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Synthesis of novel aliphatic thiourea derivatives containing s-triazine moiety as potential antimicrobial agents

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A new series of aliphatic thiourea and various aryl urea incorporating 1,3,5-s-triazine moiety is reported. This series has been obtained by the reaction of cyanuric chloride with thiophene-2-ethyl thiourea **1**. Thus, the prepared 2-(thiophene-2-ethyl thioureido)-4,6-dichloro-s-triazine **2** has been subsequently treated with morpholine to get 2-(thiophene-2-ethyl thioureido)-4-(morpholino)-6-chloro-s-triazine **3.** This is further treated with various aryl ureas to afford title compound **4a-j**. The library of ureido linkage containing triazinyl moiety has been tested for *in vitro* antibacterial and antifungal activity against different microorganisms. The structure of novel synthesized compounds has been established on the basis of elemental analysis, ^{1}H NMR, IR and mass spectral data.

Keywords: Aliphatic thiourea, s-triazine, aryl urea, antibacterial activity, antifungal activity

Research on new substances possessing antibacterial activity has attracted considerable attention owing to the continuous increase in bacterial resistance[1]. Further, infection caused by various micro-organisms pose a serious challenge to the medical community and need for an effective therapy has led to the search for novel antibacterial agents[2]. Substituted s-triazine derivatives are an important class of compounds having various biological activities like antibacterial[3], anticancer[4], antiviral[5] and antifungal[6].

Thiourea derivatives of aryl ethyl amine are used in the synthesis of anti-HIV[7], antitumor[8], antibacterial[9] and antifungal[10] agents. Thiourea derivatives of type **1** in **Scheme I** have been synthesized and found more potent than the other anti-viral drugs against multidrug resistant HIV-1 strain RT-MDR with a V106-A mutation[11,12].

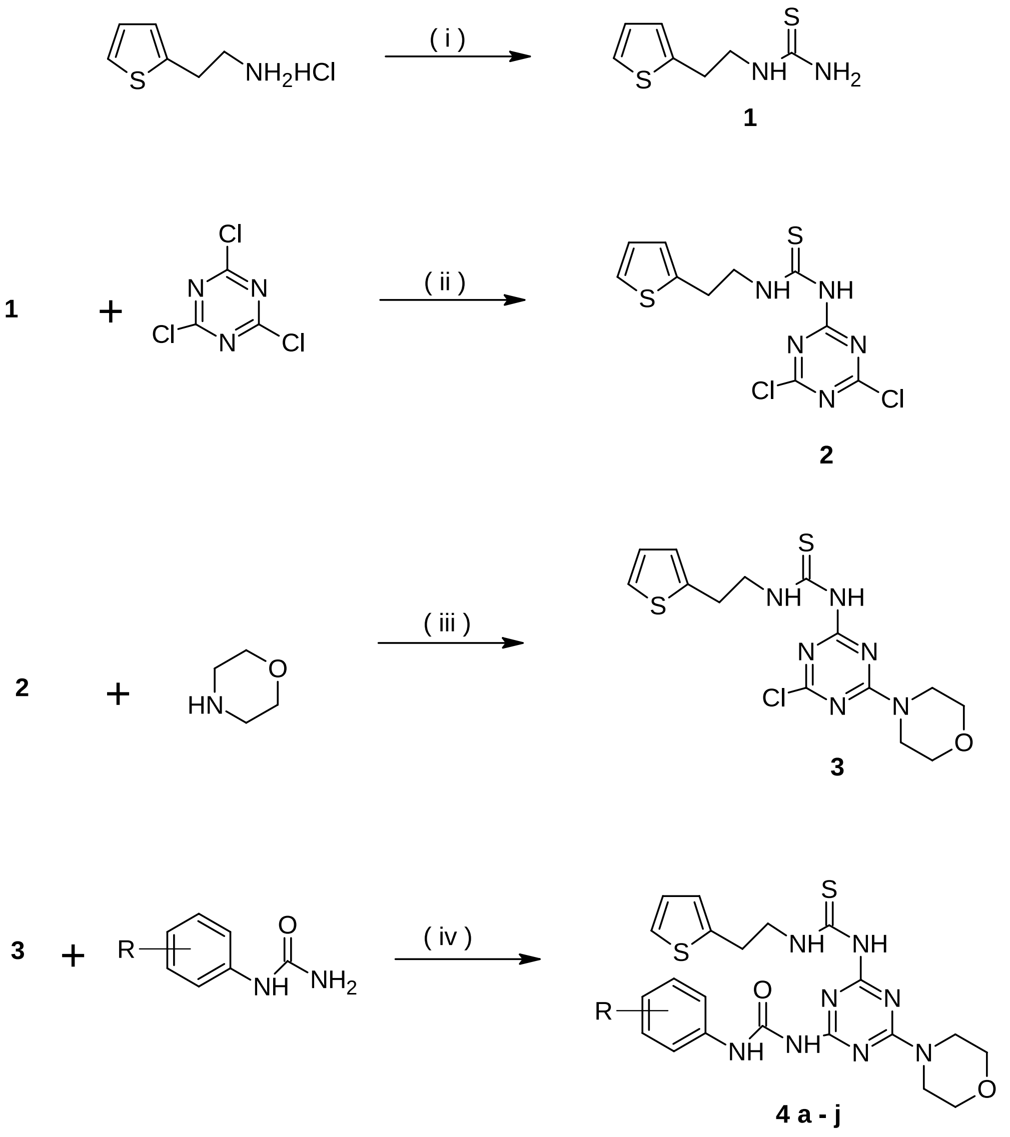
Based on the above observations and earlier investigations[13,14], herein are reported the synthesis of various 2,4,6-trisubstituted 1,3,5-s-triazine derivatives **4a-j (Scheme I, Table I)** and evaluation of their antibacterial and antifungal activity. 2-(Thiophene-2-ethylthioureido)-4,6-dichloro-s-triazine **2** was synthesized by reacting thiophene-2-ethylthiourea **1** with cyanuric chloride. Compound **1** was synthesized by the reaction of thiophene-2-ethyl amine hydrochloride with KSCN in THF. Compound **2** on treatment with morpholine gave 2-(thiophene-2-ethylthioureido)-4-(morpholino)-6-chloro-s-triazine **3** and by considering the versatile chemistry of cyanuric chloride derivative[15] and its reaction with various aryl ureas yielded title compound **4a-j**.

Experimental Section

Melting points were determined on an electrothermal apparatus (capillary method) and are uncorrected. IR spectra were recorded on FT Bommen spectrometer using KBr pellets. ^{1}H NMR spectra were obtained on Hitachi 300 MHz spectrometer. Chemical shifts are reported in δ (ppm) using TMS as internal standard. Elemental analyses (C, H, N) were carried out on Perkin-Elmer model 240C analyzer. All compounds gave satisfactory analytical results. Progress of reaction and homogeneity of products were monitored on Merck aluminum foil backed silica gel 60 F_{254} TLC plates.

Thiophene-2-ethyl thiourea, 1 (ref. 18)

To a magnetically stirred solution of thiophene-2-ethyl amine hydrochloride (7 g, 0.0427 mole) in THF (150 mL) was added KSCN (5 g, 0.064 mole). The mixture was heated at reflux for 24 hr at which point TLC analysis (1:1 EtOAc/hexane) indicated complete



[a]Reaction conditions (i) KSCN/THF, reflux 24 hr (ii) 0-5 °C Acetone/10% $NaHCO_3$ (iii) 40-50 °C Acetone/10% $NaHCO_3$(iii) Dioxane/10% $NaHCO_3$, reflux

Scheme I

consumption of the starting material. The mixture was diluted with water (100 mL) and extracted with EtOAc (2×100 mL). The interfacial solids and EtOAc extracts were combined and washed with 1*N* HCl (100 mL) and brine (50 mL). The organic layer was dried (anhyd. Na_2SO_4) and concentrated under reduced pressure to afford the thiourea derivative which was purified by recrystallization from ethanol to obtain yellow crystals of the title product **1**. m.p. 185°C, Yield 65%. $C_7H_{10}N_2S$, Found: C, 52.79; H, 6.25; N, 17.60; S, 20.10. Calcd. for C, 52.83; H, 6.28; N, 17.61; S, 20.12%.

2-(Thiophene-2-ethyl thioureido)-4, 6-dichloro-s-triazine, 2 (ref. 19)

To a stirred solution of cyanuric chloride (0.1 mole, 18.4 g) in acetone (100 mL) at 0-5°C, the solution of 2-thiophene ethylthiourea (0.1 mole, 18.6 g) in acetone (40 mL) and 10% $NaHCO_3$ (90.0 mL) was added dropwise in 2 hr maintaining the temperature between 0-5°C. After the completion of reaction (TLC analysis — methanol) the stirring was stopped and the solution was treated with crushed ice. The solid product obtained was filtered and dried. The crude product was purified by recrystallization from

Table I— Physical and analytical characterization data of 2-(thiophene-2-ethyl thioureido)-4-(morpholino)-6-(aryl ureido)-s-triazines, **4a-j**

Compd	R	Mol. formula	m.p. °C	Yield (%)	Calcd % (Found)		
					C	H	N
4a	H	$C_{21}H_{24}N_8O_2S_2$	217	70	52.06 (52.02	4.95 4.92	23.14 23.13)
4b	2-NO_2	$C_{21}H_{23}N_9O_4S_2$	222	55	47.63 (47.59	4.34 4.31	23.81 23.79)
4c	3-NO_2	$C_{21}H_{23}N_9O_4S_2$	232	51	47.63 (47.60	4.34 4.35	23.81 23.78)
4d	4-NO_2	$C_{21}H_{23}N_9O_4S_2$	225	53	47.63 (47.62	4.34 4.32	23.81 23.77)
4e	2-Cl	$C_{21}H_{23}N_8O_2S_2Cl$	208	75	48.60 (48.59	4.43 4.40	21.60 21.57)
4f	3-Cl	$C_{21}H_{23}N_8O_2S_2Cl$	196	60	48.60 (48.58	4.43 4.45	21.60 21.59)
4g	4-Cl	$C_{21}H_{23}N_8O_2S_2Cl$	218	75	48.60 (48.57	4.43 4.41	21.60 21.58)
4h	2-CH_3	$C_{22}H_{26}N_8O_2S_2$	227	62	53.01 (52.99	5.22 5.23	22.48 22.45)
4i	3-CH_3	$C_{22}H_{26}N_8O_2S_2$	219	67	53.01 (52.98	5.22 5.20	22.48 22.47)
4j	4-CH_3	$C_{22}H_{26}N_8O_2S_2$	198	69	53.01 (52.97	5.22 5.19	22.48 22.46)

Table II — Antimicrobial activity of 2-(thiophene-2-ethyl thioureido)-4-(morpholino)-6-(aryl ureido)-s-triazines, 4a-j

Compd	R	Antibacterial activity[a]				Antifungal activity *C. Albicans* (μg/mL)
		Gram positive		Gram negative		
		B. subtillis	*S. aureus*	*S. typhi*	*E. coli*	
4a	H	08	11	10	12	--
4b	2-NO_2	--	--	09	10	--
4c	3- NO_2	10	10	11	--	--
4d	4- NO_2	--	08	--	--	200
4e	2-Cl	--	--	10	10	--
4f	3-Cl	--	14	12	--	--
4g	4-Cl	10	10	--	12	100
4h	2-CH_3	--	14	12	11	--
4i	3-CH_3	--	09	14	11	200
4j	4-CH_3	--	10	13	10	--
Tetracycline		21	19	24	15	--
Chloroamphenicol		20	25	24	18	--
Miconazole		--	--	--	--	6.25

[a] Zone of inhibition in mm measured at 50μg/mL concentration.

acetone to get the title compound **2**. m.p. 210-12°C, Yield 72%. $C_{10}H_9N_5S_2Cl_2$, Found: C, 35.89; H, 2.65; N, 20.85; S, 19.10. Calcd. for C, 35.92; H, 2.69; N, 20.91; S, 19.16%.

2-(Thiophene-2-ethyl thioureido)-4-(morpholino)-6-chloro-s-triazine, 3

Morpholine (0.1 mole, 8.17 mL) solution in acetone (100 mL) was slowly added to a well-stirred solution of 2-[thiophene-2-ethylthioureido]-4,6-dichloro-s-triazine (0.1 mole, 33.4 g) in acetone (100 mL) maintaining the temperature at 35°C. The *p*H was adjusted to neutral by the dropwise addition of 10% $NaHCO_3$. The temperature was gradually raised to 45°C during 2 hr. After the completion of reaction (TLC analysis — methanol/hexane, 9:1) the resultant solution was poured into ice cold water. The product obtained was filtered and dried. The crude product was purified by recrystallization from acetone/chloroform (1:1) to get the title compound **3**. m.p. 170°C, Yield 65%. $C_{14}H_{13}N_6S_2Cl$, Found: C, 46.00; H, 3.57; N, 23.02; S, 9.61. Calcd. for C, 46.09; H, 3.59; N, 23.04; S, 9.62%.

2-(Thiophene-2-ethylthioureido)-4-(morpholino)-6-(aryl ureido)-s-triazine, 4a-j (ref. 15)

A mixture of 2-(thiophene-2-ethylthioureido)-4-(morpholino)-6-chloro-s-triazine (0.005 mole, 1.9 g) and aryl urea (0.005 mole) in dioxane (50 mL) was refluxed on a heating mantle for 3 hr. The *p*H was adjusted to neutral by the dropwise addition of 10% $NaHCO_3$. After the completion of reaction (TLC analysis — methanol/hexane, 9:1) the contents were added to ice cold water. The solid product obtained was filtered and dried. The crude product was purified by crystallization from absolute alcohol. The physical and analytical characterization data of novel compounds **4a-j** are shown in **Table I**. The spectral data of the novel synthesized compounds **4a-j** are given below.

2-(Thiophene-2-ethylthioureido)-4-(morpholino)-6-(phenylureido)-s-triazine, 4a

IR (KBr): 3395 (-NH), 1555 (C=N), 1615 (C=S), 1128 cm^{-1} (CH_2-O-CH_2); ^{1}H NMR (DMSO-d_6): δ 2.63 (2H, t, -NHCH_2), 2.87 (4H, m, CH_2-O-CH_2), 2.97 (4H, m, CH_2-N-CH_2), 6.61 (1H, s, Ar-NH-C=O),7.28 (1H, s, -NH-C=O), 9.09 (1H, s, ArNH-C=S), 8.82 (1H, -NH-C=S), 7.70-8.12 (8H, m, Ar-H); MS: *m/z* 485 [M]$^+$.

2-(Thiophene-2-ethyl thioureido)-4-(morpholino)-6-(2-nitro phenyl ureido)-s-triazine, 4b

IR (KBr): 3388 (-NH), 1556 (C=N), 1617 (C=S), 1126(CH_2-O-CH_2), 1340 cm^{-1} (N=O); ^{1}H NMR (DMSO-d_6): δ 2.69 (2H, t, -NHCH_2), 2.92 (4H, m,CH_2-O-CH_2), 3.04 (4H, m, CH_2-N-CH_2), 6.63 (1H, s, Ar-NH- C=O), 7.33 (1H, s, -NH-C=O), 9.09 (1H, s, Ar-NH-C=S), 8.86 (1H, -NH-C=S), 7.70-8.22 (8H, m, Ar-H); MS: *m/z* 529 [M]$^+$.

2-(Thiophene-2-ethyl thioureido)-4-(morpholino)-6-(3-nitro phenyl ureido)-s-triazine, 4c

IR (KBr): 3390 (-NH), 1558 (C=N), 1613 (C=S), 1129 (CH_2-O-CH_2), 1345 cm^{-1} (N=O); ^{1}H NMR (DMSO-d_6): δ 2.71 (2H, t, -NHCH_2), 2.97 (4H, m, CH_2-O-CH_2), 3.12 (4H, m, CH_2-N-CH_2), 6.62 (1H, s, Ar-NH- C=O), 7.29 (1H, s, -NH-C=O), 9.19 (1H, s, Ar-NH-C=S), 8.84 (1H, -NH-C=S), 7.72-8.19 (8H, m, Ar-H); MS: *m/z* 529 [M]$^+$.

2-(Thiophene-2-ethyl thioureido)-4-(morpholino)-6-(4-nitro phenyl ureido)-s-triazine, 4d

IR (KBr): 3392 (-NH), 1557 (C=N), 1618 (C=S), 1130 (CH_2-O-CH_2), 1343 cm^{-1} (N=O); ^{1}H NMR (DMSO-d_6): δ 2.72 (2H, t, -NHCH_2), 2.93 (4H, m, CH_2-O-CH_2), 3.14 (4H, m, CH_2-N-CH_2), 6.65 (1H, s, Ar-NH- C=O), 7.31 (1H, s, -NH-C=O), 9.18 (1H, s, Ar-NH-C=S), 8.82 (1H, -NH-C=S), 7.80-8.16 (8H, m, Ar-H); MS: *m/z* 529 [M]$^+$.

2-(Thiophene-2-ethylthioureido)-4-(morpholino)-6-(2-chlorophenyl ureido)-s-triazine, 4e

IR (KBr): 3389 (-NH), 1555 (C=N), 1616 (C=S), 1127 (CH_2-O-CH_2), 731 cm^{-1} (C-Cl); ^{1}H NMR (DMSO-d_6): δ 2.63 (2H, t, -NHCH_2), 2.87 (4H, m, -O-CH_2), 2.97 (4H, m, CH_2-N-CH_2), 6.61 (1H, s, Ar-NH- C=O), 7.28 (1H, s, -NH-C=O), 9.09 (1H, s, Ar-NH-C=S), 8.85 (1H, -NH-C=S), 7.81-8.19 (8H, m, Ar-H); MS: *m/z* 519 [M]$^+$.

2-(Thiophene-2-ethylthioureido)-4-(morpholino)-6-(3-chlorophenyl ureido)-s-triazine, 4f

IR (KBr): 3394 (-NH), 1558 (C=N),1615 (C=S), 1131 (CH_2-O-CH_2), 750 cm^{-1} (C-Cl); ^{1}H NMR (DMSO-d_6): δ 2.62 (2H, t, -NHCH_2), 2.84 (4H, m, -O-CH_2), 2.99 (4H, m, CH_2-N-CH_2), 6.69 (1H, s, Ar-NH- C=O), 7.26 (1H, s, -NH-C=O)\, 9.12 (1H, s, Ar-NH-C=S), 8.83 (1H, -NH-C=S), 7.70-7.85 (8H, m, Ar-H); MS: *m/z* 519 [M]$^+$.

2-(Thiophene-2-ethylthioureido)-4-(morpholino)-6-(4-chlorophenyl ureido)-s-triazine, 4g

IR (KBr): 3393 (-NH), 1556 (C=N), 1619 (C=S), 1129 (CH_2-O-CH_2), 731 cm^{-1} (C-Cl); ^{1}H NMR (DMSO-d_6): δ 2.66 (2H, t, -NHCH_2), 2.87 (4H, m, -O-CH_2), 2.99 (4H, m, CH_2-N-CH_2), 6.61 (1H, s, Ar-NH- C=O), 7.25 (1H, s, -NH-C=O), 9.09 (1H, s, Ar-NH-C=S), 8.79 (1H, -NH-C=S), 7.72-8.01 (8H, m, Ar-H); MS: *m/z* 519 [M]$^+$.

2-(Thiophene-2-ethylthioureido)-4-(morpholino)-6-(2-methylphenyl ureido)-s-triazine, 4h

IR (KBr): 3397 (-NH) 1559 (C=N), 1617(C=S), 1128 cm^{-1} (CH_2-O-CH_2); ^{1}H NMR (DMSO-d_6): δ 2.64 (2H, t, -NHCH_2), 2.85 (4H, m, CH_2-O-CH_2), 2.18 (3H, s, Ar-CH_3), 2.97 (4H, m, CH_2-N-CH_2), 6.63 (1H, s, Ar-NH-C=O), 7.22 (1H, s, -NH-C=O), 9.19 (1H, s, Ar-NH-C=S), 8.84 (1H, -NH-C=S), 7.72-7.96 (8H, m, Ar-H); MS: *m/z* 498 $[M]^+$.

2-(Thiophene-2-ethylthioureido)-4-(morpholino)-6-(3-methylphenyl ureido)-s-triazine, 4i

IR (KBr): 3395 (-NH), 1555 (C=N), 1615 (C=S), 1126 cm^{-1} (CH_2-O-CH_2); ^{1}H NMR (DMSO-d_6): δ 2.67 (2H, t, -NHCH_2), 2.84 (4H, m, CH_2-O-CH_2), 2.12 (3H, s, Ar-CH_3), 2.92 (4H, m, CH_2-N-CH_2), 6.61 (1H, s, Ar-NH-C=O), 7.28 (1H, s, -NH-C=O), 9.11 (1H, s, Ar-NH-C=S), 8.81 (1H, -NH-C=S), 7.40-8.04 (8H, m, Ar-H); MS: m/z 498 $[M]^+$.

2-(Thiophene-2-ethylthioureido)-4-(morpholino)-6-(4-methylphenyl ureido)-s-triazine, 4j

IR (KBr): 3390 (-NH), 1557 (C=N), 1619 (C=S), 1132 cm^{-1} (CH_2-O-CH_2); ^{1}H NMR (DMSO-d_6): δ 2.63 (2H, t, -NHCH_2), 2.83 (4H, m, CH_2-O-CH_2), 2.04 (3H, s, Ar-CH_3), 2.97 (4H, m, CH_2-N-CH_2), 6.65 (1H, s, Ar-NH-C=O), 7.29 (1H, s, -NH-C=O), 9.16 (1H, s, Ar-NH-C=S), 8.85 (1H, -NH-C=S), 7.70-7.95 (8H, m, Ar-H); MS: *m/z* 498 $[M]^+$.

Results and Discussion

The antibacterial activity was determined *in vitro* by filter paper disc diffusion method[16] by measuring zone of inhibition in mm. All the compounds were screened for their antibacterial activity against Gram positive bacterial strains such as *S. aureus* and, *B. subtilis,* and Gram-negative bacterial strain *S. typhi* and *E. coli* using tetracycline and chloramphenicol as standard drugs, respectively for comparison. Some of them were screened for *in vitro* antifungal screening[17] against *C. albicans* using miconazole as standard drug. These results are shown in **Table II**.

SAR Studies

Ten compounds were synthesized in this series. All the compounds along with starting material s-triazine were subjected to various screening programmes. This series contains three types of chemical linkages; thioureido linkage with thiophene-2-ethyl thiourea, morpholine linkage through nitrogen and ureido linkage with substituted phenyl ring. s-Triazine showed moderate activity against *E. coli*, *S. aureus*, *S. typhi* and *B. subtillis*. It was also found active against *C. albicans.*

In case of thioureido linkage (derived from thiophene-2-ethylamine) as well as morpholine linkage with s-triazine through nitrogen not much deviation of activity from the parent molecule has been observed but the incorporation of ureido linkage showed moderate to good activity.

All these linkages had the effect of increasing the biological activity as compared to their parent compound. Compounds **4a** and **4g** for *E. coli*, Compound **4f**, and **4h** for *S. aureus*, **4i** for *S. typhi* and **4c** and **4g** for *B. subtillis* exhibited maximum zone of inhibition *i.e.* 12 mm, 12 mm, 14 mm, 14 mm, 14 mm, 10 mm and 10 mm, respectively (**Table II**). Out of 10 compounds synthesised, five compounds as mentioned in **Table I** were screened for antifungal activity against *C. albicans.* Two compounds **4d** and **4i** showed poor activity at 200 μg/mL while compound **4g** showed moderate activity at 100 μg/mL. As usual, hyperconjugation effect of methyl group may favour the increase in activity of lead molecules. The structure variation such as methyl, and halo groups at *ortho, meta* and *para* positions to the ureido linkage against all micro-organisms resulted in increase in antibacterial activity as compared to the parent compound.

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