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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antibacterial evaluation of series of novel tri-substituted-*s*-triazine derivatives

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Abstract Two novel series of *s*-triazine derivatives (**6a–e** and **7a–f**) were synthesized with various aromatic and heterocyclic amines. The synthesized compounds were subsequently evaluated for their in vitro antibacterial activity against three gram-positive viz. *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), *Staphylococcus aureus* (NCIM-2079) and gram-negative bacteria viz. *Pseudomonas aeruginosa* (NCIM-2036), *Escherichia coli* (NCIM-2065) and *Klebseilla pneumoniae* (NCIM-2706) by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) using streptomycin as reference standard. Structures of the synthesized compounds were elucidated on the basis of elemental analyses and spectral data.

Keywords *s*-triazines · Amines · Antibacterial · MIC

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Introduction

Since the discovery of chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eradication of infectious disease. But these agents are now returning in new resistant forms to antibiotic therapies (Qadri *et al.,* 2005, Devasia *et al.,* 2006). The global emergence of multi-drug resistant microbial strains is increasing and has become the limiting factor to the effectiveness of current drugs. It has resulted in significant morbidity and mortality from treatment failures and increased health care cost (Projan and Bradford, 2007). Development of new, cost effective and innovative drugs is one of the most common scientific strategies to combat drug resistant microorganisms.

s-Triazine derivatives have been focus of attention for the chemists and biologists for long time due to their wide array of biological activities associated with them, such as, antiplasmodial (Klenke *et al.*, 2003), antibacterial (Srinivas *et al.,* 2006), anticancer (Mandal *et al.,* 2007) and carbonic anhydrase inhibitors (Garaj *et al.,* 2005). The purpose of this study was to synthesize various substituted *s*-triazine derivatives attached with substituted thiazoles as bioactive scaffold (Khalil *et al.,* 2009, Bharti *et al.,* 2010) in search of exploring the potential of this skeleton as antibacterial drugs against some gram-positive and gram-negative microorganisms.

Materials and method

Structural investigation

All chemicals were of analytical grade and used directly. Melting points were determined in open capillary tubes

with electrothermal melting point apparatus (MP-1) and are uncorrected. The completion of reaction was checked by thin layer chromatography (TLC) using silica gel-G coated Al-plates (0.5 mm thickness; Merck) and the plates were illuminated under UV (254 nm) and evaluated in iodine vapor. The solubility of all the compounds were tested by using water, chloroform, ethanol, DMF, DMSO, benzene, acetic acid, ethyl acetate, and dilute acids. FT-IR (in 2.0 cm^{-1}, flat, smooth, abex, KBr) spectra were recorded on Perkin Elmer-Spectrum RX-I spectrometer. Elemental analysis was carried out on a Vario EL III CHNOS elementor analyzer. ^{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). Mass spectra were obtained on VG-AUTOSPEC spectrometer. Solvents were purified prior to use according to standard procedure.

Biological evaluation

All synthesized compounds were screened for their minimum inhibitory concentration (MIC, μg/mL) against selected gram-positive organisms viz. *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), *Staphylococcus aureus* (NCIM-2079) and gram-negative organisms viz. *Pseudomonas aeruginosa* (NCIM-2036), *Escherichia coli* (NCIM-2065) and *Klebseilla pneumoniae* (NCIM-2706) bacteria by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 1982) with minor modifications. Streptomycin was used as standard antibacterial agent. Solutions of the test compounds and reference drug were prepared in dimethylsulfoxide (DMSO) at concentrations of 100, 50, 25, 12.5, 6.25, 3.125 μg/ml. Eight tubes were prepared in duplicate with the second set being used as MIC reference controls (16–24 h visual). After sample preparation, the controls were placed in a 37°C incubator and read for macroscopic growth (clear or turbid) the next day.

Into each tube, 0.8 ml of nutrient broth was pipetted (tubes 2–7), tube 1 (negative control) received 1.0 ml of nutrient broth and tube 8 (positive control) received 0.9 ml of nutrient. Tube 1, the negative control, did not contain bacteria or antibiotic. The positive control, tube 8, received 0.9 ml of nutrient broth since it contained bacteria but not antibiotic. The test compound is dissolved in dimethylsulphoxide (100 μg/ml), 0.1 ml of increasing concentration of the prepared test compounds which are serially diluted from tube 2 to tube 7 from highest (100 μg/ml) to lowest (3.125 μg/ml) concentration (tube 2–7 containing 100, 50, 25, 12.5, 6.25, 3.125 μg/ml). After this process, each tube was inoculated with 0.1 ml of the bacterial suspension whose concentration corresponded to 0.5 McFarland scale (9×10^8 cells/ml) and each bacterium was incubated on a rotary shaker at 37°C for 24 h at 150 rpm. The final volume in each tube was 1.0 ml. The incubation chamber was kept humid. 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.

Result

The molecular modification of all the compounds is shown in Table 1. The physicochemical properties, R_f values (Table 2), Calculated log P values (ClogP) (Table 3), and elemental composition of all the elements are reported (Table 4). The entire spectral data are of individual compound reported in Table 5. Antibacterial investigation of this new series of *s*-triazines derivatives (**6a–e**, **7a–f**) showed moderate to excellent MIC against tested gram-positive and gram-negative organisms. Their inhibition values are included in Table 1.

Discussion

From the structural investigation, FT-IR spectra showed the stretching frequency range between region 1550–1200 cm^{-1} due to –C=C–, –C=N stretching. ^{1}H-NMR spectra of class of compounds showed a singlet at δ 4.4–4.6 due to –NH group of phenyl thiazolyl-2-amine groups and aromatic C–NH lies at about 4.3–3.3 ppm, disappearance of primary amine peak associated with substituted phenyl thiazole-2-amine further confirmed the formation of this class of derivatives. Estimated elemental compositions were with in ±0.4% of the calculated values. The antibacterial screening of all compounds showed an excellent to no activity against both grampositive and gram-negative bacteria tested in comparison to standard streptomycin. The di-(**6a–e**) and tri-substituted derivatives (**7a–f**) of *s*-triazines series exhibited stronger inhibition of gram-negative organism compared with gram-positive and the tri-substituted derivatives showed better results than di-substituted derivatives.

As depicted in Table 1, none of the synthesized compounds proved to be effective antibacterial against tested microorganisms, except a few tri-substituted *s*-triazine derivatives. Among the compounds (**7a–f**), **7c** was found to be equipotent to the standard drug streptomycin in the case of all microorganisms, except *Bacillus cereus*. The compound **7b** was found to be equipotent to the standard against *Pseudomonas aeruginosa* and was found posses mild to moderate activity against tested microorganisms. In terms of *Klebseilla pneumoniae*, **7f**, is the only compound

Table 1 Molecular modification and MIC value of individual compounds against gram-positive and gram-negative bacteria

Compounds	R_1	R_2	MIC (μg ml^{-1})					
			Gram-positive bacteria			Gram-negative bacteria		
			Bacillus subtilis	*Bacillus cereus*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebseilla pneumoniae*
3a	Cl	–	NP	NP	NP	NP	NP	NP
3b	NO_2	–	NP	NP	NP	NP	NP	NP
6a	–	3-Chloro,4-fluoro	100	100	50	100	100	100
6b	–	4-Nitro	100	100	50	25	50	100
6c	–	4-Bromo	100	100	100	100	100	100
6d	–	4-Fluoro	50	50	100	100	50	50
6e	–	4-Chloro	50	100	50	50	25	25
7a	Cl	3-Chloro,4-fluoro	50	100	25	25	12.5	12.5
7b	NO_2	3-Chloro,4-fluoro	100	25	50	3.125	12.5	25
7c	NO_2	4-Nitro	6.25	6.25	3.125	3.125	6.25	6.25
7d	NO_2	4-Bromo	100	100	100	50	100	100
7e	NO_2	4-Fluoro	50	100	50	25	25	50
7f	NO_2	4-Chloro	25	12.5	25	6.25	12.5	6.25
Streptomycin			6.25	3.125	3.125	3.125	6.25	6.25

NP not performed

found equipotent to the standard drug like **7c**. Nearly all synthesized derivatives from both the series, viz. (**6a–e**, **7a–f**) were found inactive against *Bacillus subtilis*. Rest of the compounds was found either inactive or presented MIC value more than 100 μg/ml.

LogP value is a measure of hydrophobic/lipophilic balance of the molecule. It was believed that the lipophilic nature of the compound plays a very vital role for generation of antimicrobial effect by increasing the membrane permeation and hence, a strong correlation exists between lipophilicity and activity. In this context, lipophilicity of compound expressed as ClogP (octanol/water partition coefficient) value explains the main predictor of activity and was calculated by using the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors as shown in Table 3. The method is very robust and is able to process practically all organic and most organometallic molecules.

On close inspection of ClogP value and MIC of test compound, it was observed that ClogP is the main factor influencing physico-chemical parameter for generation of antibacterial activity. Pronounced antibacterial activity was observed in compound with having high ClogP, e.g., tri-substituted derivatives were found more active than di-substituted derivatives. And it was also confirmed that the inhibition pattern of tri-substituted derivative on gram-negative organism were more influencing than di-substitution due to high ClogP values.

In the light of above structure activity relationship studies, it could be suggested that thiazole amine pendent is well tolerated at 6-position of tri-substituted triazine. By close perlustration of the in vitro antibacterial activity, we proceed to survey that electron withdrawing groups (NO_2, Cl, Br, F) are necessary for compounds to be effective antibacterials. Further, the presence of nitro group leads to increased in activity in comparison to the other electron withdrawing groups. In next instance, we can see that the presence of chloro is the second most influencing electron withdrawing group, which can further be confirmed by antibacterial profile of compound **7b** (3-chloro, 4-fluoro benzyl amine) against *P. aeruginosa*. The importance of electron withdrawing group in enhancing the antimicrobial activity was supported by similar results observed by Sharma *et al.,* 2004.

These atoms (electron withdrawing groups) are very useful to modulate the steric effect on phenyl ring of drug and which in turn alter the ease of penetration of molecule in bacterial cell wall and this may be the plausible cause for variation in antibacterial profile of substituted derivatives. Moreover, these atoms may also influence hydrophilic–hydrophobic balance of the target molecules which would further evidenced by SAR analysis.

Conclusion

In our study, we have synthesized various di- and tri-substituted derivatives of *s*-triazine, which again proves the novelty of biological efficacy of new *s*-triazines derivatives as synthetic antibiotics. On the basis of result obtained, it has been concluded that tri-substituted *s*-triazine provided a

Table 2 Physicochemical properties and R_f values of individual compounds

Compounds	Physicochemical properties											R_f values	Percentage yield (%)
	Molecular formula	Molecular weight	Melting point (°C)	Solubility									
				Water	Chloroform	Ethanol	DMF	DMSO	Benzene	Acetic acid	Ethyl acetate	Dilute acids	
3a	$C_9H_7ClN_2S$	210.68	164–166	++	++	++	++	+++	+++	−	++	+++	0.52	82
3b	$C_9H_7N_3O_2S$	221.24	265–267	++	++	++	++	+++	+++	−	++	+++	0.53	88
6a	$C_{15}H_8Cl_3F_2N_5$	402.61	86–88	++		+++		+++	++	++		++	0.62	85
6b	$C_{15}H_{10}ClN_7O_4$	387.74	143–145	++	++	+++	−	+++	−	++	++	++	0.55	78
6c	$C_{15}H_{10}Br_2ClN_5$	455.53	169–170	−	−	+++	−	+++	++	++	+++	+++	0.35	66
6d	$C_{15}H_{10}ClF_2N_5$	333.72	124–125	++	−	+++	−	+++	++	++	−	−	0.42	85
6e	$C_{15}H_{10}Cl_3N_5$	366.63	135–137	−	++	+++	−	+++		++	−	−	0.48	83
7a	$C_{24}H_{14}C_{13}F_2N_7S$	576.84	137–139	++	++	+++	++	+++	++	−	++	+++	0.45	86
7b	$C_{24}H_{14}C_{12}F_2N_8O_2S$	587.39	152–154	++	++	+++	++	+++	++	−	++	+++	0.67	89
7c	$C_{24}H_{16}N_{10}O_6S$	572.51	130–133	++	−	+++	++	+++	++	−	−	+++	0.38	59
7d	$C_{24}H_{16}Br_2N_8O_2S$	640.31	140–144	++	−	+++	++	+++	++	++	++	+++	0.54	74
7e	$C_{24}H_{16}F_2N_8O_2S$	518.5	185–188	++	++	+++	++	+++	++	−	−	+++	0.51	87
7f	$C_{24}H_{16}Cl_2N_8O_2S$	551.41	189–192	++	−	+++	++	+++	++	−	−	+++	0.58	76

++ Sparingly soluble, +++ soluble, − not soluble

Table 3 Calculated log P (miLog P) of test compounds

Compounds	ClogP
6a	6.46
6b	4.04
6c	6.54
6d	5.25
6e	6.28
7a	8.78
7b	8.06
7c	5.63
7d	8.13
7e	6.85
7f	7.88

Table 4 Values obtained by elemental analysis for individual compounds (in %)

Compounds	Carbon		Hydrogen		Nitrogen	
	A	B	A	B	A	B
3a	51.31	51.01	3.35	3.36	13.30	13.25
3b	48.86	48.88	3.19	3.20	18.99	18.22
6a	44.75	44.79	2.00	1.97	17.39	17.38
6b	46.46	46.48	2.60	2.65	25.29	25.26
6c	39.55	39.53	2.21	2.20	15.37	15.34
6d	53.99	53.96	3.02	3.02	20.99	20.95
6e	49.14	49.17	2.75	2.77	19.10	19.15
7a	49.97	49.99	2.45	2.42	17.00	16.98
7b	49.07	49.15	2.40	2.35	19.08	19.04
7c	50.35	50.28	2.82	2.86	24.47	24.40
7d	45.02	45.10	2.52	2.55	17.50	17.48
7e	55.59	55.65	3.11	3.15	21.61	21.68
7f	52.28	52.26	2.92	2.95	20.32	20.28

(A) Calculated; (B) Found

new class of bioactive scaffolds, but in future compounds will be modified further to reduce molar mass and toxicological barriers.

This study also showed broad spectrum activity of *s*-triazines containing thiazole units, that are comparatively equipotent to the antibiotic agent (streptomycin) in the comparison test, may be due to the synergistic antibacterial effect of thiazole and triazine nuclei. Further, these molecules may act as lead for further synthetic and biological evaluation towards pursuit to discover novel class of antibacterial agents.

Experimental

The compounds were synthesized to understand the role of di- and tri-substitutions of substituted amines and 4-(4-subsitutedphenyl)-thiazole-2-amine groups on *s*-triazine moiety towards antibacterial activity. The *s*-triazine derivatives were synthesized from 2,4,6-trichloro-1,3,5-triazine by consecutive aromatic nucleophilic substitution (S_NAr) reactions under controlled conditions by treating two equivalents of substituted amine derivatives **5** with *s*-triazine **4** in presence of NaOH as activating bases to give di- substituted monochloro-*s*-triazines **6a–e**. 4-(4-substitutedphenyl)thiazole-2-amine were synthesized by cyclo-condensation reaction between substituted acetophenone **1**, and thiourea **2** with the help of bromine to give compounds **3a** and **b** (Scheme 1). And **3a** and **b** were further used as intermediate for synthesizing a series of compound by reacting with **6a–e** under harsh condition to form final corresponding compounds **7a–f** because third nucleophilic attack is difficult to perform (Scheme 2).

General procedure for synthesis of 4-(4-subsituted phenyl)thiazole-2-Yl)amine (**3a**, **b**)

Substituted acetophenone (**1**) (110 mmol) and thiourea (**2**) (220 mmol) were mixed completely; then added bromine (110 mmol) in small fractions (0.5 ml) over 3 h with continuous stirring and heated on a steam bath for 9 h. The sufficient quantity of water was added, heated until most of the solid had gone into solution and filtered while hot. The filtrate was cooled and concentrated ammonia solution was added till the precipitation ceases off. Recrystallized with pyridine–water (1:1) mixture to achieved pure products **3a** and **b**.

General procedure for synthesis of 2,4-bis(substituted amine)-6-chloro-*s*-triazine (**6a–e**)

Various substituted anilines (**5**) (0.2 mol) were added into 100 ml of acetone maintaining temperature 40–45°C. The solution of 2,4,6-tri chloro-*s*-triazine (**4**) (0.1 mol) in 25 ml acetone was added constantly, stirred for 3 h followed by drop-wise addition of NaOH solution (0.1 mol) taking care that reaction mixture does not become acidic. The product was filtered and washed with cold water and recrystallized with ethanol to afford pure products **6a–e**.

General procedure for synthesis of 2,4-bis(substituted amine)-6-(4-(4-subsituted phenyl)thiazole-2-Yl)-*s*-triazine-2,4,6-triamine (**7a–f**)

2,4-Bis(substituted amine)-6-chloro-*s*-triazine (**6a–e**) (0.1 mol) was added into 50 ml of 1,4-dioxane maintaining temperature 40–45°C. The solution of 4-(4-substituted phenyl)-thiazole-2-amine (**3a**, **b**) (0.1 mol) in 35 ml 1,4-dioxane was added constantly to above solution and stirred for 90 min followed by drop-wise addition of $NaHCO_3$

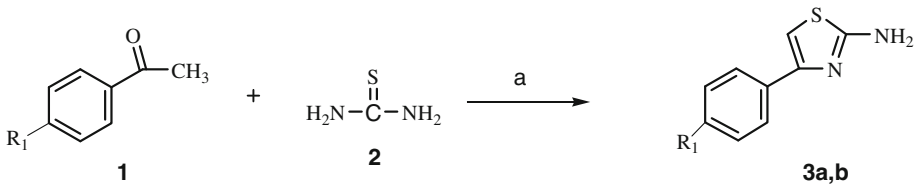
Table 5 Spectral data for individual compounds

IR wave number (in cm^{-1})	^1H NMR (in ppm)	Mass $[M + H]^+$
Compound **3a**		
v 3389.72 (NH strech, $–NH_2$), 3120.89 (aromatic C–H), 1627.11 (–C=N), 1092.77 (aromatic-Cl), 572.50 (C–S strech)	δ 7.34 (d, 2H, 3,5-CH aromatic), δ 7.46 (d, 2H, 2,6-CH aromatic), δ 6.72 (s, 1H, CH thiazolyl), δ 4.12 (s, 2H, NH amino)	211.01 (10.1%) $[M + H]^+$, 210.00 (100.0%), 212.00 (36.6%), 213.00 (4.1%)
Compound **3b**		
v 3400.39 (NH strech, $–NH_2$), 1607.97 (aromatic-C=N), 1525.08 ($–NO_2$), 1345.28 (aromatic-NO_2), 585.33 (C–S strech).	δ 8.26 (d, 2H, 3,5-CH aromatic), δ 7.75 (d, 2H, 2,6-CH aromatic), δ 6.80 (s, 1H, CH, thiazolyl), δ 4.15 (s, 2H, NH, amino).	222.03 (11.0%) $[M + H]^+$, 221.03 (100.0%), 223.02 (4.4%)
Compound **6a**		
v 3332.34 (N–H secondary), 3052.32(C–H broad), 1453.25(aromatic-C=N)	δ 6.98 (d, 2H, 2-CH aromatic), δ 6.54 (d, 2H, 2-CH aromatic), δ 6.84(d, 2H, 2-CH, aromatic), δ 4.66(d, 2H,-NH, amino)	403.90 (96.3%) $[M + H]^+$, 400.76 (100.0%), 404.09 (31.0%),
Compound **6b**		
v 3289.56 (N–H secondary), 3055.70 (C–H broad), 1548.28-1446.06 (aromatic-C=N)	δ 7.40 (t, 4H, 4=CH-aromatic), δ 7.32(t, 4H, 4=CH-aromatic), δ 3.62 (d, 2H, 2-NH aromatic)	388.04 (100.0%) $[M+H]^+$, 389.12 (34.5%), 388.09 (19.6%)
Compound **6c**		
v 3350.62 (N–H secondary), 3015.43 (C–H broad), 1656.15 (C=C stretch)	δ 7.26(d, 4H, 3,5-CH aromatic), δ 7.06 (d, 4H, 2,6-aromatic), δ 4.81 (s, 2H, NH)	456.90 (43.3%) $[M + H]^+$, 454.90 (100.0%), 452.90 (44.1%)
Compound **6d**		
3054.72(C–H broad), 1548.58 (C=N), 1448.25 (C–N aromatic)	δ 7.68 (d, 4H, 2-CH, aromatic), δ 7.14 (d, 4H, 2-CH aromatic), δ 4.84(t, 2H, -NH amino)	334.38 (18.5%) $[M + H]^+$, 333.56 (100.0%), 335.63 (32.3%)
Compound **6e**		
v 3243.26 (N–H secondary), 2965.46 (C–H broad), 1387.38 (aromatic –C=N)	δ 7.32 (d, 4H, 3,5-CH aromatic), δ 7.08 (d, 4H, 2,6-CH aromatic), δ 4.82 (s, 2H, NH)	367.15 (96.2%) $[M + H]^+$, 365.70 (100.0%), 368.99 (30.7%)
Compound **7a**		
v 3393.6 (N–H stretch, $–NH_2$), 3282.6, 3110.7 (C–H stretch, aromatic), 1626.7 (N–H bend), 1406.2 (C–N stretch), 1260.5 (C–N stretch; aromatic-NH-aromatic), 1128.9 (aromatic-F stretch), 1088.3 (aromatic-Cl stretch), 693.1, 668.8 (C–S stretch)	δ 7.93–7.91 (d, 1H, *J*=10.8 Hz, aromatic-H), δ 7.77–7.75 (d, 1H, $J = 8.8$ Hz, aromatic-H), δ 7.68 (s, 1H, NH), δ 7.55 (s, 1H, NH), δ 7.44 (s, 1H, NH), δ (s, 1H, thiazolyl), δ 7.07–7.02 (dd, 1H, $J = 20$ Hz, aromatic-H)	578.1 (1.84%) $[M+H]^+$, 274.3(100%), 331.3 (52.21%), 330.4 (45.32%)
Compound **7b**		
v 3395.2 (N–H stretch, $–NH_2$), 3110.8 (C–H stretch, aromatic), 1260.3, 1209.7 (C–N stretch, >NH), 1107.4 (aromatic-F stretch), 1055.8 (aromatic-Cl stretch), 853.8 (C–N stretch, aromatic-NO_2), 694.3 (C–S stretch)	δ 8.27–8.25 (d, 1H, $J = 7.2$ Hz, aromatic-H), δ 8.18–8.16 (d, 1H, $J = 8.8$ Hz, aromatic-H), δ 8.11–8.09 (d, 1H, $J = 8.8$ Hz, aromatic-H), δ 7.99–7.97 (d, 1H, $J = 7.2$ Hz, aromatic-H), δ 7.68 (s, 1H, NH), δ 7.60 (s, 1H, NH), δ 7.56 (s, 1H, NH), δ 7.25–7.22 (d, 1H, $J = 11.2$ Hz, aromatic-H), δ 6.53–6.51 (d, 1H, $J = 8.8$ Hz, aromatic-H)	587.03 (2.45%) $[M + H]^+$, 331.3 (100%), 228.3 (85.67%), 381.4 (84.73%)
Compound **7c**		
v 3399.3 (N–H stretch, -NH_2), 3360.2, 3217.9 (C–H stretch, aromatic), 1594.2, 1502.8 (N–H_{bend}, >NH), 1478.4, 1325.3 ($(N=O)_2$ stretch, aromatic-NO_2), 842.0 (C–N stretch, aromatic-NO_2), 698.2 (C–S stretch)	δ 8.35–8.29 (m, 1H, aromatic -H), δ 8.21–8.19 (d, 1H, $J = 8.8$ Hz, aromatic-H), δ 8.16–8.14 (d, $J = 8.8$, aromatic-H), δ 8.10–8.08 (d, 1H, $J = 9.8$ Hz, aromatic-H), δ 8.20–7.99 (d, 1H, $J = 8.4$ Hz, aromatic-H), δ 7.91–7.89 (d, 1H, $J = 8.8$ Hz, aromatic-H), δ 7.84–7.82 (d, 1H, $J = 9.2$, aromatic-H), δ 7.68 (s, 1H, NH), δ 7.55 (s, 1H, NH), δ 7.38 (s, 1H, NH), δ 7.33 (s, 1H, thiazolyl), δ 6.57–6.50 (m, aromatic-H)	573.51 (1.84%) $[M + H]^+$, 353.4 (100%), 274.3 (71%), 381.4 (67.79%), 330.4 (31.01%)
Compound **7d**		
v 3394.8 (N–H stretch, $–NH_2$), 3105.6 (C–H stretch, aromatic), 1567.8, 1512.1 (N–H bend, >NH), 1485.1, 1327.0 ($(N=O)_2$ stretch, aromatic-NO_2), 1230.4 (C–N stretch, aromatic -NH- aromatic), 645.3 (C–S stretch)	δ 8.35–8.33 (d, 1H, $J = 6.8$ Hz, aromatic–H), δ 8.30-8.28 (d, 1H, $J = 5.5$ Hz, aromatic-H), δ 8.20–8.18 (d, 1H, $J = 8$ Hz, aromatic-H), δ 8.14–8.12 (d, 1H, $J = 9.2$ Hz, aromatic-H), δ 8.01–7.99 (d, 1H, $J = 6.8$ Hz, 1H, aromatic-H), δ 7.78 (s, 1H, NH), δ 7.50 (s, 1H, NH), δ 7.43–7.411 (d, 1H, $J = 8.4$ Hz, aromatic-H), δ 7.36 (s, 1H, NH), δ 7.21 (s, 1H, thiazolyl), δ 7.02–6.98 (m, 1H, aromatic-H), δ 6.93–6.91 (d, 1H, $J = 6$ Hz, aromatic-H)	640.95 (1.84%) $[M + H]^+$, 274.3 (100%), 330.4 (36.45%), 302.4 (27.76%), 419.4 (25.73%)

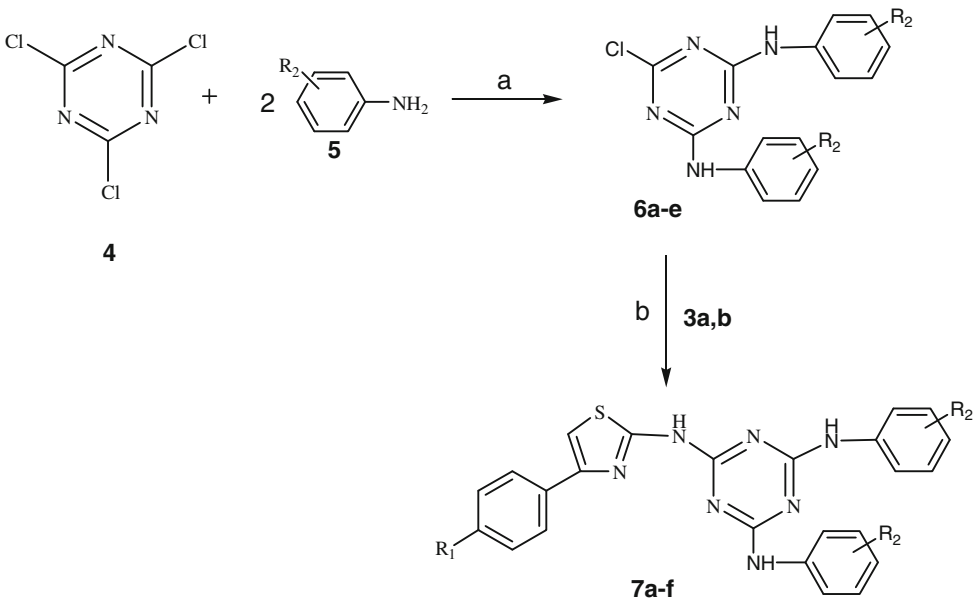
Table 5 continued

IR wave number (in cm^{-1})	^1H NMR (in ppm)	Mass $[M + H]^+$
Compound **7e**		
v 3399.3 (N–H stretch, $–NH_2$), 3124.4 (C–H stretch, aromatic), 1538.0, 1504.2 (N–H bend, >NH), 1420.3, 1385.0, 1329.2 ($(N=O)_2$ stretch, aromatic-NO_2), 1203.3, 1157.9 (aryl fluoro, aromatic-F), 1107.8, 1039.2 (C–N stretch, aromatic-NH-aromatic), 632.3 (C–S stretch)	δ 8.72–8.35 (d, 1H, $J = 8.8$ Hz, aromatic-H), δ 8.31–8.29 (d, 1H, $J = 8.4$ Hz, aromatic-H), δ 8.20-8.18 (d, 1H, $J = 8.8$ Hz, aromatic-H), δ 8.15–8.13 (d, 1H, $J = 7.2$ Hz, aromatic-H), δ 8.10-8.08 (d, 1H, $J = 8.4$ Hz, 1H, aromatic-H), δ 8.01-7.99 (d, 1H, $J = 8.8$ Hz, 1H, aromatic-H), δ 7.94–7.92 (d, 1H, $J = 8.4$ Hz, aromatic-H), δ 7.76 (s, 1H, NH), δ 7.66–7.67 (d, 1H, $J = 8.8$ Hz, aromatic-H), δ 7.56 (s, 1H, NH), δ 7.38 (s, 1H, NH), δ 7.33 (s, 1H, NH), δ 7.33 (s, 1H, thiazolyl), δ 7.10–7.07 (d, 1H, $J = 8.8$ Hz, aromatic-H)	519.1 (1.8%) $[M + H]^+$, 274.3 (100%), 331.3 (44.45%), 330.4 (30.16%), 381.4 (27.68%)
Compound **7f**		
v 3374.1 (N–H stretch, $–NH_2$), 3112.4 (C–H stretch, aromatic), 1571.5 (N–H bend, >NH), 1488.8, 1404.9, 1328.3 ($(N=O)_2$ stretch, aromatic-NO_2), 1232.7 (C-Nstretch, aromatic-NH-aromatic), 632.3 (C–S stretch).	δ 8.10–8.18 (d, 1H, $J = 8.4$ Hz, aromatic-H), δ 8.02–8.00 (d, 1H, $J = 8.4$ Hz, aromatic-H), δ 7.44 (s, 1H, NH), δ 7.60 (s, 1H, NH), δ 7.38 (s, 1H, NH), δ 7.32–7.30 (d, 1H, $J = 8.4$ Hz, aromatic-H), δ 7.21 (s, 1H, thiazolyl)	551.05 (3.4%) $[M + H]^+$, 331.3 (91.39%), 353.4 (84.73%), 228.3 (66.58%)

Scheme 1 Reagents and condition: *a* Br_2, reflux



Scheme 2 Reagents and condition: *a* NaOH, 40–50°C; *b* $NaHCO_3$, 135–145°C



(0.1 mol) and refluxed this mixture at 135–145°C for 9 h. The product was filtered and washed with cold water and recrystallized with ethanol to afford the corresponding pure products **7a–f**.

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Conflict of interest None.

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