European Journal of Medicinal Chemistry 45 (2010) 2359–2365

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of new [1,2,4]triazino[5,6-*b*] indol-3-ylthio-1,3,5-triazines and [1,2,4]triazino[5,6-*b*]indol-3-ylthio-pyrimidines against *Leishmania donovani*

Leena Gupta [a], Naresh Sunduru [a], Aditya Verma [b], Saumya Srivastava [b], Suman Gupta [b], Neena Goyal [c], Prem M.S. Chauhan [a,*]

[a] *Division of Medicinal and Process Chemistry, Central Drug Research Institute, MG Road, Lucknow 226001, U.P., India*
[b] *Division of Parasitology, Central Drug Research Institute, Lucknow, U.P., India*
[c] *Biochemistry Division, Central Drug Research Institute, Lucknow, U.P., India*

ARTICLE INFO

Article history:
Received 20 August 2009
Received in revised form
29 January 2010
Accepted 4 February 2010
Available online 12 February 2010

Keywords:
Leishmaniasis
Triazino[5,6-*b*]indole
Pyrimidine
1,3,5-Triazine

ABSTRACT

A series of [1,2,4]triazino[5,6-*b*]indol-3-ylthio-1,3,5-triazines and [1,2,4]triazino[5,6-*b*]indol-3-ylthio-pyrimidines were synthesized and screened for their *in vitro* antileishmanial activity against *Leishmania donovani*. Among all, 8 compounds have shown more than 90% inhibition against promastigotes and IC_{50} in the range of 4.01–57.78 μM against amastigotes. Compound **5**, a triazino[5,6-*b*]indol-3-ylthio-1,3,5-triazine derivative was found to be the most active and least toxic with 20- & 10-fold more selectivity (S.I. = 56.61) as compared to that of standard drugs pentamidine and sodium stibogluconate (SSG), respectively.

1. Introduction

Leishmaniasis is vector-borne disease caused by blood and tissue dwelling protozoan parasite species belonging to the genus *Leishmania*. It is the second-largest parasitic killer of humankind with its three clinical forms; Cutaneous Leishmaniasis and Muco-cutaneous Leishmaniasis, which are less severe form of the disease with usually self-healing ulcers. While Visceral Leishmaniasis, the most severe form of the disease, can result in 100% mortality of infected patients if not treated. They affect 12 million people in 88 countries, with 1.5–2 million new cases and 70000 deaths each year [1]. The chemotherapy against leishmaniasis is mainly based on antimony agents like sodium stibogluconate and meglumine antimoniate, amphotericin B, miltefosine and paromomycin, which is disappointing due to their serious side effects of toxicity [2,3]. And the emerged resistance against to antimonials [4], AIDS and other immunosuppressive conditions have also escalated the risk of infection and contributed to the appearance of new severe clinical forms of the disease [5]. Thus, affordable alternative drugs against leishmaniasis are desperately needed and the search for them is still a challenging task.

Dihydrofolate reductase (DHFR) has successfully been used as a drug target in the area of parasitic diseases. But most of the clinically used DHFR inhibitors show less selectivity for leishmanial enzymes [6]. This is due to the over expression of the gene pteridine reductase (*PTR1*) in some leishmanial mutants. This *PTR1* has ability to provide reduced pterins and folates and has the potential to act as a by-pass or modulator of DHFR inhibition. Thus to stop the folate biosynthesis that is essential for the survival of infection, both *PTR1* and DHFR have to be inhibited [7]. Selective inhibitors of *PTR1* or a single inhibitor that acts on both enzymes would constitute a rational approach for new antileishmanial agents.

Earlier, pyrimidines were synthesized and evaluated as inhibitors of leishmanial and trypanosomal dihydrofolate reductase [8], while triazine class of compounds being the inhibitors of DHFR [9–11] have also been identified as potential antileishmanial agents [12–14]. While on other side, triazino[5,6-*b*]indole derivatives have aroused considerable interest as a result of their broad spectrum of antibacterial, antifungal and antiparasitic activities [15–17]. Based on these observations we have designed and synthesized the two classes of hybrid molecules, one the hybrids of [1,2,4]triazino[5,6-*b*]

* Corresponding author. Tel.: +91 522 2262411X4470; fax: +91 522 2623405.
E-mail addresses: prem_chauhan_2000@yahoo.com, premsc58@hotmail.com (P.M.S. Chauhan).

0223-5234/$ – see front matter © 2009 Published by Elsevier Masson SAS.
doi:10.1016/j.ejmech.2010.02.015

indole with 1,3,5-triazine and the other one is of [1,2,4]triazino[5,6-*b*]indole with pyrimidine. These compounds were screened for their antileishmanial profile and have shown encouraging results against *Leishmania donovani*.

2. Chemistry

Synthesis of [1,2,4]triazino[5,6-*b*]indole-triazine derivatives (**5**–**15**) and [1,2,4]triazino[5,6-*b*]-pyrimidine derivatives (**16**–**26**) has been synthesized as outlined in the Scheme 1. *N*-methylisatin **1** was achieved by protecting NH of isatin using dimethyl sulfate in methanolic KOH [18], which was cyclised by thiosemicarbazide [17] to give 5-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**2**) The intermediates 3-(4,6-dichloro-1,3,5-triazin-2-ylthio)-5-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indole (**3**) and 3-(2-chloro-6-methylpyrimidin-4-ylthio)-5-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indole (**4**) were synthesized by reacting compound **2** with 2,4,6-trichloro-1,3,5-triazine and 6-methyl-2,4-dichloropyrimidine, respectively. Intermediate **3** was subjected to nucleophilic substitution with various amines (Table 1) in THF to yield targeted compounds (**5**–**15**), while intermediate **4** produced the final compounds (**16**–**26**) on nucleophilic substitution with various amines at second position of pyrimidine in the presence of K_2CO_3 in DMF [19]. All the above synthesized compounds were well characterized by spectroscopic methods such as IR, mass, NMR and elemental analyses.

3. Biological activities

3.1. Material and method

3.1.1. Antipromastigote activity

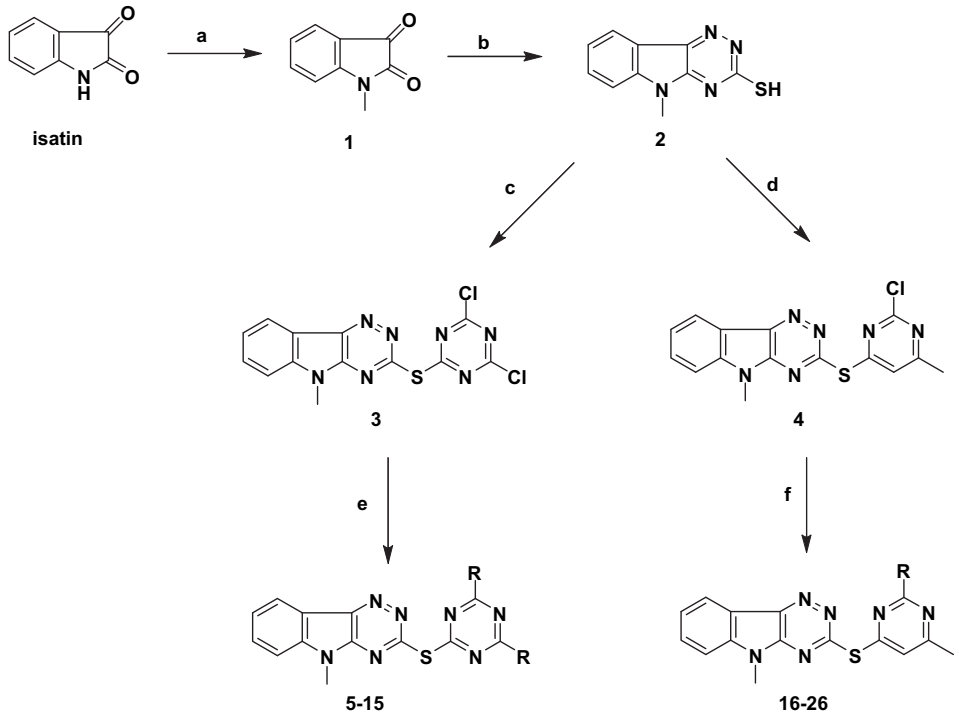
The *L. donovani* promastigotes (MHOM/IN/Dd8; originally obtained from Imperial college, London) were transfected with firefly luciferase gene and the transfectants were maintained in medium 199 (Sigma chemical Co., USA) supplemented with 10% foetal calf serum (GIBCO) and 1% penicillin (50 U/mL), streptomycin (50 μg/mL) solution (Sigma) under pressure of G418 (Sigma). The *in vitro* effect of the compounds on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after treatment. The transgenic promastigotes of late log phase were seeded at 5×10^5/100 μL medium 199 well in 96-well flat-bottomed microtiter (MT) plates (CELLSTAR) and incubated for 72 h in medium alone or in the presence of serial dilutions of drugs (1–10 μg/mL) in DMSO [20]. Parallel dilutions of DMSO were used as controls. After incubation, an aliquot (50 μg/mL) of promastigote suspension was aspirated from each well of a 96-well plate and mixed with an equal volume of steady Glo® reagent (Promega) and luminescence was measured by a luminometer. The values were expressed as relative luminescence unit (RLU). The inhibition of parasitic growth is determined by comparison of the luciferase activity of drug-treated parasites with that of untreated controls by the general formula:

$$\text{Percentage Inhibition} = \frac{N - n \times 100}{N}$$

where N is average relative luminescence unit (RLU) of control wells, and n is average RLU of treated wells.

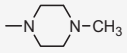
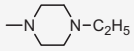
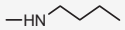
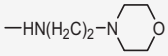
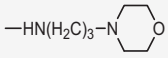
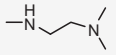
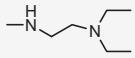
3.1.2. Antiamastigote activity

In vitro activity: for assessing the activity of compounds against the amastigote stage of the parasite, mouse macrophage cell line (J774A.1) infected with promastigotes expressing luciferase firefly reporter gene was used. Cells were seeded in a 96-well plate (1.5×10^4 cell/100 μL/well) in RPMI-1640 containing 10% foetal calf serum and the plates were incubated at 37 °C in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary-phase promastigotes (2.25×10^5/100 μL/well). Promastigotes invades the macrophage and are transformed into



Scheme 1. Reagents and conditions: (a) Dimethyl sulfate, MeOH, 10% methanolic KOH, rt.; (b) thiosemicarbazide, K_2CO_3, water, reflux; (c) 2,4,6-trichloro-1,3,5-triazine, THF, rt; (d) 6-methyl-2,4-dichloropyrimidine, DIPEA, DMF, reflux; (e) various amines, K_2CO_3, THF, reflux; (f) different amines, K_2CO_3, DMF, reflux.

Table 1
Antileishmanial in vitro activity against luciferase-promastigote system.

R	Comp. No.	Percent inhibition (at 40 μg/mL) promastigotes	Comp. No.	Percent inhibition (at 40 μg/mL) promastigotes
	**5**	75.56	**16**	99.31
	**6**	43.13	**17**	NI
	**7**	NI	**18**	99.90
	**8**	99.95	**19**	99.99
	**9**	93.17	**20**	99.73
	**10**	97.41	**21**	66.72
	**11**	99.27	**22**	99.25
	**12**	77.11	**23**	51.11
	**13**	NI	**24**	95.10
	**14**	68.53	**25**	100
	**15**	49.33	**26**	99.93
Pentamidine®[a]				
SSG® (Sodium stibogluconate)[b]				

NI: no inhibition.
[a] Pentamidine shows 85–90% inhibition against promastigotes at 0.5 μg/ml.
[b] SSG shows 40–50% inhibition against promastigotes at 940 μg/ml.

amastigote. The test material in appropriate concentrations (0.25–10 μg/mL) in complete medium was added after replacing the previous medium and the plates were incubated at 37 °C in a CO_2 incubator for 72 h. After incubation, the drug containing medium was decanted and 50 μL PBS was added in each well and mixed with an equal volume of steady Glo® reagent. After gentle shaking for 1–2 min, the reading was taken in a luminometer [20]. The inhibition of parasitic growth is determined by comparison of the luciferase activity of drug-treated parasites with that of untreated controls as described above.

3.1.3. Data analysis

IC_{50} and IC_{90} were calculated by Probit analysis [21]. Compounds with more than 15 μg/mL IC_{50} were considered as inactive while compounds with IC_{50} between 15 and 5 μg/mL were considered as moderately active and less than 5 μg/mL as highly active compounds. These values were expressed in micromolar (μM) concentrations using their respective molecular weights.

3.1.4. Cytotoxicity assay

The cell viability was determined using the MTT assay as described by Tempone et al., 2005 [22] using the J774A.1 cell line, which was obtained from Tissue Culture Laboratory, Central Drug Research Institute, India. J774A.1 cell line was maintained in RPMI medium (Sigma), supplemented with 10% foetal calf serum and 40 mg/mL gentamycin. Exponentially growing cells (1×10^4 cells/100 μL/well) were incubated with different drug concentrations for 72 h and were incubated at 37 °C in a humidified mixture of CO_2 and 95% air in an incubator. Stock solutions of compounds were initially dissolved in DMSO and further diluted with fresh complete medium. After incubation, 25 μL of MTT reagent (5 mg/mL) in PBS medium, followed by syringe filtration were added to each well and incubated at 37 °C for 2 h. At the end of the incubation period, the supernatant were removed by tilting plate completely without disturbing cell layer and 150 μL of pure DMSO are added to each well. After 15 min of shaking the readings were recorded as absorbance at 544 nm on a microplate reader. The cytotoxic effect were expressed as 50% lethal dose, i.e., as the concentration of a compound which provoked a 50% reduction in cell viability compared to cell in culture medium alone. CC_{50} values were estimated through the preformed template as described by Huber and Koella (1993) [23].

4. Results and discussion

A series of 11 hybrids of 1,3,5-triazine (a DHFR inhibitor) and same number of hybrids of pyrimidine (also a DHFR inhibitor) were synthesized to compare the structure activity relationship by incorporating both of them separately with [1,2,4]triazino[5,6-*b*] indole moiety. The *in vitro* biological activity of [1,2,4]triazinoindole-triazine and [1,2,4]triazinoindole-pyrimidine derivatives has shown interesting results against *L. donovani* and clearly suggests that the [1,2,4]triazinoindole-triazines are better inhibitors in comparison to [1,2,4]triazinoindole-pyrimidines.

The percentage inhibition of these compounds against promastigotes has been shown in Table 1. Among all 11 hybrids of [1,2,4]triazinoindole-triazines, four compounds **8**, **9**, **10** and **11** have shown more than 90% inhibition against promastigotes, while eight compounds **16**, **18**, **19**, **20**, **22**, **24**, **25** and **26** of [1,2,4]triazinoindole-pyrimidine hybrids shown same percentage of inhibition against promastigotes. Compounds which have shown more than 75% of inhibition against promastigotes, were further screened against amastigote model and their IC_{50}, MIC, CC_{50} and selectivity index (SI) were given in Table 2. Compound **5**, a [1,2,4]triazinoindole-[1,3,5] triazine derivative with **R** = piperidine, found to be the most active among all the synthesized compounds with an IC_{50} of 4.01 μM and a MIC of 20.54 μM. It was also found to be the least toxic with a CC_{50} of 227.04 μM and thus made it to 20- & 10-fold more selective than the standard drugs Pentamidine and Sodium stibogluconate (SSG), respectively, with a SI of 56.57. On the other hand, compound **16** which is a [1,2,4]triazinoindole-pyrimidine derivative having same **R** showed IC_{50} of 25.70 μM and CC_{50} of 173.29 μM, led to its drop off in selectivity in comparison to compound **5**, but comparable to that of standard drug SSG. Similarly, compound **8** of [1,2,4]triazinoindole-triazine hybrid with **R** = *N*-ethylpiperazine showed IC_{50} of 15.51 μM, MIC of 26.55 μM and CC_{50} of 93.17 μM, with SI of value 6.74, which is comparable to that of standard drug SSG. While compound **19** of [1,2,4]triazinoindole-pyrimidine hybrid having same **R,** exhibited decreased potency of IC_{50} 26.31 μM and poor selectivity. Compound **11**, a [1,2,4]triazinoindole-triazine derivative having isopropyl as **R**, also exhibited better IC_{50} of 17.38 μM as compared to its respective [1,2,4]triazinoindole-pyrimidine analogue **22** (IC_{50} = 29.64 μM) with almost same selectivity. While compound **20** showed good IC_{50} of 18.33 μM among all pyrimidine

Table 2
In vitro activity against MQ-amastigotes and cytotoxicity.

Comp. No.	*In vitro* activity against amastigotes		Cytotoxicity against J774A.1 cell lines CC_{50} (μM)	SI (selectivity index) CC_{50}/IC_{50}
	IC_{50} (μM)	MIC (μM)[a]		
5	4.01	20.54	227.04	56.61
8	15.51	26.55	93.17	6.00
9	NI	ND	17.07	NA
10	NI	ND	ND	NA
11	17.38	90.53	64.49	3.71
12	NI	ND	ND	NA
16	25.70	138.82	173.29	6.74
18	57.78	68.69	29.21	0.50
19	26.30	90.80	28.14	1.06
20	18.33	110.21	88.39	4.82
22	29.64	93.75	108.71	3.66
24	NI	ND	ND	NA
25	NI	ND	ND	NA
26	NI	ND	ND	NA
Pentamidine®	20.43	>168.71	52.82	2.58
SSG®	71.90	163.89	398.26	5.53

NI: no inhibition at 40 μg/mL; ND: not done; NA; not available.
[a] MIC: minimum inhibitory concentration required for more than 90% inhibition.

derivatives, with improved selectivity on replacing **R** with *n*-butylamine. On the basis of above discussion it is evident that [1,2,4]triazinoindole-[1,3,5]triazine hybrids are more potent anti-leishmanial agents as compared to their, respective, [1,2,4]triazinoindole-pyrimidine hybrids.

In conclusion, Leishmania is influencing the humankind with its morbidity and mortality. The development of efficient chemotherapy is urgently required but still kept aside due to lack of interest and financial support. As our research is oriented to the development of novel heterocycles as anti-infectious agents, we synthesized hybrids of 1,3,5-triazine and pyrimidine as anti-leishmanial agents in the past and optimization of these hybrids is still producing the promising results against leishmania. Therefore, carry-over of research on these heterocycles is trustworthy for generating efficient antileishmanials.

5. Experimental

IR spectra were recorded on Beckman Aculab-10, PerkinElmer 881 and FTIR 8210 PC, Schimadzu spectrophotometers either on KBr discs or in neat. Nuclear magnetic resonance (NMR) spectra were recorded on either Bruker Avance DRX-300 MHz or Bruker DPX 200 FT spectrometers using TMS as an internal reference. EI mass spectra were recorded on JEOL JMS-D-300 spectrometer with the ionization potential of 70 eV and ES mass spectra on Quantro-II, micromass. Chemical analysis was carried out on Carlo-Erba-1108 instrument. The melting points were recorded on an electrically heated melting point apparatus and are uncorrected.

5.1. Synthesis of compound *1*

To a suspension of (0.1 mol) of isatin in 200 ml of anhydrous methanol, 100 ml of 10% methanolic potassium hydroxide solution was added in portions with stirring for 30 min. To this purple mixture, 15 ml of dimethyl sulfate was added and after 1 h, the solution was filtered to remove potassium methyl sulfate. After removal of about 250 ml of solvent under reduced pressure, 40 ml of warm water was added to the residue obtained. On cooling, orange precipitate occurred which was filtered and dried to obtain compound **1**.

5.1.1. 1-Methylindoline-2,3-dione (*1*)

Yield: 75%; mp: 80–82 °C; MS: 162 (M + 1); IR (KBr): 3029, 1725, 1617, 1490, 1464, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.43–7.35(*m*, 2H), 7.07(*m*, 1H), 6.84(*m*, 1H), 3.21(*s*, 3H). ^{13}C ($CDCl_3$, 50 MHz): 171.6, 152.7, 144.2, 131.2, 125.3, 124.6, 123.2, 108.9, 25.4. Anal. Calc. for $C_9H_7NO_2$: C: 67.07, H: 4.38, N: 8.69. Found: C: 67.45, H: 4.66, N: 8.45.

5.2. Synthesis of compound *2*

A mixture of **1** (0.1 mol), thiosemicarbazide (0.1 mol) and K_2CO_3 (0.15 mol) in 500 mL of water was refluxed with stirring for 3 h. On cooling the mixture was filtered and precipitated by acidification with acetic acid. The solid was washed with water and dried to obtain **2** as yellow solid.

5.2.1. 5-Methyl-5H-[1,2,4]triazino[5,6-b]indole-3-thiol (*2*)

Yield: 72%; mp > 250 °C (Decompose); MS: 217 (M + 1); IR (KBr): 3108, 2935, 2856, 1565, 1441, 1076 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.85(bs, 1H), 8.46(*d*, 1H, *J* = 7.14 Hz), 7.68(m, 1H), 7.51–7.42(m, 2H), 3.91(*s*, 3H). ^{13}C ($CDCl_3$, 50 MHz): 141.6, 132.2, 131.4, 123.8, 122.7, 121.7, 117.5, 110.4, 27.7. Anal. Calc. for $C_{10}H_8N_4S$: C: 55.54, H: 3.73, N: 25.91. Found: C: 55.37, H: 3.88, N: 25.65.

5.3. Synthesis of compound *3*

To a stirred solution of cyanuric chloride (1.2 equiv.) in dry THF, compound **2** (1 equiv.) was added slowly and stirred for 3 h. at rt. After the completion of reaction, the reaction mixture was evaporated to dryness and the solid residue obtained was purified with column chromatography using silica gel as an adsorbent to obtain desired compound **3**.

5.3.1. 3-(4,6-Dichloro-1,3,5-triazin-2-ylthio)-5-methyl-5H-[1,2,4]triazino[5,6-b]indole (*3*)

Yield: 60%; mp: 152–155 °C; MS: 363 (M + 1); IR (KBr): 3116, 2931, 2851, 1583, 1540, 1435, 1298, 1182, 1064, 721 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.40(*d*, 1H, *J* = 7.24 Hz), 7.84(*m*, 1H), 7.56 (*m*, 2H), 3.85(*s*, 3H). ^{13}C ($CDCl_3$, 50 MHz): 163.9, 147.8, 142.5, 141.8, 132.1, 123.4, 122.5, 117.5, 110.2, 27.7. Anal. Calc. for $C_{13}H_7Cl_2N_7S$: C: 42.87, H: 1.94, N: 26.92. Found: C: 42.99, H: 1.85, N: 26.74.

5.4. Synthesis of compound *4*

To a solution of **2** (1 equiv.) in dry DMF, DIPEA (1.5 equiv.) was added and stirred at rt. for 1 h. After that 6-methyl-2,4-dichloropyrimidine (1.2 equiv.) was added and allowed to stir at rt. for 4 h.

After the completion of reaction, the mixture was evaporated to dryness and the solid obtained was dissolved in water and extracted with DCM. The organic layer was washed with brine solution (three times), with water (three times), and dried over anhydrous Na_2SO_4 and concentrated to obtain the intermediate **4**.

*5.4.1. 3-(2-Chloro-6-methylpyrimidin-4-ylthio)-5-methyl-5H-[1,2,4]triazino[5,6-b]indole (**4**)*

Yield: 62%; mp: 144–146 °C; MS: 343 (M + 1); IR (KBr): 3116, 2931, 2851, 1583, 1540, 1435, 1298, 1182, 1064, 721 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.46(*d*, 1H, *J* = 8.10 Hz), 7.74(*t*, 1H, *J* = 7.48 Hz), 7.55–7.49(*m*, 2H), 6.98(*s*, 1H), 3.84(*s*, 3H), 2.32(*s*, 3H). ^{13}C ($CDCl_3$, 50 MHz): 171.4, 169.6, 163.2, 160.7, 146.3, 142.5, 132.1, 123.8, 123.4, 119.7, 118.5, 110.2, 27.7, 24.4. Anal. Calc. for $C_{15}H_{11}ClN_6S$: C: 52.55, H: 3.23, N: 24.52. Found: C: 52.76, H: 3.14, N: 24.61.

*5.5. General procedure for the synthesis of compounds **5**–**15***

A mixture of **3** (1 equiv.) and respective amine (2 equiv.) as described in Table 1 and K_2CO_3 (1.2 equiv.) in dry THF was refluxed with stirring for 7 h. After the completion of reaction, mixture was evaporated to dryness and the solid residue was purified with column chromatography using silica gel as an adsorbent to obtain final targeted compounds **5**–**15**.

*5.5.1. 3-(4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-ylthio)-5-methyl-5H-[1,2,4]triazino[5,6-b]indole (**5**)*

Yield: 55%; mp: 178–180 °C; MS: 462 (M + 1); IR (KBr): 3054, 2933, 2850, 1544, 1491, 1444, 1295, 1229, 1175, 1066 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.46(*d*, 1H, *J* = 7.68 Hz), 7.85(*t*, 1H, *J* = 7.42 Hz), 7.58–7.55(*m*, 2H), 3.91(*s*, 3H), 3.71(*m*, 8H), 1.55–1.47 (*m*, 12H). ^{13}C ($CDCl_3$, 50 MHz): 164.7, 146.8, 142.6, 141.3, 132.4, 123.8, 122.1, 117.5, 110.4, 50.9, 27.3, 26.2, 25.5. Anal. Calc. for $C_{23}H_{27}N_9S$: C: 59.85, H: 5.90, N: 27.31. Found: C: 59.76, H: 5.88, N: 27.40.

*5.5.2. 4,4'-(6-(5-Methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1,3,5-triazine-2,4-diyl)dimorpholine (**6**)*

Yield: 58%; mp: 218–220 °C; MS: 466 (M + 1); IR (KBr): 3061, 2934, 2856, 1540, 1493, 1445, 1297, 1230, 1166, 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.54(*d*, 1H, *J* = 7.24 Hz), 7.82(*t*, 1H, *J* = 7.58 Hz), 7.59–7.51(*m*, 2H), 3.93(*s*, 3H), 3.83–3.66(*m*, 16H). ^{13}C ($CDCl_3$, 50 MHz): 164.3, 145.9, 142.6, 141.5, 132.4, 123.5, 122.6, 117.8, 110.3, 67.5, 50.2, 27.2. Anal. Calc. for $C_{21}H_{23}N_9O_2S$: C: 54.18, H: 4.98, N: 27.08. Found: C: 54.20, H: 4.99, N: 27.06.

*5.5.3. 3-(4,6-Bis(4-methylpiperazin-1-yl)-1,3,5-triazin-2-ylthio)-5-methyl-5H-[1,2,4]triazino[5,6-b]indole (**7**)*

Yield: 64%; mp: 198–200 °C; MS: 492 (M + 1); IR (KBr): 3108, 2928, 2845, 1537, 1484, 1440, 1293, 1235, 1162, 1082 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.31(*d*, 1H, *J* = 5.20 Hz), 7.64(*t*, 1H, *J* = 5.37 Hz), 7.43–7.33(*m*, 2H), 3.97(*s*, 3H), 3.58(*m*, 8H), 2.77(*m*, 8H), 2.45(*s*, 6H). ^{13}C ($CDCl_3$, 50 MHz): 163.2, 146.8, 142.7, 141.5, 132.1, 123.9, 122.7, 117.6, 110.4, 53.2, 49.2, 43.6, 27.7. Anal. Calc. for $C_{23}H_{29}N_{11}S$: C: 56.19, H: 5.95, N: 31.34. Found: C: 56.34, H: 5.77, N: 31.42.

*5.5.4. 3-(4,6-Bis(4-ethylpiperazin-1-yl)-1,3,5-triazin-2-ylthio)-5-methyl-5H-[1,2,4]triazino[5,6-b]indole (**8**)*

Yield: 61%; mp: 230–232 °C; MS: 520 (M + 1); IR (KBr): 3072, 2926, 2861, 1547, 1485, 1443, 1295, 1228, 1177, 1074 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.46(*d*, 1H, *J* = 7.04 Hz), 7.78–7.75(*m*, 1H), 7.57–7.48(*m*, 2H), 3.89(*s*, 3H), 3.45–3.24(*m*, 16H), 2.75(*m*, 4H), 1.37(*t*, 6H, *J* = 6.65 Hz). ^{13}C ($CDCl_3$, 50 MHz): 164.3, 146.8, 142.5, 141.8, 131.7, 123.1, 122.7, 117.5, 110.2, 53.4, 52.8, 50.7, 27.7, 12.5. Anal. Calc. for $C_{25}H_{33}N_{11}S$: C: 57.78, H: 6.40, N: 29.65. Found: C: 57.99, H: 6.62, N: 29.16.

*5.5.5. N^2,N^4-dibutyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1,3,5-triazine-2,4-diamine (**9**)*

Yield: 68%; mp: 138–140 °C; MS: 438 (M + 1); IR (KBr): 3348, 3112, 2928, 2875, 1542, 1490, 1452, 1287, 1232, 1174, 1068 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.52(*d*, 1H, *J* = 7.54 Hz), 7.77(m, 1H), 7.55–7.50(*m*, 2H), 3.94(*s*, 3H), 3.28–3.06(*m*, 4H), 1.49–1.29(*m*, 8H), 0.93(*t*, 3H, *J* = 5.53 Hz). ^{13}C ($CDCl_3$, 50 MHz): 164.3, 146.8, 142.3, 141.9, 132.6, 123.1, 122.5, 117.6, 110.1, 40.3, 31.4, 27.8, 19.9, 13.4. Anal. Calc. for $C_{21}H_{27}N_9S$: C: 57.64, H: 6.22, N: 28.81. Found: C: 57.34, H: 6.12, N: 28.98.

*5.5.6. N^2,N^4-di-tert-butyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1,3,5-triazine-2,4-diamine (**10**)*

Yield: 55%; mp: 176–178 °C; MS: 438 (M + 1); IR (KBr): 3342, 3079, 2931, 2852, 1546, 1489, 1445, 1290, 1219, 1183, 1075 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.67(*d*, 1H, *J* = 7.21 Hz), 7.92(*m*, 1H), 7.69–7.66(*m*, 2H), 3.03(*s*, 3H), 1.14(*s*, 18H). ^{13}C ($CDCl_3$, 50 MHz): 164.5, 146.8, 142.4, 141.8, 131.6, 123.1, 122.7, 117.6, 110.1, 51.9, 29.7, 27.5. Anal. Calc. for $C_{21}H_{27}N_9S$: C: 57.64, H: 6.22, N: 28.81. Found: C: 57.66, H: 6.25, N: 28.79.

*5.5.7. N^2,N^4-diisopropyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1,3,5-triazine-2,4-diamine (**11**)*

Yield: 59%; mp: 148–150 °C; MS: 410 (M + 1); IR (KBr): 3385, 3056, 2934, 2852, 1543, 1490, 1434, 1285, 1230, 1178, 1076 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.45(*d*, 1H, *J* = 6.96 Hz), 7.87(*m*, 2H), 7.55(*m*, 1H), 4.02(bs, 2H), 3.99–3.95(*m*, 2H), 3.85(*s*, 3H), 1.06(*d*, 12H, *J* = 3.96 Hz). ^{13}C ($CDCl_3$, 50 MHz): 163.9, 146.8, 142.3, 141.8, 132.2, 123.1, 122.7, 117.5, 110.2, 47.2, 27.7, 24.3. Anal. Calc. for $C_{19}H_{23}N_9S$: C: 55.73, H: 5.66, N: 30.78. Found: C: 55.66, H: 5.74, N: 30.95.

*5.5.8. 6-(5-Methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-N^2,N^4-bis(2-morpholinoethyl)-1,3,5-triazine-2,4-diamine (**12**)*

Yiled: 67%; 210–212 °C; MS: 552 (M + 1); IR (KBr): 3442, 3064, 2930, 2856, 1541, 1490, 1444, 1293, 1231, 1174, 1082 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.47(*d*, 1H, *J* = 7.12 Hz), 7.76(*m*, 1H), 7.56 (*m*, 2H), 3.88(*s*, 3H), 3.51–3.35(*m*, 12H), 2.69(*m*, 8H), 2.13–2.09(*m*, 4H). ^{13}C ($CDCl_3$, 50 MHz): 164.3, 146.8, 142.3, 141.9, 132.5, 123.6, 122.7, 117.5, 110.2, 67.3, 57.4, 53.1, 42.6, 27.7. Anal. Calc. for $C_{25}H_{33}N_{11}O_2S$: C: 54.43, H: 6.03, N: 27.93. Found: C: 54.75, H: 6.24, N: 27.70.

*5.5.9. 6-(5-Methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-N^2,N^4-bis(3-morpholinopropyl)-1,3,5-triazine-2,4-diamine (**13**)*

Yield: 64%; mp: 232–234 °C; MS: 580 (M + 1); IR (KBr): 3436, 3045, 2935, 2860, 1550, 1492, 1443, 1280, 1231, 1185, 1067 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.43(*d*, 1H, *J* = 6.98 Hz), 7.72(*m*, 1H), 7.49(*m*, 2H), 6.82(bs, 2H), 3.86(*s*, 3H), 3.59–3.40(*m*, 12H), 2.36 (*m*, 12H), 1.72–1.69(*m*, 4H). ^{13}C ($CDCl_3$, 50 MHz): 164.5, 146.8, 142.2, 141.8, 132.6, 123.2, 122.7, 117.5, 110.1, 66.4, 61.3, 54.1, 30.2, 27.6, 24.6. Anal. Calc. for $C_{27}H_{37}N_{11}O_2S$: C: 55.94, H: 6.43, N: 26.58. Found: C: 55.66, H: 6.24, N: 26.69.

*5.5.10. N^1,$N^{1'}$-(6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1,3,5-triazine-2,4-diyl)bis(N^2,N^2-dimethylethane-1,2-diamine) (**14**)*

Yield: 59%; mp: 189–191 °C; MS: 468 (M + 1); IR (KBr): 3435, 3061, 2932, 2854, 1547, 1482, 1295, 1228, 1174, 1056 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.41(*d*, 1H, *J* = 7.26 Hz), 7.82(*m*, 1H), 7.55 (*m*, 2H), 3.91(*s*, 3H), 3.49(*m*, 4H), 2.58(*m*, 4H), 2.32(*s*, 12H). ^{13}C ($CDCl_3$, 50 MHz): 163.9, 145.6, 142.4, 141.7, 132.2, 131.4, 123.9, 122.7,

117.5, 110.3, 58.7, 45.1, 42.2, 27.8. Anal. Calc. for $C_{21}H_{29}N_{11}S$: C: 53.94, H: 6.25, N: 32.95. Found: C: 53.83, H: 6.47, N: 32.82.

*5.5.11. N^1,$N^{1'}$-(6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1,3,5-triazine-2,4-diyl)bis(N^2,N^2-diethylethane-1,2-diamine) (**15**)*

Yield: 56%; mp: 201–203 °C; MS: 524 (M + 1); IR (KBr): 3447, 3052, 2934, 2855, 1546, 1479, 1289, 1238, 1175, 1066 cm^{-1}; ^1H NMR ($CDCl_3$ 200 MHz): δ (ppm) 8.47(*d*, 1H, *J* = 6.80 Hz), 7.89(*m*, 1H), 7.57 (*m*, 2H), 3.88(*s*, 3H), 3.54(*m*, 4H), 3.13(*m*, 12H), 1.4(*t*, 12H, *J* = 6.75 Hz). ^{13}C ($CDCl_3$, 50 MHz): 164.1, 145.5, 142.3, 141.4, 132.2, 131.3, 123.6, 122.7, 117.1, 110.2, 52.2, 47.2, 46.8, 27.7, 12.2. Anal. Calc. for $C_{25}H_{37}N_{11}S$: C: 57.34, H: 7.12, N: 29.42. Found: C: 57.53, H: 7.02, N: 29.54.

*5.6. General procedure for the synthesis of compounds **16**–**26***

To a mixture of **4** (1 equiv.) and K_2CO_3 (1.5 equiv.) in dry DMF, respective amine (1.2 equiv.) as described in Table 1, was added and this reaction mixture was refluxed for 8 h. After the completion of reaction, mixture was evaporated to dryness and the solid residue was purified with column chromatography using silica gel as an adsorbent to obtain final targeted compounds **16**–**26**.

*5.6.1. 5-Methyl-3-(6-methyl-2-(piperidin-1-yl)pyrimidin-4-ylthio)-5H-[1,2,4]triazino[5,6-b]indole (**16**)*

Yield: 61%; mp: 169–171 °C; MS: 392 (M + 1); IR (KBr): 3111, 2930, 2852, 1583, 1536, 1438, 1289, 1188, 1071 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.48(*d*, 1H, *J* = 7.98 Hz), 7.75(*t*, 1H, *J* = 7.24 Hz), 7.54–7.47(*m*, 2H), 6.85(*s*, 1H), 3.86(*s*, 3H), 3.74(*m*, 4H), 2.35(*s*, 3H), 1.59(*m*, 6H). ^{13}C ($CDCl_3$, 50 MHz): 170.5, 167.5, 165.1, 161.2, 146.9, 142.4, 131.8, 123.6, 123.2, 118.2, 110.5, 109.1, 45.2, 27.8, 26.2, 25.3, 24.7. Anal. Calc. for $C_{20}H_{21}N_7S$: C: 61.36, H: 5.41, N: 25.04. Found: C: 61.43, H: 5.52, N: 25.14.

*5.6.2. 4-(4-Methyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)pyrimidin-2-yl)morpholine (**17**)*

Yield: 70%; mp: 203–205 °C; MS: 394 (M + 1); IR (KBr): 3115, 2928, 2864, 1580, 1543, 1436, 1288, 1178, 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.41(*d*, 1H, *J* = 5.62 Hz), 7.71–7.65(*m*, 1H), 7.45–7.40(*m*, 2H), 6.88(*s*, 1H), 3.79(*s*, 3H), 3.68–3.62(*m*, 8H), 2.26(*s*, 3H). ^{13}C ($CDCl_3$, 50 MHz): 171.5, 167.4, 165.1, 146.8, 142.4, 131.8, 123.6, 123.3, 118.3, 110.6, 109.1, 67.6, 51.5, 27.7, 24.6. Anal. Calc. for $C_{19}H_{19}N_7OS$: C: 58.00, H: 4.87, N: 24.92. Found: C: 58.22, H: 4.52, N: 24.85.

*5.6.3. 5-Methyl-3-(6-methyl-2-(4-methylpiperazin-1-yl)pyrimidin-4-ylthio)-5H-[1,2,4]triazino[5,6-b]indole (**18**)*

Yield: 63%; mp: 238–240 °C; MS: 407 (M + 1); IR (KBr): 3124, 2929, 2854, 1585, 1540, 1438, 1291, 1183, 1075 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.43(*d*, 1H, *J* = 6.98 Hz), 7.74 (*t*, 1H, *J* = 7.42 Hz), 7.52–7.44(*m*, 2H), 6.87(*s*, 1H), 3.83(*s*, 3H), 3.01–2.89(*m*, 7H), 2.61(*m*, 4H), 2.28(*s*, 3H). ^{13}C ($CDCl_3$, 50 MHz): 171.5, 169.4, 163.5, 1602, 146.7, 142.8, 132.3, 123.9, 123.3, 119.6, 118.1, 110.6, 55.8, 51.2, 46.6, 27.8, 24.4. Anal. Calc. for $C_{20}H_{22}N_8S$: C: 59.09, H: 5.45, N: 27.56. Found: C: 59.12, H: 5.40, N: 27.45.

*5.6.4. 3-(2-(4-Ethylpiperazin-1-yl)-6-methylpyrimidin-4-ylthio)-5-methyl-5H-[1,2,4]triazino[5,6-b]indole (**19**)*

Yield: 60%; mp: 220–222 °C; MS: 421 (M + 1); IR (KBr): 3117, 2930, 2862, 1584, 1537, 1444, 1288, 1185, 1069 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.40(*d*, 1H, *J* = 4.81 Hz), 7.71 (*t*, 1H, *J* = 5.06 Hz), 7.49–7.42(*m*, 2H), 6.79(*s*, 1H), 3.81(*s*, 3H), 3.57–3.28(*m*, 8H), 2.41(*m*, 2H), 2.24(*s*, 3H), 1.04(*t*, 3H, *J* = 6.54 Hz). ^{13}C ($CDCl_3$, 50 MHz): 170.6, 169.2, 163.6, 160.2, 146.8, 142.9, 132.1, 123.8, 123.4, 119.5, 118.1, 109.9, 53.4, 52.8, 50.8, 27.7, 24.6, 12.3. Anal. Calc. for $C_{21}H_{24}N_8S$: C: 59.98, H: 5.75, N: 26.65. Found: C: 59.92, H: 5.70, N: 26.45.

*5.6.5. N-butyl-4-methyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)pyrimidin-2-amine (**20**)*

Yield: 56%; mp: 153–155 °C; MS: 380 (M + 1); IR (KBr): 3453, 3115, 2927, 2856, 1580, 1546, 1434, 1288, 1187, 1067 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.48(*d*, 1H, *J* = 8.12 Hz), 7.76 (*t*, 1H, *J* = 7.72 Hz), 7.54–7.47(*m*, 2H), 6.93(*s*, 1H), 5.45(bs, 1H), 3.87(*s*, 3H), 3.31(*m*, 2H), 2.33(*s*, 3H), 1.47(*m*, 2H), 1.25(*m*, 2H), 0.85(*t*, 3H, *J* = 6.88 Hz). ^{13}C ($CDCl_3$, 50 MHz): 170.4, 169.6, 163.5, 160.2, 146.8, 142.9, 132.6, 123.7, 123.4, 119.6, 118.1, 110.6, 45.8, 33.2, 27.7, 24.5, 20.6, 14.24. Anal. Calc. for $C_{19}H_{21}N_7S$: C: 60.14, H: 5.58, N: 25.84. Found: C: 60.54, H: 5.67, N: 25.91.

*5.6.6. N-tert-butyl-4-methyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)pyrimidin-2-amine (**21**)*

Yield: 52%; mp: 207–209 °C; MS: 380 (M + 1); IR (KBr): 3448, 3120, 2935, 2844, 1579, 1542, 1435, 1287, 1182, 1072 cm^{-1}; ^1H NMR ($CDCl_3$ 200 MHz): δ (ppm) 8.48(*d*, 1H, *J* = 7.44 Hz), 7.78 (*t*, 1H, *J* = 7.41 Hz), 7.55–7.52(*m*, 2H), 6.98(*s*, 1H), 3.88(*s*, 3H), 2.53(*s*, 3H), 1.25(*s*, 9H). ^{13}C ($CDCl_3$, 50 MHz): 170.4, 169.7, 163.5, 160.4, 146.8, 142.9, 132.3, 123.9, 123.3, 119.6, 118.1, 110.6, 51.3, 30.1, 28.2, 24.6. Anal. Calc. for $C_{19}H_{21}N_7S$: C: 60.14, H: 5.58, N: 25.84. Found: C: 60.22, H: 5.69, N: 25.57.

*5.6.7. N-isopropyl-4-methyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)pyrimidin-2-amine (**22**)*

Yield: 67%; mp: 97–99 °C; MS: 366 (M + 1); IR (KBr): 3452, 3123, 2930, 2854, 1587, 1555, 1436, 1281, 1185, 1062 cm^{-1}; ^1H NMR ($CDCl_3$ 200 MHz): δ (ppm) 8.41(*d*, 1H, *J* = 5.20 Hz), 7.71(*t*, 1H, *J* = 4.86 Hz), 7.49–7.42(*m*, 2H), 6.68(*s*, 1H), 3.85(*s*, 3H), 3.64–3.60 (*m*, 1H), 2.28(*s*, 3H), 1.55(*d*, 6H, *J* = 3.83 Hz). ^{13}C ($CDCl_3$, 50 MHz): 170.5, 169.6, 163.8, 160.4, 146.8, 142.9, 132.3, 123.9, 123.4, 119.5, 118.1, 110.7, 47.3, 27.8, 24.4, 24.3. Anal. Calc. for $C_{18}H_{19}N_7S$: C: 59.16, H: 5.24, N: 26.83. Found: C: 59.23, H: 5.34, N: 26.52.

*5.6.8. 4-Methyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-N-(2-morpholinoethyl)pyrimidin-2-amine (**23**)*

Yield: 62%; mp: 216–218 °C; MS: 437 (M + 1); IR (KBr): 3448, 3122, 2927, 2848, 1580, 1565, 1434, 1283, 1187, 1065 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.47(*d*, 1H, *J* = 7.30 Hz), 7.76(*t*, 1H, *J* = 7.44 Hz), 7.53–7.51(*m*, 2H), 6.96(*s*, 1H), 5.79(bs, 1H), 3.87(*s*, 3H), 3.53(*m*, 4H), 3.25(*m*, 2H), 2.61(*m*, 6H), 2.32(*s*, 3H). ^{13}C ($CDCl_3$, 50 MHz): 171.2, 169.5, 163.5, 160.4, 146.6, 142.8, 132.1, 123.9, 123.8, 119.5, 118.1, 110.6, 67.2, 57.4, 53.5, 41.3, 27.8, 24.4. Anal. Calc. for $C_{21}H_{24}N_8OS$: C: 57.78, H: 5.54, N: 25.67. Found: C: 57.70, H: 5.46, N: 25.74.

*5.6.9. 4-Methyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-N-(3-morpholinopropyl)pyrimidin-2-amine (**24**)*

Yield: 61%; mp: 235–237 °C; MS: 451 (M + 1); IR (KBr): 3456, 3112, 2935, 2854, 1580, 1537, 1436, 1290, 1198, 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.41(*d*, 1H, *J* = 7.78 Hz), 7.84(*m*, 2H), 7.54 (*d*, 1H, *J* = 4.08 Hz), 7.25(bs, 1H), 6.82(*s*, 1H), 3.82(*s*, 3H), 3.66–3.52 (*m*, 6H), 2.37–2.19(*m*, 9H), 1.62–1.52(*m*, 2H). ^{13}C ($CDCl_3$, 50 MHz): 171.2, 169.4, 163.4, 160.4, 146.8, 142.9, 132.3, 123.8, 123.3, 119.4, 118.2, 110.6, 65.9, 61.2, 54.1, 30.2, 27.7, 24.6, 24.5. Anal. Calc. for $C_{22}H_{26}N_8OS$: C: 58.65, H: 5.82, N: 24.87. Found: C: 56.86, H: 5.74, N: 24.89.

*5.6.10. N^1,N^1-dimethyl-N^2-(4-methyl-6-(5-methyl-5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)pyrimidin-2-yl)ethane-1,2-diamine (**25**)*

Yield: 54%; mp: 197–199 °C; MS: 395 (M + 1); IR (KBr): 3436, 3106, 2940, 2848, 1582, 1547, 1435, 1285, 1190, 1067 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.41(*d*, 1H, *J* = 7.64 Hz), 7.86(*m*, 2H), 7.54–7.53(*m*, 1H), 7.23(bs, 1H), 6.89(*s*, 1H), 3.82(*s*, 3H), 3.35(*m*, 2H),

2.82(*m*, 2H), 2.39(*s*, 3H), 2.22(*s*, 6H); ^{13}C ($CDCl_3$, 50 MHz): 172.1, 169.6, 164.5, 161.1, 147.5, 142.9, 132.4, 123.7, 123.6, 119.5, 118.6, 110.9, 58.7, 45.2, 42.3, 27.7, 24.5. Anal. Calc. for $C_{19}H_{22}N_8S$: C: 57.85, H: 5.62, N: 28.40. Found: C: 57.65, H: 5.43, N: 28.49.

*5.6.11. N^1,N^1-diethyl-N^2-(4-methyl-6-(5-methyl-5H-[1,2,4]triazino [5,6-b]indol-3-ylthio)pyrimidin-2-yl)ethane-1,2-diamine (**26**)*

Yield: 55%; mp: 217–219 °C; MS: 423 (M + 1); IR (KBr): 3442, 3109, 2925, 2862, 1585, 1537, 1435, 1290, 1186, 1072 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.43(*d*, 1H, *J* = 7.64 Hz), 7.88(*m*, 1H), 7.52–7.46(*m*, 2H), 6.85(*s*, 1H), 3.86(*s*, 3H), 3.36(*m*, 2H), 3.12(*m*, 6H), 2.34(*s*, 3H), 1.05(*t*, 6H, *J* = 6.78 Hz). ^{13}C ($CDCl_3$, 50 MHz): 171.3, 169.6, 163.2, 160.7, 146.8, 142.7, 132.4, 123.9, 123.4, 119.8, 118.5, 110.1, 52.2, 47.3, 46.9, 27.7, 24.3, 12.2. Anal. Calc. for $C_{21}H_{26}N_8S$: C: 56.69, H: 6.20, N: 26.52. Found: C: 59.75, H: 6.03, N: 26.66.

Acknowledgement

L.G. thanks the University Grant Commission, New Delhi and N. S. thanks the Council of Scientific and Industrial Research (India) for the award of Senior Research Fellowship. We are also thankful to S. A.I.F. Division, CDRI, Lucknow for providing spectroscopic data. Thanks are also due to Mr. M. P. S. Negi, Division of Biometry & Statistics, CDRI for data analysis. CDRI communication No: 7823.

References

[1] WHO, Available at: <http://www.who.int/leishmaniasis/burden/en>.
[2] S.L. Croft, G.H. Coombs, Trends. Parasitol. 19 (2003) 502–508.
[3] M. Ouellette, J. Drummelsmith, B. Papadopoulou, Drug. Resist. Updat. 7 (2004) 257–266.
[4] S.L. Croft, S. Sundar, A.H. Fairlamb, Clin. Microbiol. Rev. 19 (2006) 111–126.
[5] G. Harms, H. Feldmeier, Trop. Med. Int. Health. 7 (2002) 479–488.
[6] R.A. Neal, S.L. Croft, J. Antimicrob. Chemother. 14 (1984) 463–475.
[7] B. Nare, J. Luba, L.W. Hardy, S. Beverly, Parasitology 114 (1997) S101–S110.
[8] S.F. Chowdhury, V.B. Villamor, R.H. Guerrero, I. Leal, R. Brun, S.L. Croft, J. M. Goodman, L. Maes, L.M. Ruiz-Perez, D.G. Pacanowska, I.H. Gilbert, J. Med. Chem. 42 (1999) 4300–4312.
[9] H. Lee, W. Chui, Bioorg. Med. Chem. 7 (1999) 1255–1262.
[10] Y. Yuthavong, T. Vilaivan, N. Chareonsethakul, S. Kamchonwongpaisan, W. Sirawaraporn, R. Quarrell, G. Lowe, J. Med. Chem. 43 (2000) 2738–2744.
[11] S. Kamchonwongpaisan, R. Quarrell, N. Charoensetakul, R. Ponsinet, T. Vilaivan, J. Vanichtanankul, B. Tarnchompoo, W. Sirawaraporn, G. Lowe, Y. Yuthavong, J. Med. Chem. 47 (2004) 673–680.
[12] A. Kumar, S.B. Katiyar, S. Gupta, P.M.S. Chauhan, Eur. J. Med. Chem. 41 (2006) 106–113.
[13] N. Sunduru, A. Agarwal, S.B. Katiyar, N.N. Goyal, S. Gupta, P.M.S. Chauhan, Bioorg. Med. Chem. 14 (2006) 7706–7715.
[14] N.N. Sunduru, S. Palne, P.M.S. Chauhan, S. Gupta, Eur. J. Med. Chem. 44 (2009) 2473–2481.
[15] V.J. Ram, Arch. Pharm. 313 (1980) 108–113.
[16] J.M.Z. Gladych, R. Hornby, J.H. Hunt, D. Jack, J.J. Boyle, R.J. Ferlauto, R.F. Haff, C. G. Kormendy, F.J. Stanfield, R.C. Stewart, J. Med. Chem. 15 (1972) 277–281.
[17] J.L. Kgokong, P.P. Smith, G.M. Matsabisa, Bioorg. Med. Chem. 13 (2005) 2935–2942.
[18] R.C. Elederfield, H.H. Rembges, J. Org. Chem. 32 (1967) 3809–3814.
[19] W. Zhang, Org. Lett. 5 (2003) 1011–1013.
[20] A.S. Gupta, R.S. Sundar, N. Goyal, Antimicrobial. Agents. Chemother. 49 (2005) 3776–3783.
[21] D.J. Finney, Probit Analysis, third ed. Cambridge University Press, 1971.
[22] A.G. Tempone, A.C.M.P. da Silva, C.A. Brandt, F.S. Martinez, S.E.T. Borborema, M. A.B. da Silveira, H.F. de Andrade Jr., Antimicrobial. Agents. Chemother. 49 (2005) 1076–1080.
[23] W. Huber, J.C. Koella, Acta. Trop. 55 (1993) 257–261.