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ORIGINAL ARTICLE

Synthesis, SAR and antibacterial activity of hybrid chloro, dichloro-phenylthiazolyl-*s*-triazines

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KEYWORDS

s-Triazines;
Amines;
Linker bridge;
MIC;
Broth dilution assay

Abstract A series of hybrid novel chloro (**1a**–**9a**) and dichloro (**10b**–**18b**) phenylthiazolyl-s-triazine were synthesized and subsequently subjected to their antibacterial activity against three gram positive *viz*. *Lactobacillus casei* (NCIM-2651); *Bacillus cereus* (NCIM-2458); *Staphylococcus aureus* (NCIM-2120) and three gram negative viz *Salmonella typhimurium* (NCIM-2501); *Escherichia coli* (NCIM-2065); *Klebsiella aerogenes* (NCIM-2098). The SAR studies around the lead compound revealed that introduction of electron withdrawing groups and amino (–NH–) and mercapto (–S–) linker bridge seemed more promising towards antibacterial activity. Moreover, the virtual Molinspiration screenings are in compliance with Ghose's rule.

1. Introduction

Brewing public health crisis has been obsessed by two key factors, the emergence of antibacterial resistance in imperative pathogenic species and a downturn in the figure of new antibacterial agents coming through pharmaceutical company pipelines (News and Analysis, 2010), with only six new antibiotics approved since 2003 (Fox, 2006). Consistent with historical data, the preponderance of antibiotics under clinical development are natural products or derivatives thereof (Donadio et al., 2010). However, WHO and many experts recommend policies encouraging the research and development of new, cost effective and innovative drugs (WHO, 2005).

s-Triazine analogues have been already gained substantial attention due to their cost effectiveness and diverse

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antimicrobial activities such as antimalarial (Manohar et al., 2010; Kumar et al., 2010), antibacterial (Nishigaki et al., 1969; Zhou et al., 2006; Srinivas et al., 2006), antitumor (Saczewski and Bulakowska, 2006; Matsuno et al., 2010), antitubercular (Sunduru et al., 2010) etc. Previously we have reported several triazine derivatives bearing pendent thiazole motif showing considerable antibacterial activity by the virtue of the presence of different lipophillic and electron withdrawing groups (Singh et al., 2010; Gahtori et al., 2009, 2010). Further, we have recognized a lead fragment pertaining to structural feature necessary for hybrid thiazole-*s*-triazine to act as potent antibacterials. On the basis of these results and as a part of our endeavor on novel s-triazine antibacterial agents, we decided to focus our attention around our identified lead and disclose synthesis, SAR and antibacterial activities of newer hybrid thiazole-*s*-triazine antibacterials, by assimilation of various pharmacophoric groups.

2. Chemistry

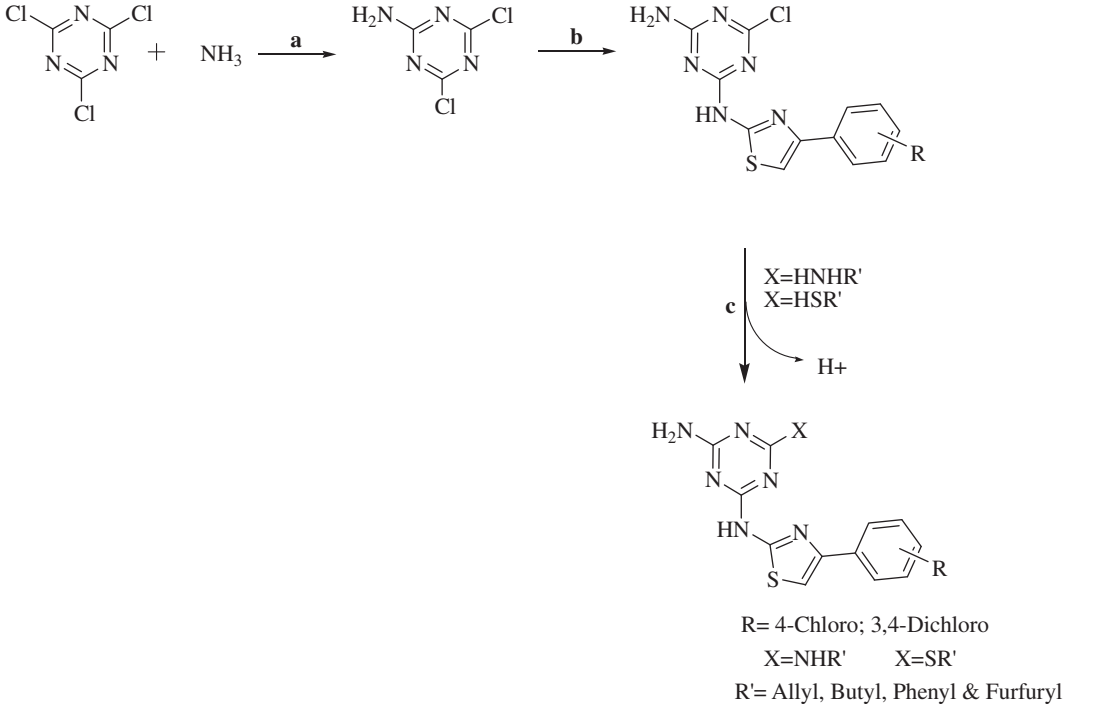
Two novel series of hybrid chloro (Series A) and dichloro (Series B) phenylthiazolyl-*s*-triazine derivatives were synthesized to comprehend the role of electron withdrawing viz. chloro and di-chloro substitution on phenylthiazolyl-s-triazine as well as the influence of amino (–NH–) and mercapto (–S–) linker bridge between phenylthiazole and s-triazine, towards antibacterial activity. Synthetic methods adopted for making compounds are illustrated in Scheme 1. Synthesis of targeted compounds was performed by means of nucleophilic displacement of third chlorine atom of di-substituted-1,3,5-triazine in the presence of $NaHCO_3$ in dioxane under nitrogen atmosphere to furnish clubbed chloro and di-chloro phenylthiazolyl-*s*-triazine derivatives (Fig. 1).

3. Antibacterial efficacy

All the targeted compounds (**1a**–**9a**) and (**10b**–**18b**) were screened for their minimum inhibitory concentration (MIC) against three selected gram-positive organisms viz. *Lactobacillus casei* (NCIM-2651); *Bacillus cereus* (NCIM-2458); *Staphylococcus aureus* (NCIM-2120) and three gram negative viz *Salmonella typhimurium* (NCIM-2501); *Escherichia coli* (NCIM-2065); *Klebsiella aerogenes* (NCIM-2098) by the broth dilution method as recommended by the EUCAST 2000 procedure (EUCAST, 2000), with minor modifications using Streptomycin and Penicillin as standard antibacterial agents. Solutions of the tested compounds and reference drugs were dissolved in dimethylsulfoxide (DMSO) at prepared concentrations of 100, 50, 25, 12.5, 6.25, 3.125 μg/ml. The chemical compound-broth medium in serial test tube dilution was inoculated with equal amount of each bacterium and was incubated on a rotary shaker at 37 °C for 24 h at 150 rpm. The incubation chamber was kept humid and at the end of the incubation period, MIC values were recorded as the lowest concentration of the substance that gave no visible turbidity, i.e., no growth of inoculated bacteria. In an effort to order the scope of antibacterial activity in detail, a computational study of all titled compounds was performed by using Molinspiration online property calculation toolkit (http://www.molinspiration.com/cgi-bin/properties).

4. Results and discussion

Two series of chloro (**A**) and dichloro (**B**) phenylthiazolyl-s-triazine derivatives were synthesized and found in agreement with spectroscopic analysis, e.g., IR spectra of all the products



R= 4-Chloro; 3,4-Dichloro

X=NHR' X=SR'

R'= Allyl, Butyl, Phenyl & Furfuryl

Scheme 1 Reagents and condition: (a) $KHCO_3$, aq Dioxane 0–5 °C, (b) 4-chloro phenylthiazole-2-amine and 3,4-dichloro phenylthiazolyl $NaHCO_3$, aq dioxane 45–50 °C (c) $NaHCO_3$, dioxane 90–100 °C under nitrogen atmosphere.

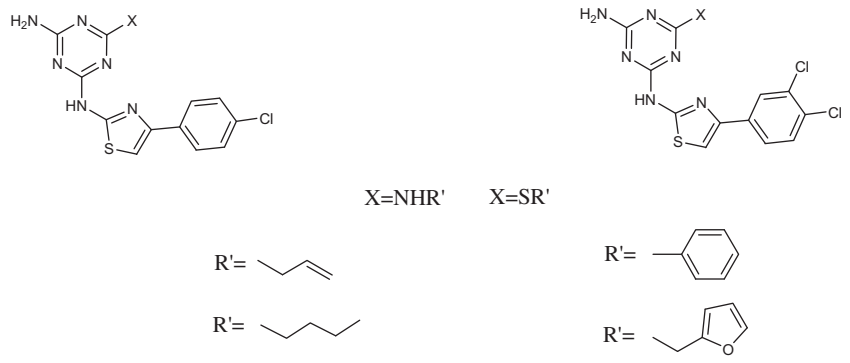
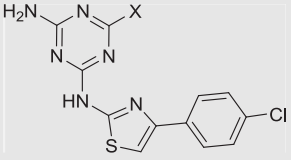


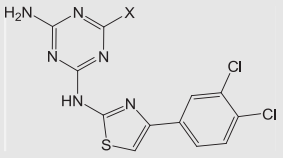
Figure 1 Chloro (**1a**–**9a**) and dichloro (**10b**–**18b**) phenylthiazolyl-*s*-triazine derivatives.

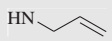
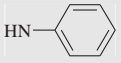
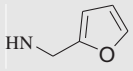
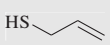
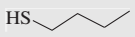
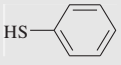
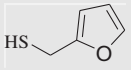
Table 1 Structural modification and MIC of mono-chloro phenylthiazolyl-*s*-triazine derivatives (Series A).



Compounds	X	MIC (μg/ml)					
		Gram positive			Gram negative		
		Lactobacillus casei (NCIM-2651)	Bacillus cereus (NCIM-2458)	Staphylococcus aureus (NCIM-2120)	Salmonella typhimurium (NCIM-2501)	Escherichia coli (NCIM-2065)	Klebsiella aerogenes (NCIM-2098)
1a	Cl	>200	100	50	>200	100	100
2a	HN	50	50	25	50	100	100
3a	HN	25	50	25	25	25	50
4a	HN	3.125	6.25	3.125	6.25	12.5	50
5a	HN	25	25	12.5	50	50	100
6a	HS	50	50	25	50	100	100
7a	HS	25	25	12.5	25	25	50
8a	HS	3.125	6.25	3.125	6.25	6.25	50
9a	HS	25	50	25	50	25	50
Penicillin		1.562	1.562	3.125	12.5	12.5	12.5
Streptomycin		12.5	6.25	6.25	1.562	3.125	1.562

Table 2 Structural modification and MIC of di-chloro phenylthiazolyl-*s*-triazine derivatives (Series B).



Compounds	X	MIC (μg/ml)					
		Gram positive			Gram negative		
		Lactobacillus casei (NCIM-2651)	Bacillus cereus (NCIM-2458)	Staphylococcus aureus (NCIM-2120)	Salmonella typhimurium (NCIM-2501)	Escherichia coli (NCIM-2065)	Klebsiella aerogenes (NCIM-2098)
10b	Cl	100	50	50	>200	100	100
11b		25	25	12.5	50	25	100
12b		12.5	25	12.5	25	50	100
13b		3.125	3.125	6.25	6.25	6.25	50
14b		12.5	25	12.5	50	25	100
15b		50	100	25	100	50	200
16b		12.5	25	12.5	50	50	200
17b		3.125	3.125	6.25	6.25	12.5	25
18b		25	25	25	50	50	100
Penicillin		1.562	1.562	3.125	12.5	12.5	12.5
Streptomycin		12.5	6.25	6.25	1.562	3.125	1.562

1a–**9a** and **10b**–**18b** nearer at 3450 and 3350 cm^{-1} attributable to bonded primary amino groups is observed, where as secondary –NH linker between thiazole and *s*-triazine appears in the region 3320–3140 cm^{-1}. Many strong absorption bands at 850–670 cm^{-1} confirm the existence of aromatic ring. Stronger band at 920, 995 cm^{-1} and medium band at 1650 cm^{-1} are indicative of vinyl groups. The ^1H NMR spectrums reveal a signal corresponding to the NH_2 group at 4.12–4.48 ppm. Further, these compounds are subsequently subjected to their in vitro antibacterial efficacy in terms of minimum inhibitory concentration, MIC (Tables 1 and 2). The preliminary SAR screening of these series indicates that many compounds exhibited significant to almost no activity against tested microorganisms, among them compounds **4a, 8a, 13b** and **17b** displayed interesting activities for some Gram positive and Gram negative bacterium in comparison to standard drugs, penicillin and streptomycin. The results also divulge more susceptibility of these compounds for selected Gram positive bacteria viz. *Lactobacillus casei* (NCIM-2651); *Bacillus cereus* (NCIM-2458) and *Staphylococcus aureus* (NCIM-2120).

Compounds **4a, 8a, 13b** and **17b** displayed more potent activities than streptomycin against *L. casei*, **12, 14b** and **16b** are substantially active, where as **3a, 5a, 7a, 9a, 11b** and **18b** are moderately active and rest of the compounds were found weakly active for the same strain. More potent activities are also reported against *B. cereus* by **13b** and **17b** where as **4a** and **8a** exhibited equipotent activity, while rest of the compounds were found moderate to weakly active for the same in comparison to streptomycin. *S. aureus* has documented the most susceptibilities towards synthesized compounds

Table 3 Molinspiration calculations (Series A & B).

Sr. no.	miLogP	TPSA	natoms	MW	nON	nOHNH	nrotb	Volume	nViolations	MOA*
1a	3.82	89.62	21	339.21	6	3	3	251.71	0	GPCRL: −0.11, ICM: −1.41, KI: −0.10, NCL: −2.03
2a	4.07	101.65	24	359.85	7	4	6	295.11	0	GPCRL: −0.22, ICM: −1.20, KI: −0.09, NCL: −1.48
3a	4.86	101.65	25	375.89	7	4	7	317.54	0	GPCRL: −0.06, ICM: −1.02, KI 0.05, NCL: −1.20
4a	5.14	101.65	27	395.88	7	4	5	321.99	1	GPCRL: −0.12, ICM: −0.87, KI 0.08, NCL: −1.45
5a	4.08	114.79	27	399.87	8	4	6	320.35	0	GPCRL: −0.40, ICM: −1.07, KI: −0.09, NCL: −2.09
6a	4.94	89.62	24	376.90	6	3	6	300.84	0	GPCRL: −0.62, ICM: −1.48, KI: −0.65, NCL: −1.79
7a	5.74	89.62	25	392.94	6	3	7	323.27	1	GPCRL: −0.39, ICM: −1.22, KI: −0.68, NCL: −1.51
8a	5.28	89.62	27	412.93	6	3	5	327.71	1	GPCRL: −0.45, ICM: −1.19, KI: −0.14, NCL: −1.77
9a	5.15	102.76	27	416.92	7	3	6	326.08	1	GPCRL: −0.55, ICM: −1.34, KI: −0.44, NCL: −2.25
10b	4.43	89.62	22	373.66	6	3	3	265.25	0	GPCRL: −0.10, ICM: −1.33, KI: −0.10, NCL: −1.91
11b	4.67	101.65	25	394.29	7	4	6	308.65	0	GPCRL: −0.21, ICM: −1.14, KI: −0.10, NCL: −1.41
12b	5.47	101.65	26	410.33	7	4	7	331.08	1	GPCRL: −0.06, ICM: −0.97, KI 0.03, NCL: −1.13
13b	5.75	101.65	28	430.32	7	4	5	335.52	1	GPCRL: −0.12, ICM: −0.82, KI 0.07, NCL: −1.39
14b	4.69	114.79	28	434.31	8	4	6	333.89	0	GPCRL: −0.39, ICM: −1.02, KI: −0.10, NCL: −2.01
15b	5.55	89.62	25	411.34	6	3	6	314.37	1	GPCRL: −0.60, ICM: −1.41, KI: −0.64, NCL: −1.70
16b	6.34	89.62	26	427.39	6	3	7	336.80	1	GPCRL: −0.37, ICM: −1.16, KI: −0.67, NCL: −1.44
17b	5.88	89.62	28	447.38	6	3	5	341.25	1	GPCRL: −0.43, ICM: −1.13, KI: −0.15, NCL: −1.70
18b	5.76	102.76	28	451.36	7	3	6	339.62	1	GPCRL: −0.53, ICM: −1.28, KI: −0.43, NCL: −2.15
Penicillin G	1.82	86.71	23	320.37	6	2	4	287.55	0	GPCRL: −0.56, ICM: −0.41, KI: −0.74, NCL: −0.45
r^2 value#	0.17	0.07	0.57	0.27	0.22	0.10	0.07	0.51		

* G-protein coupled receptor ligand: GPCRL; Ion channel modulator: ICM; Kinase inhibitor: KI; Nuclear receptor Ligand: NCL.
\# Average MIC.

(MIC = 3.125–50 μg/ml), among them compounds **4** and **8** displayed equipotent activity to Penicillin. *S. typhimurium* achieved the moderate to weak activity by these compounds, excluding **4a, 8a, 13b** and **17b**. Compounds **8a** and **13b** displayed potent activities than penicillin for Gram negative *E. coli*, **4a** and **17b** were found equipotent where as rest of the compounds were found weakly active for the same strains. Along with promising activities, the results also unveil that *K. aerogenes* (MIC = 25–200 μg/ml) has shown no activity towards synthesized compounds.

Analogues were synthesized in which chlorine of **1a** was replaced by various aliphatic and aromatic (heterocyclic) fragments like allyl, butyl, phenyl and methyl furanyl bearing different amine (–NH–) and mercapto (–S–) as a linker bridge. From series A, **4a** and **8a** was found to be the most active analogue. The phenyl fragment appears to be an indispensable trait for optimal activity and the more prominent activity was also observed for gram negative bacteria when amine (**4a**) was replaced by mercapto bridge (**8a**) and it was found more potent to the standard drug penicillin for some gram negative bacteria such as *S. typhimurium* and *E. coli*. The **4a** and **8a** were found equipotent to penicillin and more potent than streptomycin for gram positive bacteria *S. aureus*.

Similarly analogues of compound **10b** (Series B) having dichloro phenyl thiazole portion were synthesized by replacing chlorine of *s*-triazine by various aliphatic and aromatic (heterocyclic) groups by incorporating amine (–NH–) and mercapto (–S–) as a linker bridge. Among them the most active triazine were **13b** and **17b**, again the phenyl fragment was characterized as vital facet for antibacterial activity and the more pronounced activity was also observed for gram negative bacteria when amine (**13b**) was replaced by mercapto bridge (**17b**) and it was found equipotent to the standard drug penicillin for *E. coli*. Besides that, presence of intact chlorine (**10b**) does not have any marked influence on antibacterial activity. Further, activity flourishes upon replacement of chlorine of analogue **1** by other aliphatic and aromatic including heterocyclic moieties. But, slight increase in activity was observed in furan fragment connected by amine bridge rather than their corresponding mercapto bridge in a comparison test. On comparing the MICs of allyl fragments (**11b** and **15b**), presence of amine bridge leads to increase in activity (**11b**) with their corresponding mercapto bridge (**15b**) but could not translate into significant antibacterial activity.

It is quite ambiguous from the virtual studies, that title compounds show plausible mode of antibacterial action by nuclear receptor ligand binding domain (Table 3), which trigger key steps during development, and induce or inhibit cellular proliferation, differentiation and death. It is apparent with accordance of natoms (r^2 = 0.57) and volume (r^2 = 0.51) maximize the number of hydrophobic contacts, thus contributing to the stability of the complex and the selectivity of the pocket for the cognate ligand. Whilst the violation of Lipinski's rule for lipophilicity showed no selectivity, as the corresponding phenyl groups showed maximum efficacy indicating the applicability of Ghose rule for these set of compounds.

5. Conclusion

As concluding remark, we can see that comparatively strong activity is demonstrated by di-chloro phenylthiazolyl-*s*-triazine than monochloro phenylthiazolyl-*s*-triazine derivatives. Introduction of more electronic withdrawing groups like chlorine to phenylthiazole may improve the antibacterial activities in case of some amino derivatives. Further, by changing the substitution pattern with allyl group from butyl improves the antibacterial activity in a significant manner, which in turn also flourishes by methyl furanyl group but the strongest activity is demonstrated by phenyl groups, among them compounds **4a, 8a, 13b** and **17b** were found more potent than streptomycin for some gram positive microorganism. Ultimately, it will recommend that presence of –S– linker bridge between *s*-triazine

and phenylthiazole lessen the antibacterial activity than their amine bridge equivalents.

6. Experimental

All chemicals were of analytical grade and used without further purification. Melting points were determined on a Veego, Model No. MPI open capillary melting point apparatus and were uncorrected. The completion of reaction was checked by thin layer chromatography (TLC) using silica gel-GF254 (0.5 mm thickness, Benzene, Ethyl acetate and 0.1 M alcoholic KOH solvent system) and spots were visualized under ultraviolet (UV) radiation. FTIR (in KBr) were recorded on Perkin Elmer-Spectrum RX-I spectrophotometer. ^{1}H NMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer in DMSO-d_6 using TMS as internal standard (chemical shift in δ, ppm). Elemental analysis was carried out on a Vario EL III elemental analyzer and was within $\pm 0.4\%$ of the theoretical values.

7. General procedure for synthesis of mono-chloro phenylthiazolyl-*s*-triazine derivatives (1a–9a)

The synthesis of targeted compounds **1a**–**9a** is shown in Scheme 1. The 2-Amino-4,6-dichloro-1,3,5-triazine (**1**) was prepared according to published procedure (Thruston et al., 1951), under ammonia stream at constantly maintained temperature 0–5 °C with diethyl cellosolve, filtered under vacuum and recrystallized from water. The resulting mass **1** (1 mol) and synthesized (4′-chlorophenyl)thiazolyl-2-amine (1 mol) mixture were heated and $NaHCO_3$ (1 mol) was added slowly at room temperature 40–45 °C under aq. Dioxane with constant stirring for 10 mins, filtered immediately under vacuum to furnish (4′-Chlorophenyl)thiazolyl-*s*-triazine (**1a**). The various substituted amines (0.1 mmol) and substituted thiols (0.1 mmol) were added to **1a** in 100 ml dioxane under nitrogen atmosphere in $NaHCO_3$ (0.1 mmol) at 120 °C for 6 h. The product was filtered, washed with water, and purified by flash column chromatography (Silica gel mesh size 100–200) using methanol/ethyl acetate (1:20 v/v) as eluent to afford pure mono-chloro phenylthiazolyl-*s*-triazine derivatives **2a**–**9a**.

*7.1. 6-Chloro-N^2-(4-(4-chlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4-diamine (**1a**)*

Physical state: Brownish yellow solid; %Yield: 91.65; mp: 158–160 °C; R_f (Silica gel GF254): 0.2758 (Benzene: Ethyl acetate: 0.1 M alco. KOH 2:3:5); FTIR (KBr, cm^{-1}): 1353 (C–N), 1694 (C═N), 1665 (C═C), 1473 (C═C Ar bending), 3323 & 3456 (NH_2), 3310 (NH), 3030 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 4.33 (s, 2H, NH_2), 4.82 (s, 1H, NH), 6.50 (s, 1H, 1-CH thiazole), 7.05 (d, 2H, Ar–H), 7.25 (d, 2H, Ar–H); Anal. Calcd for $C_{12}H_8C_{12}N_6S$: C-42.49, H-2.38, N-24.78, Found C-42.62, H-2.37, N-24.47.

*7.2. N^2-allyl-N^4-(4-(4-chlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**2a**)*

Physical state: Light brown solid; %Yield: 84.22; mp: 94–96 °C; R_f (Silica gel GF254): 0. 8012 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}): 1350 (C–N), 1688 (C═N), 1669 (C═C cis/vinyl), 1474 (C═C Ar bending), 3324, 3458 (NH_2), 3314 (NH), 3037 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 3.72 (t, 2H, CH_2), 4.12 (s, 2H, NH_2), 4.45 (m, 1H, NH), 4.68 (s, 1H, NH), 5.14 (d, 2H, $J = 9$, CH_2═C ethylene), 5.79–5.93 (m, 1H, CH═C ethylene), 6.38 (s, 1H, 1-CH thiazole), 7.02 (d, 2H, Ar–H), 7.20 (d, 2H, Ar–H); Anal. Calcd for $C_{15}H_{14}ClN_7S$: C-50.07, H-3.92, N-27.25 Found C-49.98, H-3.97, N-27.10.

*7.3. N^2-butyl-N^4-(4-(4-chlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**3a**)*

Physical state: Brownish Yellow solid; %Yield: 64.77; mp: 146–148 °C; R_f (Silica gel GF254): 0.7867 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}): 1103 (Ar–Cl), 1361 (C–N), 1687 (C═N), 1475 (C═C Ar bending), 2880 (CH_2 Stretch), 3320, 3454 (NH_2), 3317 (N–H), 3023 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 1.05 (t, 3H, –CH_3), 1.38 (m, 2H, –CH_2–), 1.58 (m, 2H, –CH_2–), 3.21 (m, 2H, N–CH_2–), 3.52 (s, 2H, NH_2), 4.23 (m, 1H, NH), 4.76 (s, 1H, NH), 6.62 (s, 1H, 1-CH thiazole), 6.92 (d, 2H, Ar–H), 7.11 (d, 2H, Ar–H); Anal. Calcd for $C_{16}H1_8ClN_7S$: C-51.13, H-4.83, N-26.08 Found C-51.36, H-4.86, N-26.28.

*7.4. N^2-(4-(4-chlorophenyl)thiazol-2-yl)-N^4-phenyl-1,3,5-triazine-2,4,6-triamine (**4a**)*

Physical state: Light yellow solid; %Yield: 62.54; mp: 112–114 °C; R_f (Silica gel GF254): 0.7142 (Benzene: Ethyl acetate: 0.1 M KOH 3:3:4); FTIR (KBr, cm^{-1}): 1033 (Ar–Cl), 1252 (C–N), 1639 (C═N), 1445 (C═C, Ar bending), 3230, 3410 (NH_2, two peaks), 3324 (N–H broad peak), 3041(Ar–C–H); ^{1}H NMR (400 mHz, δ ppm) 4.22 (s, 2H, NH_2), 4.58 (s, 1H, NH), 4.81 (s, 1H, NH), 6.42 (s, 1H, 1-CH thiazole), 6.89 (d, 2H, Ar–H), 7.04 (d, 2H, Ar–H), 7.22 (d, 1H, Ar–H), 7.39 (t, 2H, Ar–H), 7.52 (d, 2H, Ar–H); Anal. Calcd for $C_{18}H_{14}ClN_7S$: C-54.61, H-3.56, N-24.77 Found C-54.12, H-3.78, N-24.79.

*7.5. N^2-(4-(4-chlorophenyl)thiazol-2-yl)-N^4-(furan-2-ylmethyl)-1,3,5-triazine-2,4,6-triamine (**5a**)*

Physical state: Whitish brown solid; %Yield: 68.25; mp: 49–51 °C; R_f (Silica gel GF254): 0.8216 (Benzene: Ethyl acetate: 0.1 M KOH 3:3:4); FTIR (KBr, cm^{-1}) 1063 (Ar–Cl), 1352 (C–N), 1683 (C═N), 1470 (C═C Ar bending), 3322, 3456 (NH_2), 3312 (NH), 3032 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 3.32 (s, 2H, –CH_2), 4.27 (s, 2H, NH_2), 4.87 (s, 1H, NH), 4.94 (s, 1H, NH), 5.88 (d, 1H, 3′CH-furan), 6.18 (t, 1H, 4′CH-furan), 6.44 (s, 1H, 1-CH thiazole), 6.76 (d, 1H, 5′CH-furan), 7.04 (d, 2H, Ar–H), 7.23 (d, 2H, Ar–H); Anal. Calcd for $C_{17}H_{13}ClN_6OS_2$: C-48.98, H-3.14, N-20.16 Found C-48.50, H-3.33, N-20.49.

*7.6. 6-(Allylthio)-N^2-(4-(4-chlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4-diamine (**6a**)*

Physical state: Dark Brown solid; %Yield: 52.36; mp: 91–93 °C; R_f (Silica gel GF254): 0.7661 (Benzene: Ethyl acetate: 0.1 M KOH 3:3:4); FTIR(KBr, cm^{-1}): 1053 (Ar–Cl), 1252 (C–N), 1639 (C═N), 1445 (C═C Ar bending), 3230, 3410

(NH_2, two peaks), 3041 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 3.68 (t, 2H, CH_2), 4.10 (d,2H,NH_2), 4.36 (s, 1H, NH), 5.10 (d, 2H, J = 9, CH_2═C ethylene), 5.68 (m, 1H, CH═CH_2 ethylene), 6.34 (s, 1H, 1-CH thiazole), 6.99 (d, 2H, Ar–H), 7.07 (d, 2H, Ar–H); Anal. Calcd for $C_{15}H_{13}ClN_6S_2$: C-47.80, H-3.48, N-22.30 Found C-47.62, H-3.57, N-22.67.

*7.7. 6-(Butylthio)-N^2-(4-(4-chlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4-diamine (**7a**)*

Physical state: Light brown liquid; %Yield: 75.24; mp: 10–12 °C; R_f (Silica gel GF254): 0.7602 (Benzene: Ethyl acetate: 0.1 M KOH 3:3:4); FTIR(KBr, cm^{-1}): 1052 (Ar–Cl), 1246 (C–N), 1628 (C═N), 1435 (C═C Ar bending), 2850 (CH_2 stretching) 3250, 3453(NH_2, two peaks), 3022 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm) 1.01 (t, 3H, –CH_3), 1.30 (m, 2H, –CH_2–), 1.39 (m, 2H, –CH_2–), 3.12 (m, 2H, N–CH_2–), 3.42 (s, 2H, NH_2), 6.52 (s, 1H, 1-CH thiazole), 6.87 (d, 2H, Ar–H), 7.08 (d, 2H, Ar–H); Anal. Calcd for $C_{16}H_{17}ClN_6S_2$: C-48.91, H-4.36, N-21.39 Found C-48.32, H-4.98, N-21.36.

*7.8. N^2-(4-(4-chlorophenyl)thiazol-2-yl)-6-(phenylthio)-1,3,5-triazine-2,4-diamine (**8a**)*

Physical state: Yellow solid; %Yield: 74.67; mp: 135–137 °C; R_f (Silica gel GF254): 0. 7576 (Benzene: Ethyl acetate: 0.1 M KOH 3:3:4); FTIR (KBr, cm^{-1}): 1039 (Ar–Cl), 1242 (C–N), 1654 (C═N), 1437 (C═C Ar bending), 3300, 3457 (NH_2, two peaks), 3064 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 4.19 (s, 2H, NH_2), 4.90 (s, 1H, NH), 6.35 (s, 1H, 1-CH thiazole), 7.02(d, 2H, Ar–H), 7.18 (d, 2H, Ar–H), 7.22 (t, 1H, Ar–H), 7.27 (d, 2H, Ar–H), 7.32 (d, 2H, Ar–H); Anal. Calcd for $C_{16}H_{17}ClN_6S_2$: C-48.91, H-4.36, N-21.39 Found C-48.32, H-3.98, N-22.06.

*7.9. N^2-(4-(4-chlorophenyl)thiazol-2-yl)-6-(furan-2-ylmethylthio)-1,3,5-triazine-2,4-diamine (**9a**)*

Physical state: Dark brown solid; %Yield: 58.88; mp: 87–89 °C; R_f (Silica gel GF254): 0.8142 (Benzene: Ethyl acetate: 0.1 M KOH 3:3:4); FTIR (KBr, cm^{-1}): 1076 (Ar–Cl), 1266 (C–N), 1612 (C═N), 1482 (C═C Ar bending), 3289, 3458 (NH_2, two peaks), 3056 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 3.24 (s, 2H, –CH_2), 3.31 (s, 2H, NH_2), 4.47 (s, 1H, NH), 5.76 (d, 1H, 3′CH-furan), 6.09 (t, 1H, 4′CH-furan), 6.22 (s, 1H, 1-CH thiazole), 6.65 (d, 1H, 5′CH-Furan), 6.84 (d, 2H, Ar–H), 6.88 (d, 2H, Ar–H); Anal. Calcd for $C_{17}H_{14}ClN_7OS$: C-51.06, H-3.53, N-24.52 Found C-50.82, H-3.67, N-22.78

8. General procedure for synthesis of di-chloro phenylthiazolyl-s-triazine derivatives (10b–18b)

The previously prepared **1** (1 mol) was heated with synthesized (3′,4′-Dichlorophenyl)thiazolyl-2-amine (1 mol) and $NaHCO_3$(1 mol) mixture at constant temperature 40–45 °C with uniform stirring for 10 mins, filtered immediately under vacuum to yield (3′,4′-dichlorophenyl)thiazolyl-*s*-triazine (**10b**). The various substituted amines (0.1 mmol) and substituted thiols (0.1 mmol) were added to **10b** into 100 ml dioxane under nitrogen atmosphere in $NaHCO_3$ (0.1 mmol) at 120 °C for 6 h. The product was filtered, washed with water, and purified by flash column chromatography(Silica gel mesh size 100–200) using methanol/ethyl acetate (1:20 v/v) as eluent to furnish pure di-chloro phenylthiazolyl-*s*-triazine derivatives **11b–18b**.

*8.1. 6-Chloro-N^2-(4-(3,4-dichlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4-diamine (**10b**)*

Physical state: Whitish Yellow solid; %Yield: 73.33; mp: 158–160 °C; R_f (Silica gel GF254): 0.5714 (Benzene: Ethyl acetate: 0.1 M alco. KOH 2:3:5); FTIR (KBr, cm^{-1}): 1043 (Ar–Cl), 1271 (C–N), 1678 (C═N), 1455 (C═C Ar bending), 3289, 3499 (NH_2, two peaks), 3326 (N–H broad peak), 3098 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 4.48 (s, 2H, NH_2), 4.98 (s, 1H, NH), 6.67 (s, 1H, 1-CH thiazole), 7.29 (d, 1H, Ar–H), 7.49 (d, 1H, Ar–H), 7.55 (s, 1H, Ar–H); Anal. Calcd for $C_{12}H_7Cl_3N_6S$: C-38.57, H-1.89, N-22.49 Found C-38.48, H-1.93, N-22.11.

*8.2. N_2-allyl-N_4-(4-(3,4-dichlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**11b**)*

Physical state: Brownish yellow solid; %Yield: 64.17; mp: 54–56 °C; R_f (Silica gel GF254): 0.7517 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}) 1103 (Ar–Cl), 1252 (C–N), 1654 (C═N), 1679 (C═C), 1492 (C═C Ar bending), 3279, 3477 (NH_2, two peaks), 3198 (N–H broad peak), 3024 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 3.86 (t, 2H, N–CH_2), 5.27 (d, 2H, CH_2═CH_2), 5.85 (m, 1H, C–HC═CH_2), 4.31 (s, 2H, NH_2), 4.62 (m, 1H,NH), 6.60 (s, 1H, 1-CH thiazole), 7.26 (d, 1H, 3-ArH), 7.31 (d, 1H, 2-Ar–H), 7.49 (s, 1H, 5-Ar–H); Anal. Calcd for $C_{15}H_{13}Cl_2N_7S$: C-45.69, H-3.32, N-24.87 Found C-45.48, H-3.30, N-24.95.

*8.3. N2-butyl-N^4-(4-(3,4-dichlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**12b**)*

Physical state: Whitish brown solid; %Yield: 59.34; mp: 122–124 °C; R_f (Silica gel GF254): 0.7342 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, ν_{max} cm^{-1}), 1053 (Ar–Cl), 1152 (C–N), 1645 (C═N), 1475 (C═C Ar bending), 3239& 3409 (NH_2, two peaks), 3201 (N–H broad peak), 2856 (CH_2 stretching), 3041 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 1.27 (t, 3H, CH_3), 1.53 (m, 2H, CH_2), 1.80 (m, 2H, CH_2), 3.49 (m, 2H, N–CH_2), 3.77 (s, 2H, NH_2), 4.78 (m, 1H, NH), 6.53 (s, 1H, 1-CH thiazole), 7.31 (d, 1H, 3-Ar–H), 7.51 (d, 1H, 2-Ar–H), 7.47 (s, 1H, 5-Ar–H); Anal. Calcd for $C_{16}H_{17}Cl_2N_7S$: C-46.83, H-4.18, N-23.89 Found C-46.48, H-4.22, N-23.88.

*8.4. N^2-(4-(3,4-dichlorophenyl)thiazol-2-yl)-N^4-phenyl-1,3,5-triazine-2,4,6-triamine (**13b**)*

Physical state: Light brown solid; %Yield: 70.12; mp: 113–115 °C; R_f (Silica gel GF254): 0.6613 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}) 1079 (Ar–Cl), 1264 (C–N), 1683 (C═N), 1412 (C═C Ar bending), 3340, 3480 (NH_2, two peaks), 3324 (N–H broad peak), 3086 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm) 4.34 (s, 2H, NH_2), 4.64 (s, 1H, NH), 5.01 (s,1H,NH), 6.54 (s,1H,1-CH Thiazole), 7.29 (d,2H,Ar′H), 7.31 (t,1H,Ar′H), 7.44 (t,2H,Ar′H), 7.61

(d, 1H, 3-Ar–H), 7.76 (d, 1H, 2-Ar–H), 7.89 (s, 1H,5-Ar–H); Anal. Calcd for $C_{18}H_{13}Cl_2N_7S$: C-50.24, H-3.05, N-22.78 Found C-50.02, H-3.06, N-22.75.

*8.5. N^2-(4-(3,4-dichlorophenyl)thiazol-2-yl)-N^4-(furan-2-ylmethyl)-1,3,5-triazine-2,4,6-triamine (**14b**)*

Physical state: Yellow solid; %Yield: 71.27; mp: 107–109 °C; R_f (Silica gel GF254): 0.6724 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}) 1049 (Ar–Cl), 1259 (C–N), 1629 (C═N), 1444 (C═C Ar bending), 3357, 3468 (NH_2, two peaks), 3304 (N–H broad peak), 3101 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 4.44 (s, 2H, NH_2), 4.65 (m, 1H, NH), 4.82 (s, 1H, NH), 6.12 (d, 1H, 3′CH-furan), 6.24 (m, 1H, 4′CH-furan), 6.46 (s, 1H, 1-CH thiazole), 7.28 (d, 1H, 5′CH-furan), 7.30 (d, 1H, 3-Ar–H), 7.37 (d, 1H, 2-Ar–H), 7.46 (s, 1H, 5-Ar–H); Anal. Calcd for $C_{17}H_{13}Cl_2N_7OS$: C-47.01, H-3.02, N-22.58 Found C-46.89, H-2.98, N-22.50.

*8.6. 6-(Allylthio)-N^2-(4-(3,4-dichlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4-diamine (**15b**)*

Physical state: Yellowish black solid; %Yield: 46.69; mp: 92–94 °C; R_f (Silica gel GF254): 0.7196 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}): 1054 (Ar–Cl), 1256 (C–N), 1669 (C═N), 1668 (C═C), 1476 (C═C Ar bending), 3356, 3421 (NH_2, two peaks), 3307 (N–H broad peak), 3123 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 3.60 (d, 2H, S–CH_2), 5.03 (d, 2H, C═CH_2), 5.96 (m, 1H, HC═C), 4.25–4.29 (m, 2H, NH_2), 4.46 (m, 1H, NH), 6.62 (s, 1H, 1-CH thiazole), 7.24 (d, 1H, 3-Ar–H), 7.26 (d, 1H, 2-Ar–H), 7.44 (s, 1H, 5-Ar–H); Anal. Calcd for $C_{15}H_{12}Cl_2N_6S_2$:C-43.80, H-2.94, N-20.43 Found C-43.52, H-2.80, N-20.45.

*8.7. 6-(Butylthio)-N^2-(4-(3,4-dichlorophenyl)thiazol-2-yl)-1,3,5-triazine-2,4-diamine (**16b**)*

Physical state: Yellowish brown solid; %Yield: 82.34; mp: 76–78 °C; R_f (Silica gel GF254): 0.7098 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}): 1045 (Ar–Cl), 1267 (C–N), 1654 (C═N), 1429 (C═C Ar bending), 3367, 3406 (NH_2, two peaks), 3310 (N–H broad peak), 3066 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 0.94 (t, 3H, CH_3), 1.26 (m, 2H, CH_2), 1.32 (m, 2H, CH_2), 2.94 (m, 2H, S–CH_2), 4.26 (s, 2H, NH_2), 4.65 (s, 1H, NH), 6.44 (s, 1H, 1-CH thiazole), 7.22 (d, 1H, 3-Ar–H), 7.30 (d, 1H, 2-Ar–H), 7.41 (s, 1H, 5-Ar–H); Anal. Calcd for $C_{16}H_{16}Cl_2N_6S_2$: C-44.97, H-3.77, N-19.66 Found C-43.88, H-3.50, N-19.83.

*8.8. N^2-(4-(3,4-dichlorophenyl)thiazol-2-yl)-6-(phenylthio)-1,3,5-triazine-2,4-diamine (**17b**)*

Physical state: Brownish yellow solid; %Yield: 65.89; mp: 92–94 °C; R_f (Silica gel GF254): 0.6984 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}) 1094 (Ar–Cl), 1242 (C–N), 1672 (C═N), 1445 (C═C Ar bending), 3320, 3411 (NH_2, two peaks), 3301 (N–H broad peak), 3083 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 4.25 (s, 2H, qaNH_2), 4.44 (s, 1H, NH), 6.55 (s, 1H, 1-CH thiazole), 7.10 (m, 2H, Ar′–H), 7.08 (m, 1H, Ar′–H), 7.21 (m, 2H, Ar′–H), 7.33 (d, 1H, 3-Ar–H), 7.41 (d, 1H, 2-Ar–H), 7.47 (s, 1H, 5-Ar–H); Anal. Calcd for $C_{18}H_{12}Cl_2N_6S_2$: C-48.33, H-2.70, N-18.79 Found C-47.98, H-2.91, N-18.56.

*8.9. N^2-(4-(3,4-dichlorophenyl)thiazol-2-yl)-6-(furan-2-ylmethylthio)-1,3,5-triazine-2,4-diamine (**18b**)*

Physical state: Greenish brown semisolid; %Yield: 55.88; mp: 55–57 °C; R_f (Silica gel GF254): 0.7684 (Benzene: Ethyl acetate: 0.1 M alco. KOH 3:3:4); FTIR (KBr, cm^{-1}) 1076 (Ar–Cl), 1248 (C–N), 1669 (C═N), 1447 (C═C Ar bending), 3330, 3479 (NH_2, two peaks), 3310 (N–H broad peak), 3057 (Ar–C–H); ^{1}H NMR (400 mHz, δ ppm): 4.29 (m, 2H, N–CH_2), 4.10 (s, 2H, NH_2), 4.38 (m, 2H, NH), 6.08 (d, 1H, 3′CH-furan), 6.24 (m, 1H, 4′CH-furan), 6.41 (s, 1H, 1-CH thiazole), 7.28 (d, 1H, 5′CH-furan), 7.34 (d, 1H, 3-Ar–H), 7.40 (d, 1H, 2-Ar–H), 7.48 (s, 1H, 5-Ar–H); Anal. Calcd for $C_{17}H_{13}Cl_2N_7OS$: C-47.01, H-3.02, N-22.58 Found C-46.89, H-3.47, N-22.50.

Conflict of interest

The authors declare that there is no conflict of interest.

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