Design, synthesis and antimicrobial evaluation of s-triazinyl urea and thiourea derivatives

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Abstract

A series of urea and thiourea derivatives of s-triazine have been developed based on high yielding nucleophilic substitution of 2,4,6-trichloro-1,3,5-triazine by 4-hydroxy coumarin, cyclopropylamine and ammonia at suitable conditions. These were further treated with various substituted aryl isocyanate and aryl isothiocyanate. All the synthesized compounds were evaluated for their antibacterial activities against various Gram-positive and Gram-negative strains of bacteria. A few compounds showed good to superior *in vitro* antibacterial activity against *S.aureus, B.subtilis, E.coli* and *P.aeruginosa* respectively. The new synthesized compounds were characterized using IR, ^{1}H-NMR and elemental analysis.

Keywords: s-Triazine, urea, thiourea, 4-hydroxy coumarin, antibacterials

Introduction

The exploration of heterocycles as privileged structures in drug discovery is, beyond doubt, one of the major areas in medicinal chemistry. These privileged structures represent a class of molecules that act as ligands for various biological receptors with a high degree of binding affinity. Problems of multi-drug resistant microorganisms have reached on alarming level in many countries around the world. A numbers of recent clinical reports describe the increasing occurrence of meticillin-resistant *S. aureus* and other antibiotic-resistant human pathogenic microorganisms in United State and European countries. Infections caused by those microorganisms pose a serious challenge to the medical community and the need for an effective therapy has led to a search for novel antimicrobial agents. Exploitation of these molecules should allow us to rapidly discover new biologically active compounds across a broad range of therapeutic areas in a shorter time scale.

In this work, we report the synthesis and biological activity of some 1,3,5-triazinyl urea and thiourea analogues a class of privileged structures that have a wide range of biological properties. Among the compound having good antimicrobial properties, s-triazine derivatives constitute an important class of compounds possessing diverse pharmacological activities including broadly active as herbicidal [1] and antimicrobial.[2] Some are also used for the treatment of HIV infection.[3-5] Several workers investigated the s-triazine nucleus in the scope of potential therapeutic agents for diseases due to bacteria [6-9] cancer,[10-12] antitumor [13-14] and malaria.[15-16] The above literature survey led us to consider the s-triazine nucleus as a possible scaffold.

Coumarins constitute an important class of compounds with several types of pharmaceutical agents possessing anticancer, anti-HIV, anticoagulant and antiproliferative activity.[17] 4-Hydroxy coumarin has specific space among other coumarin derivatives, it possess anticonvulsant, antibacterial, antifungal and antitumor activities.[18-19]

In the design of new compounds, development of hybrid molecules through the combination of different pharmacophores in one structure may lead to compounds with increased antimicrobial activity. Therefore, these observations prompted us to synthesize new s-triazine derivatives which were attached with coumarin ring through an oxygen bridge. Then, the synthesized compounds were tested against two Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*), two Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli*) using the broth microdilution method.

Results and Discussion

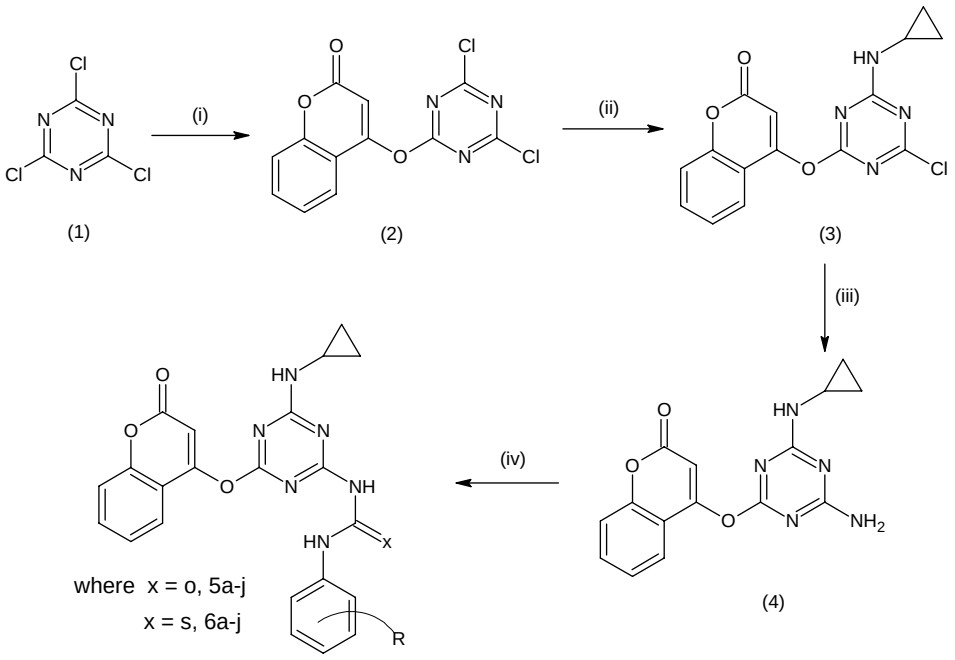
Materials and Methods

All reagents were of analytical grade and use directly. All the melting points were determined in open glass capillary and are uncorrected. Progress of reaction was monitored by thin layer chromatography (TLC) using silica gel-G coated aluminium plates (0.5 mm thickness, Merck) and spots were visualized under UV radiation, purified by recrystallisation and column chromatography. The IR spectra were recorded on BRUKER TENSOR Series using KBr pellets. ^{1}H NMR spectra were recorded on 300MHz BRUKER ULTRASHIELD using DMSO-d_6 as a solvent and TMS as an internal reference and chemical shift values were expressed in δ ppm.

Chemistry

The synthesis of the series proceeded as depicted in Schemes 1. 2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(aryl ureido)-s-triazine **(5a-j)** and 2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(aryl thioureido)-s-triazine **(6a-j)** were prepared in four steps from s-triazine. Briefly, s-triazine **(1)** was reacted with 4-hydroxy coumarin in presence of Na_2CO_3 to give 2-(Coumarinyl-4-oxy)-4,6-dichloro-s-triazine **(2)**. The structure of compound **(2)** was confirmed by the IR and ^{1}H NMR spectral analysis. It is interesting to note that in IR spectra two sharp bands in the region of 1230, 1040 cm^{-1} due to symmetrical and unsymmetrical C-O-C

group respectively. Compound **(2)** was condensed with cyclopropyl to give 2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-chloro-s-triazine **(3)**. The IR spectra of compound **(3)** shown one sharp absorption band at 3315 cm^{-1} for –NH group and PNMR spectra of compound **(3)** shown singlet at δ 3.97 of –N<u>H</u> proton. Compound **(4)** was obtained by the condensation of compound **(3)** with ammonia. The structure of compound **(4)** was confirmed by the IR and ^{1}H NMR spectral analysis. Compound (4) was condensed with various isocyanate and isothiocyanate to afforded **(5a-j)** and **(6a-j)** respectively. All the synthesized compounds were fully characterized by IR, ^{1}H-NMR spectroscopy and elemental analysis.



Scheme 1. Synthesis of the titled compounds. (i) 4-Hydroxy coumarin, Acetone, $Na_2CO_{3\ (aq)}$ (10%), 0-5°C, (ii) Cyclopropylamine, Acetone, $Na_2CO_{3\ (aq)}$ (10%), 30-45°C (iii) Ammonia, 1,4-dioxane, 70-80°C (iv) Ar-NCX, Ethanol, reflux.

Experimental Section

2-(Coumarinyl-4-oxy)-4,6-dichloro-s-triazine (2). To a stirred solution of cyanuric chloride **(1)** (4 g, 0.021 mol) in acetone (50 mL) at 0-5°C, the solution of 4-hydroxy coumarin (3.5 g, 0.021 mol) in 10% Na_2CO_3 (25 mL) was added drop wise in 1 hour. The stirring was continued at 0-5°C for 4 hours. The progress of reaction was monitored by TLC using toluene: methanol (7:3) as eluent. After the completion of reaction, the content was poured into crushed ice water. Product was filtered, washed with cold water and dried to give **(2)**, yield 78%, mp 208-210°C.

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-chloro-s-triazine (3). To a stirred solution of **2** (4 g, 0.013 mol) in acetone (50 mL) at 30-45°C, cyclopropylamine (0.74 g, 0.9 mL, 0.013 mol) was added drop wise. The pH was adjusted neutral by the addition of 10% Na_2CO_3 solution. The temperature was gradually raised to 45°C during 2 hours and further maintained for 2 hours. The progress of reaction was monitored by TLC using toluene: acetone (6:4) as eluent. After the completion of reaction, the content was poured into crushed ice. Product was filtered, washed with cold water and recrystallized from ethyl acetate to give **(3)** yield 60%, mp 198-200°C.

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(amino)-s-triazine (4). A mixture of **(3)** (3 g, 0.009 mol) and 28% aqueous ammonia (1.2 mL, 0.018 mol) in 1, 4-dioxane (40 mL) was heated at 70-80^{o}C on water bath for 5 hours. The progress of reaction was monitored by TLC using toluene: acetone (5:5) as eluent. After the completion of reaction, the content was poured into ice water. Product was filtered, washed with cold water and recrystallized from ethanol to give **(4)** yield 65%, mp 178-182°C.

General procedure for the preparation of 2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(aryl ureido)-s-triazine (5a-j)

A mixture of (**4)** (1.5 g, 0.005 mol) and aryl isocyanate (0.005 mol) in ethanol (30 mL) was refluxed for 5-6 hours. The progress of reaction was monitored by TLC using toluene: acetone (7:3) as eluent. After the completion of reaction, the solvent was removed by distillation and the resulting solid was recrystallized from methanol.

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(phenyl ureido)-s-triazine (5a). Yield 64%, mp 122-124°C. Anal. Calcd for $C_{22}H_{18}O_4N_6$, Calculeted: C 61.39, H 4.22, N 19.53, Found : C 61.33, H 4.21, N 19.51. IR(KBr)cm^{-1}, 3315(N-H str), 1660(C=O urea), 1340(C-O-C), 1730(C=O δ-lactone), 825(C-N), 2990(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.49-0.63(m, 4H, 2 CH_2), 1.24-1.53(m, 1H, CH), 3.97(s, 1H, NH), 5.97(s, 1H, Ar-H), 6.85(d, 1H, Ar-H), 7.12-8.0(m, 8H, Ar-H), 8.72(s, 1H, Ar-NHCO), 9.67(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(2-chloro penyl ureido)-s-triazine (5b). Yield 70%, mp 242-244°C, Anal. Calcd for $C_{22}H_{17}O_4N_6Cl$, Calculeted : C 56.84, H 3.69, N 18.08, Found : C 56.81, H 3.68, N 18.05. IR(KBr) cm^{-1}, 3255(N-H str), 1685(C=O urea), 1355(C-O-C), 1720(C=O δ-lactone), 770(C-Cl str), 835(C-N), 3010(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.48-0.61(m, 4H, 2 CH_2), 1.22-1.51(m, 1H, CH), 3.92(s, 1H, NH), 5.89(s, 1H, Ar-H), 6.7-7.9(m, 8H, Ar-H), 8.79(s, 1H, Ar-NHCO), 9.54(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(3-chloro phenyl ureido)-s-triazine (5c). Yield 55%, mp 162-164°C, Anal. Calcd for $C_{22}H_{17}O_4N_6Cl$, Calculeted : C 56.84, H 3.69, N 18.08, Found : C 56.79, H 3.72, N 18.03. IR(KBr) cm^{-1}, 3160(N-H str), 1645(C=O urea), 1347(C-O-C), 1730(C=O δ-lactone), 785(C-Cl str), 828(C-N), 3005(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.50-0.66(m, 1H, 2CH_2), 1.30-1.58(m, 1H, CH), 3.91(s, 1H, NH), 5.80(s, 1H, Ar-H), 6.61-7.8(m, 8H, Ar-H), 9.20(s, 1H, Ar-NHCO), 9.72(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(4-chloro phenyl ureido)-s-triazine (5d). Yield 59%, mp 172-174°C, Anal. Calcd for $C_{22}H_{17}O_4N_6Cl$, Calculeted : C 56.84, H 3.69, N

18.08, Found : C 56.81, H 3.66, N 18.04. IR(KBr) cm^{-1}, 3250(N-H str), 1712(C=O urea), 1352(C-O-C), 1732(C=O δ-lactone), 690(C-Cl str), 825(C-N), 3028(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.46-0.63(m, 4H, $2CH_2$), 1.28-1.52(m, 1H, CH), 3.86(s, 1H, NH), 5.78(s, 1H, Ar-H), 6.48(d, 2H, Ar-H), 6.9-7.84(m, 6H, Ar-H), 8.75(s, 1H, Ar-NHCO), 9.40(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(2-methyl phenyl ureido)-s-triazine (5e). Yield 64%, mp 158-162°C, Anal. Calcd for $C_{23}H_{20}O_4N_6$, Calculeted : C 62.36, H 4.54, N 18.90, Found : C 62.32, H 4.51, N 18.86. IR(KBr) cm^{-1}, 3287(N-H str), 1694(C=O urea), 1335(C-O-C), 1740(C=O δ-lactone), 832(C-N), 3025(C-H). ^{1}H NMR (DMSO- d_6, δ) ppm, 0.48-0.64(m, 4H, $2CH_2$), 1.27-1.49(m, 1H, CH), 3.82(s, 1H, NH), 5.87(s, 1H, Ar-H), 2.24(3H, s, Ar-CH_3), 6.84-7.82(m, 8H, Ar-H), 8.95(s, 1H, Ar-NHCO), 9.62(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(3-methyl phenyl ureido)-s-triazine (5f). Yield 62%, mp 126-128°C, Anal. Calcd for $C_{23}H_{20}O_4N_6$, Calculeted : C 62.36, H 4.54, N 18.90, Found : C 62.12, H 4.52, N 18.87. IR(KBr) cm^{-1}, 3298(N-H str), 1705(C=O urea), 1330(C-O-C), 1755(C=O δ-lactone), 818(C-N), 3037(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.46-0.67(m, 4H, $2CH_2$), 1.25-1.46(m, 1H, CH), 3.88(s, 1H, NH), 5.94(s, 1H, Ar-H), 2.35(3H, s, Ar-CH_3), 6.57(s, 1H, Ar-H), 6.9-7.9(m, 7H, Ar-H), 8.82(s, 1H, Ar-NHCO), 9.58(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(4-methyl phenyl ureido)-s-triazine (5g). Yield 68%, mp 112-114°C, Anal. Calcd for $C_{23}H_{20}O_4N_6$, Calculeted : C 62.36, H 4.54, N 18.90, Found : C 62.12, H 4.52, N 18.88. IR(KBr) cm^{-1}, 3257(N-H str), 1710(C=O urea), 1364(C-O-C), 1728(C=O δ-lactone), 810(C-N), 3009(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.48-0.68(m, 4H, $2CH_2$), 1.23-1.42(m, 1H, CH), 3.92(s, 1H, NH), 5.78(s, 1H, Ar-H), 2.23(s, 3H, Ar-CH_3), 6.57(d, 2H, Ar-H), 7.08(d, 2H, Ar-H), 7.30-7.74(m, 4H, Ar-H), 9.02(s, 1H, Ar-NHCO), 9.47(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(2-methoxy phenyl ureido)-s-triazine (5h). Yield 72%, mp 172-174°C, Anal. Calcd for $C_{23}H_{20}O_5N_6$, Calculeted : C 61.39, H 4.38, N 18.25, Found : C 61.34, H 4.35, N 18.22. IR(KBr) cm^{-1}, 3320(N-H str), 1698(C=O urea), 1305(C-O-C), 1742(C=O δ- lactone), 842(C-N), 3048(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.50-0.70(m, 4H, $2CH_2$), 1.21-1.54(m, 1H, CH), 3.95(s, 1H, NH), 5.85(s, 1H, Ar-H), 3.48(3H, s, Ar-OCH_3), 6.9-8.1(m, 8H, Ar-H), 9.30(s, 1H, Ar-NHCO), 9.51(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(4-methoxy phenyl ureido)-s-triazine (5i). Yield 68%, mp 186-188°C, Anal. Calcd for $C_{23}H_{20}O_5N_6$, Calculeted : C 61.39, H 4.38, N 18.25, Found : C 61.37, H 4.36, N 18.23. IR(KBr) cm^{-1}, 3322(N-H str), 1695(C=O urea), 1380(C-O-C), 1720(C=O δ- lactone), 845(C-N), 3072(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.51-0.74(m, 4H, $2CH_2$), 1.25-1.63(m, 1H, CH), 4.21(s, 1H, NH), 5.69(s, 1H, Ar-H), 3.87(3H, s, Ar-OCH_3), 6.44(d, 2H, Ar-H), 7.28(d, 2H, Ar-H), 7.43-7.77(m, 6H, Ar-H), 9.18(s, 1H, Ar-NHCO), 9.62(s,1H, CONH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(3-nitro phenyl ureido)-s-triazine (5j). Yield 56%, mp 88-90°C, Anal. Calcd for $C_{22}H_{17}O_6N_7$, Calculeted : C 55.58, H 3.60, N 20.62, Found : C 55.55, H 3.57, N 20.58. IR(KBr) cm^{-1}, 3280(N-H str), 1660(C=O urea), 1358(C-O-C), 1736(C=O δ- lactone), 890(C-N), 1527(N=O), 3040(C-H). ^{1}H NMR (DMSO-d_6, δ) ppm, 0.44-

0.56(m, 4H, $2CH_2$), 1.24-1.41(s, 1H, CH), 4.0(s, 1H, NH), 5.64(s, 1H, Ar-H), 8.39(s, 1H, Ar-H), 7.0-8.1(m, 7H, Ar-H), 8.72(s, 1H, Ar-NHCO), 9.25(s,1H, CONH).

General procedure for the preparation of 2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(phenyl thioureido)-s-triazine (6a-j)

A mixture of (**4)** (1.5 g, 0.005 mol) and aryl isothiocyanate (0.005 mol) in ethanol (30 mL) was refluxed for 5-6 hours. The progress of reaction was monitored by TLC using toluene: acetone (6:4) as eluent. After the completion of reaction, the solvent was removed by distillation and the resulting solid was recrystallized from methanol.

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(phenyl thioureido)-s-triazine (6a). Yield 72%, mp 80-82°C, Anal. Calcd for $C_{22}H_{18}O_3N_6S$, Calculeted : C 59.18, H 4.02, N 18.82, Found : C 59.15, H 4.01, N 18.79. IR(KBr) cm^{-1}, 3246(N-H str), 1400(C=S), 1265(C-O-C), 1699(C=O δ-lactone), 785(C-N), 2992(C-H). ^{1}H NMR (DMSO-d_6 δ) ppm, 0.30-0.54(m, 4H, $2CH_2$), 1.22-1.50(s, 1H, CH), 3.8(s, 1H, NH), 5.72(s, 1H, Ar-H), 6.8(dd, 1H, Ar-H), 7.2-7.9(m, 7H, Ar-H), 10.9(s, 1H, Ar-NHCS), 11.18(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(2-chloro phenyl thioureido)-s-triazine (6b). Yield 66%, mp 94-96°C, Anal. Calcd for $C_{22}H_{17}O_3N_6SCl$, Calculeted : C 54.94, H 3.56, N 17.47, Found : C 54.91, H 3.52, N 17.44. IR(KBr) cm^{-1}, 3285(N-H str), 1440(C=S), 1204(C-O-C), 1735(C=O δ-lactone), 810(C-N), 3045(C-H). ^{1}H NMR (DMSO- d_6 δ) ppm, 0.41-0.56(m, 4H, $2CH_2$), 1.20-1.48(m, 1H, CH), 4.1(s, 1H, NH), 5.86(s, 1H, Ar-H), 6.8-7.89(m, 8H, Ar-H), 11.01(s, 1H, Ar-NHCS), 11.4(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(3-chloro phenyl thioureido)-s-triazine (6c). Yield 59%, mp 92-94°C, Anal. Calcd for $C_{22}H_{17}O_3N_6SCl$, Calculeted : C 54.94, H 3.56, N 17.47, Found : C 54.91, H 3.52, N 17.44. IR(KBr) cm^{-1}, 3318(N-H str), 1510(C=S), 1228(C-O-C), 1750(C=O δ-lactone), 880(C-N), 3025(C-H).^{1}H NMR (DMSO- d_6 δ) ppm, 0.37-0.54(m, 4H, $2CH_2$), 1.23-1.51(m, 1H, CH), 3.8(s, 1H, NH), 5.91(s, 1H, Ar-H), 6.69(1H, s, Ar-H), 7.1-8.2(m, 7H, Ar-H), 10.87(s, 1H, Ar-NHCS), 11.25(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(4-chloro phenyl thioureido)-s-triazine (6d). Yield 63%, mp 74-76°C, Anal. Calcd for $C_{22}H_{17}O_3N_6SCl$, Calculeted : C 54.94, H 3.56, N 17.47, Found : C 54.92, H 3.51, N 17.44. IR(KBr) cm^{-1}, 3280(N-H str), 1435(C=S), 1205(C-O-C), 1698(C=O δ-lactone), 852(C-N), 3060(C-H). ^{1}H NMR (DMSO- d_6 δ) ppm, 0.39-0.53(m, 4H, $2CH_2$), 1.23-1.35(s, 1H, CH), 3.97(s, 1H, NH), 5.79(s, 1H, Ar-H), 6.58(d, 2H, Ar-H), 7.38(d, 2H, Ar-H), 7.50-7.64(m, 4H, Ar-H) 10.87(s, 1H, Ar-NHCS), 11.09(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(2-methyl phenyl thioureido)-s-triazine (6e). Yield 69%, mp 102-104°C, Anal. Calcd for $C_{23}H_{20}O_3N_6S$, Calculeted : C 59.99, H 4.38, N 18.25, Found : C 59.94, H 4.36, N 18.21. IR(KBr) cm^{-1}, 3318(N-H str), 1510(C=S), 1225(C-O-C), 1722(C=O δ-lactone), 870(C-N), 3076(C-H). ^{1}H NMR (DMSO- d_6 δ) ppm, 0.37-0.52(m, 4H, $2CH_2$), 1.22-1.52(s, 1H, CH), 4.0(s, 1H, NH), 5.82(s, 1H, Ar-H), 2.3(s, 3H, Ar-CH_3), 6.8-7.92(m, 8H, Ar-H), 10.72(s, 1H, Ar-NHCS), 11.2(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(3-methyl phenyl thioureido)-s-triazine (6f). Yield 58%, mp 114-116°C, Anal. Calcd for $C_{23}H_{20}O_3N_6S$, Calculeted : C 59.99, H 4.38, N 18.25, Found : C 59.97, H 4.34, N 18.21. IR(KBr) cm^{-1}, 3257(N-H str), 1350(C=S), 1272(C-O-C), 1706(C=O δ-lactone), 856(C-N), 3012(C-H). ^{1}H NMR (DMSO- d_6 δ) ppm, 0.38-0.53(m, 4H, $2CH_2$), 1.21-1.52(m, 1H, CH), 3.97(s, 1H, NH), 5.82(s, 1H, Ar-H), 2.13(s, 3H, Ar-CH_3), 6.58(s, 1H, Ar-H), 6.9-8.0(m, 7H, Ar-H), 10.9(s, 1H, Ar-NHCS), 11.42(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(4-methyl phenyl thioureido)-s-triazine (6g). Yield 65%, mp 78-80°C, Anal. Calcd for $C_{23}H_{20}O_3N_6S$, Calculeted : C 59.99, H 4.38, N 18.25, Found : C 59.96, H 4.34, N 18.21. IR(KBr) cm^{-1}, 3362(N-H str), 1490(C=S), 1198(C-O-C), 1688(C=O δ-lactone), 905(C-N), 3040(C-H). ^{1}H NMR (DMSO-d_6 δ) ppm, 0.4-0.54(m, 4H, $2CH_2$), 1.2-1.5(m, 1H, CH), 3.72(s, 1H, NH), 6.0(s, 1H, Ar-H), 2.3(s, 3H, Ar-CH_3), 6.49(d, 2H, Ar-H), 6.55(d, 2H, Ar-H), 6.8-7.9(m, 4H, Ar-H), 10.8(s, 1H, Ar-NHCS), 11.3(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(2-methoxy phenyl thioureido)-s-triazine (6h). Yield 70%, mp 102-104°C, Anal. Calcd for $C_{23}H_{20}O_4N_6S$, Calculeted : C 57.97, H 4.23, N 17.64, Found : C 57.95, H 4.22, N 17.61. IR(KBr) cm^{-1}, 3300(N-H str), 1510(C=S), 1140(C-O-C), 1725(C=O δ-lactone), 835(C-N), 3020(C-H). ^{1}H NMR (DMSO-d_6 δ) ppm, 0.37-0.51(m, 4H, $2CH_2$), 1.3-1.6(m, 1H, CH), 3.92(s, 1H, NH), 5.82(s, 1H, Ar-H), 3.32(3H, s, Ar-OCH_3), 6.9-8.1(m, 8H, Ar-H), 11.13(s, 1H, Ar-NHCS), 11.54(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(4-methoxy phenyl thioureido)-s-triazine (6i). Yield 74%, mp 84-86°C, Anal. Calcd for $C_{23}H_{20}O_4N_6S$, Calculeted : C 57.97, H 4.23, N 17.64, Found : C 57.93, H 4.21, N 17.60. IR(KBr) cm^{-1}, 3310(N-H str), 1425(C=S), 1190(C-O-C), 1704(C=O δ-lactone), 892(C-N), 3075(C-H). ^{1}H NMR (DMSO-d_6 δ) ppm, 0.47-0.53(m, 4H, $2CH_2$), 1.4-1.5(m, 1H, CH), 3.82(s, 1H, NH), 5.9(s, 1H, Ar-H), 3.42(s, 3H, Ar-OCH_3), 6.63(d, 2H, Ar-H), 6.82(d, 2H, Ar-H), 7.0-8.1(m, 4H, Ar-H), 10.92(s, 1H, Ar-NHCS), 11.42(s, 1H, CSNH).

2-(Coumarinyl-4-oxy)-4-(cyclopropylamino)-6-(4-nitro phenyl thioureido)-s-triazine (6j). Yield 54%, mp 86-88°C, Anal. Calcd for $C_{23}H_{17}O_5N_7S$, Calculeted : C 53.76, H 3.49, N 19.95, Found : C 53.75, H 3.47, N 19.92. IR(KBr) cm^{-1}, 3360(N-H str), 1470(C=S), 1218(C-O-C), 1710(C=O δ-lactone), 878(C-N), 3060(C-H), 1520(N=O).^{1}H NMR (DMSO-d_6 δ) ppm, 0.49-0.54(m, 4H, $2CH_2$), 1.3-1.47(m, 1H, CH), 3.94(s, 1H, NH), 5.76(s, 1H, Ar-H), 6.59(d, 2H, Ar-H), 6.9-7.8(m, 6H, Ar-H), 10.84(s, 1H, Ar-NHCS), 11.32(s, 1H, CSNH).

Antibacterial activity

The minimum inhibitory concentration (MIC) of the synthesized compound were tested against two representative Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) and two Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards[20] with gradual dilution starting from (100, 50, 25, 12.5, 6.25, 3.125) μg/mL. Penicillin and Streptomycin were used as reference antibacterial agents. Solution of the test compounds and reference drugs were dissolved in DMSO.

Table 1. Antibacterial activities

Compound	R	X	Minimum Inhibitory Concentration in μg/mL			
			Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
5a	H	O	25	50	100	25
5b	2-Cl	O	12.5	25	50	50
5c	3-Cl	O	25	6.25	-	25
5d	4-Cl	O	50	6.25	100	50
5e	2-CH_3	O	100	25	-	100
5f	3-CH_3	O	50	12.5	50	25
5g	4-CH_3	O	12.5	-	100	50
5h	2-OCH_3	O	12.5	25	50	12.5
5i	4-OCH_3	O	6.25	50	100	25
5j	3-NO_2	O	25	100	-	50
6a	H	S	12.5	50	50	25
6b	2-Cl	S	25	100	100	50
6c	3-Cl	S	12.5	25	50	50
6d	4-Cl	S	25	6.25	100	12.5
6e	2-CH_3	S	50	100	50	25
6f	3-CH_3	S	6.25	25	25	12.5
6g	4-CH_3	S	12.5	-	12.5	50
6h	2-OCH_3	S	100	50	50	25
6i	4-OCH_3	S	12.5	12.5	25	50
6j	4-NO_2	S	-	12.5	100	25
Penicillin			1.562	1.562	12.5	6.25
Streptomycin			6.25	6.25	3.125	3.125

For antibacterial activity, in present protocol 25 μg mL^{-1} is considered as moderate activity, 12.5 μg mL^{-1} is considered as good activity and 6.25 μg mL^{-1} is considered as excellent active compound to the standard drug.

Results and Discussion

The present paper is focused on the synthesis of novel heterocyclic compounds as possible antibacterial agents. The minimum inhibitory concentration (MICs, μg mL^{-1}) of tested compounds against bacteria are shown in Table I. All the synthesized compounds exhibited moderate to excellent inhibitory effect with MIC values against four strains of bacteria (Gram-positive, Gram-negative). The ureido linkage two chloro substituted (**5c** and **5d**) and methoxy substituted (**5i**) compounds showed excellent activity against Gram-positive *B. subtillus* and *S.*

aureus bacteria. Among the thioureido linkage compounds, (**6d** and **6f**) displayed excellent activity against Gram-positive *B. subtillis* and *S. aureus* organism. In general, it is concluded that the presence of electron withdrawing group increases the antibacterial activities compare to the electron donating group to the aromatic ring. Based upon this observation it becomes necessary to optimize lead compound with chloro substitution for augmenting antibacterial prob. Based upon the results, it will also be necessary to optimize by the substituting a series of electron-withdrawing groups on aromatic ring and selectively modifying the s-triazine nucleus. The substitution in the C-3 and C-4 positions of the phenyl ring seems to be very important for antibacterial effect, as well as the presence and the position of oxo-linkage in the connecting linker between the aromatic rings seems to be very important for antibacterial effect.

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References

1. Garmouna, M.; Blanchoud, H.; Teil, M.; Blanchard, M.; Chevreuil, M. *Water, Air, Soil, Poll*. **2001**, *132*, 1.
2. Jain, S.; Bhambi, D.; Sharma, R.; Talesara, L. *Ind. J. Pharm. Sci.* **2007**, *69(1)*, 28.
3. Klenke, B.; Stewart, M.; Barett, M. P.; Brun, R.; Gilbert, I. H. *J. Med. Chem.* **2001**, *44*, 3440.
4. Kukla, M. J.; Janseen, P. A. *Eur. Pat.* **1999**, *945*, 447.
5. Patel, R. B.; Chikhalia, K. H.; De clercq, E. *J. Braz. Chem. Soc.* **2007**, *18*, 2.
6. Srinivas, K.; Shrinivas, U.; Rao, V. J.; Bhanuprakash, K.; Chishore, K. H.; Murty, U. S. N. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1121.
7. Lubbers, T.; Angechm, P.; Gumunder, H.; Herzig, S.; Kulhanek, J. *Bioorg. Chem. Lett.* **2000**, *3*, 821.
8. Desai, A. D.; Mahajan, D. H.; Chikhalia, K. H. *Ind. J. Chem.* **2007**, *46B*, 1169.
9. Srinivas, K.; Srinivas, U.; Bhanuprakash, K.; Harakishore, K.; Murthy, U. S. N.; Rao, V. J. *Euro. J. Med. Chem.* **2006**, *41*, 1240.
10. Alteramn, M.; Samulsson, B. *J. Med. Chem.* **1998**, *41*, 3782.
11. Ktritzky, A. R.; Boris, V. R.; Vladimir, Y. V.; Normand, H and Behranz, F. *J. Org. Chem.* **2001**, *66*, 6797.
12. Mayumi, O.; Kawahara, N. and Santo, Y. *Cancer Res.* **1996**, *56*, 1512.
13. Brozowski, Z.; Gdaniec, M. Eur. *J. Med. Chem.* **2000**, *35*, 1053.

14. Shin-ichi, Y.; Yasuhisa, F.; Ichiro, K.; Hisashi, Y.; Toshiyuki, M.; Hiroaki, G.; Takato, Y. J. *Nat. Cancer Inst.* **2006**, *98(8)*, 545.
15. Youngyuth, Y.; Tirayut, V.; Netnapa, C.; Sumalee, K.; Gordon, L. *J. Med. Chem.* **2000**, *43*, 2738.
16. Agrwal, A.; Srivastava, K.; Puri, S. K. and Chauhan, M. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 531.
17. Irena, K.; Georgi, M.; Margarita, K. *Bioorg. Chem. Appl.* **2006**, 1.
18. Jung, J. C.; Lee, J. H.; Oh, S.; Lee, J. G. *Bioorg. Med. Chem. Lett.* **2004**, *14(22)*, 27.
19. Sueed, U. R.; Zahid, H. C.; Lunlaz, F.; Supuram, C. T. J. *Enzy. Inh. Med. Chem.* **2005**, *20(4)*, 333.
20. National Committee for Clinical Laboratory standards (NCCLS). Standard methods for dilution antimicrobial susceptibility test for bacteria which grows aerobically Nat. Comm. Clini. Lab. Stands. Villanova. **1982**, 242.