found to be most active when compared with ampicillin (MIC = 250 μg/mL) against *S. aureus* and *B. subtilis* strains. Compound **9b** showed equipotent activity against *S. aureus* when compared with ciprofloxacin (MIC = 50 μg/mL). Compounds **9a**, **9b** and **9c** revealed broad spectrum activity comparable to ampicillin. Compound **9d** had shown selectivity for Gram-negative bacteria. Modification of the parent compounds with various substituents such as halogen, hydroxyl, methoxyl, amino and cyano were performed to explore the structure–activity relationships (SAR) of theses thieno[3,2-*c*]pyridine derivatives containing acetohydrazide linkage. As observed from activity data, compounds **9a**, **9b**, **9c** and **9j** with electron withdrawing substituents at phenyl ring are more effective than compounds **9d**, **9e**, **9f**, **9g**, **9h** and **9i** with electron donating substituents against Gram-negative bacteria. Replacement 2-Cl **9a** with 4-Cl **9b** at phenyl ring increases the antibacterial activity. Introduction of 2,6-dichloro group **9c** decreases the activity.

The results of in vitro antifungal activities (Table 3) showed that synthesized compounds **9**(**a**–**j**) have moderate to good activity. Comparison of antifungal activity of compounds with that of antifungal drug miconazole (MIC = 25 μg/mL), showed that compound **9e** and **9f** (MIC = 25 μg/mL) had same antifungal profile against *C. albicans*. Compound **9g** (MIC = 12.5 μg/mL) had shown equipotent activity against *A. niger* when compared with miconazole (MIC = 12.5 μg/mL). Compounds **9g** (MIC = 37.5 μg/mL) and **9i** (MIC = 40 μg/mL) had shown moderate activity against *C. albicans* when compared with miconazole. All the synthesized compounds were found less active against *C. albicans* and *A. niger* when compared with fluconazole. Structure–activity relationship of compounds **9**(**a**–**j**) revealed that scaffold containing 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine and acetohydrazide shows considerable antifungal activity. As observed through data analysis, the compounds **9d**, **9e**, **9f**, **9g**, **9h** and **9i** with electron donating groups on phenyl ring are more effective than compounds **9a**, **9b**, **9c** and **9j** with electron withdrawing groups except for the compound **9f** where activity has reduced against *A. niger* organism. Introduction

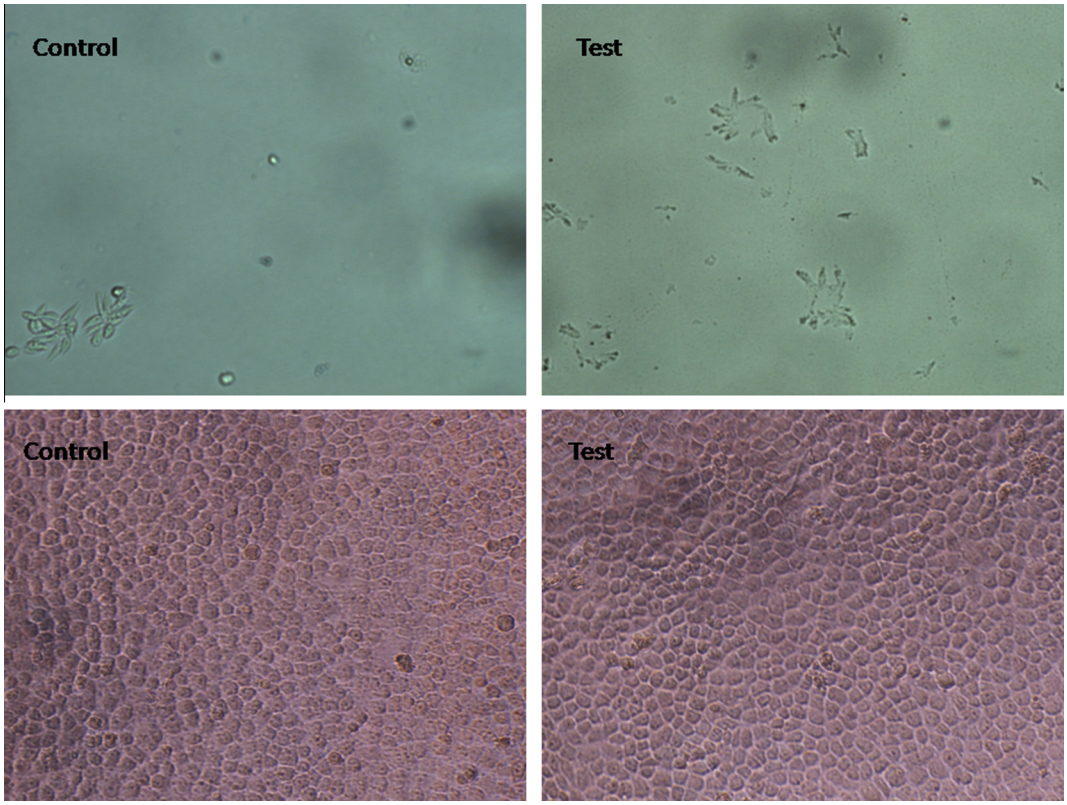


Figure 2. Antileishmanial activity (upper panel) and cytotoxic study on HeLa cell line (lower panel) of compound **9i**.

Table 3
In vitro antimicrobial evaluation of synthesized compounds **9**(**a**–**j**)

Entry	Antibacterial activity (MIC values in μg/mL)				Antifungal activity (MIC values in μg/mL)	
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
9a	25	125	100	100	60	120
9b	25	50	50	100	100	100
9c	50	150	250	250	80	160
9d	50	150	*	*	75	75
9e	125	250	75	100	25	50
9f	125	250	75	100	25	150
9g	150	200	150	150	37.5	12.5
9h	175	*	275	*	60	140
9i	50	*	100	*	40	100
9j	100	150	250	*	80	140
Ampicillin	100	100	250	250	—	—
Ciprofloxacin	25	25	50	50	—	—
Miconazole	—	—	—	—	25	12.5
Fluconazole	—	—	—	—	5	10

* No activity found up to 300 μg/mL; — denotes not tested; the data represents the mean values of three replicates; standard errors were all within 10% of the mean.

of 2,4-dimethoxy **9e** and 2,5-dimethoxy **9f** against *C. albicans* and 3,4-dimethoxy **9g** against *A. niger* on phenyl ring lead to potent compounds. Replacement of 3,4-dihydroxy **9d** with 3,4-dimethoxy **9g** of phenyl ring increases the activity.

Molecular docking study of the synthesized compounds **9**(**a**–**j**) was performed against pteridine reductase 1 enzyme of *L. donovani* (PDB ID: 2XOX)[37] to understand the binding interactions using VLife MDS 4.3 package following standard procedure[38] and docking calculation and hydrogen bond interactions are shown in Table 2. Pteridine reductase 1 (PTR1), a Leishmania enzyme responsible for salvage of pteridines is potential target for chemotherapeutic intervention.[39] The interaction energy of the compounds **9**(**a**–**j**) and their antileishmanial activity showed the corresponding results. The active compounds **9a** and **9i** showed lowest interaction energy that is −67.16 kcal/mol and −70.76 kcal/mol, respectively. The docking results indicated that the thienopyridine acetohydrazide core of these compounds **9**(**a**–**j**) held in the active pocket by combination of hydrophobic and van der Waals interactions with the protein. Major hydrophobic contacts occurred between the thienopyridine acetohydrazide core with the side chain of THR12, GLY13, ARG17, LEU18, LEU66, ASP181, ALA182, and SER185. The hydrophilic substituents (–OH, and $-OCH_3$) at phenyl ring did not form any interactions with active hydrophobic pocket of enzyme. The hydrophobic groups (–Cl, and $-N(CH_3)_2$ at phenyl ring were more favorable for hydrophobic interactions. The 4-$N(CH_3)_2$ substituent at phenyl ring of most active compound **9i** fitted well into the hydrophobic pocket and formed hydrophobic interactions with amino acid residues like

ARG17, LEU18, ASP181, and SER185. Thus, suggesting that the replacement of hydrophilic group with bulky hydrophobic group at phenyl ring would show good interactions with pteridine reductase 1 enzyme. The interactions of most active compound **9i** with enzyme is shown in Figure 3 and revealed that amino acids LYS16 (2.499 Å) and SER185 (2.249 Å) had formed hydrogen bond with nitrogen of $-N(CH_3)_2$ and oxygen of —C=O group, respectively. On the basis of activity data and docking result, it was found the compound **9i** had potential to inhibit pteridine reductase 1 enzyme.

A computational study of titled compounds **9**(**a**–**j**) was performed for prediction of ADME, molecular volume, molecular weight, miLog *P*, number of hydrogen acceptors, number properties and value obtained depicted in Table 4. Polar surface area (TPSA),[40] number of rotatable bonds of hydrogen donors and Lipinski's rule of five[41] were calculated using Molinspiration online property calculation toolkit.[42] Absorption (% ABS) was calculated by: % ABS = 109 − (0.345 × TPSA).[43] From all these parameters, it can be observed that all titled compounds exhibited a good % ABS (79.62–93.57%). Furthermore, none of the compounds violated Lipinski's rule of five and thus showing possible utility of series for developing the compound with drug like properties. A molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: log*P* (octanol–water partition coefficient) ⩽5, molecular weight ⩽500, number of hydrogen bond acceptors ⩽10 and number of hydrogen bond donors ⩽5.[40] All the synthesized compounds followed the criteria for orally active drug and therefore, these compounds can be further developed as oral drug candidates.

In conclusion, synthesis of *N*′-substitutedbenzylidene-2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetohydrazide derivatives **9**(**a**–**j**) has been presented and newly synthesized compounds investigated for antileishmanial, antibacterial and antifungal activities. Compounds **9a** with 2-Cl substituent and **9i** with 4-*N*,*N*-dimethylamino substituent on phenyl ring were most active for antileishmanial activity among the tested compounds. Compounds **9b** with 4-Chloro substituent on phenyl ring showed potent as well broad spectrum antibacterial activity. Compound **9e** with 2,4-dimethoxy substituent on phenyl ring showed promising antifungal activity. Molecular docking study showed good binding of these compounds to the active site of pteridine reductase 1 enzyme of *L. donovani*. Also none of the synthesized compounds were cytotoxic to HeLa cell line up to the concentration 300 μg/mL. Furthermore, analysis of the ADME parameters for synthesized

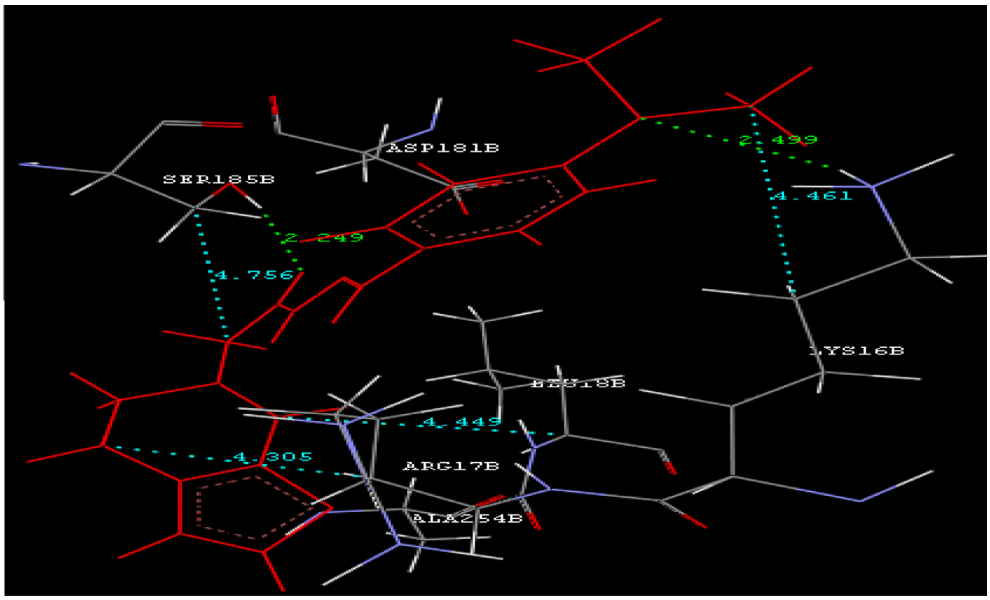


Figure 3. Docking study of compounds **9i** with pteridine reductase 1 (PDB ID: 2XOX). Ligands are shown in red color. Hydrogen bonds are shown in green color. Hydrophobic bonds are shown in sky blue color.

Table 4
Pharmacokinetic parameters important for good oral bioavailability of synthesized compounds **9**(**a**–**j**)

Entry	% ABS	TPSA (A^2)	*n*-ROTB	MV	MW	mi log*P*	*n*-ON acceptors	*n*-OHNH donors	Lipinski's violations
Rule	—	—	—	—	<500	⩽5	<10	<5	⩽1
9a	93.57	44.70	4	283.49	333.84	3.31	4	1	0
9b	93.57	44.70	4	283.49	333.84	3.36	4	1	0
9c	93.57	44.70	4	297.03	368.28	3.96	4	1	0
9d	79.62	85.15	4	285.99	331.39	1.71	6	3	0
9e	87.20	63.16	6	321.05	359.44	2.72	6	1	0
9f	87.20	63.16	6	321.05	359.44	2.33	6	1	0
9g	87.20	63.16	6	321.05	359.44	2.72	6	1	0
9h	81.41	79.95	5	306.79	344.43	2.12	6	3	0
9i	92.46	47.93	5	315.86	342.46	2.78	5	1	0
9j	85.37	68.49	4	286.81	324.40	2.43	5	1	0

% ABS: percentage absorption, TPSA: topological polar surface area, *n*-ROTB: number of rotatable bonds, MV: molecular volume, MW: molecular weight, mi log*P*: logarithm of partition coefficient of compound between *n*-octanol and water, *n*-ON acceptors: number of hydrogen bond acceptors, *n*-OHNH donors: number of hydrogen bonds donors.

compounds showed good drug like properties and can be developed as oral drug candidate. Thus, suggesting that compounds from the present series **9a** and **9i** (antileishmanial activity), **9b** (antibacterial activity) and **9e** (antifungal activity) can be further optimized and developed as a lead molecule.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.035.

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