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ORIGINAL PAPER

Synthesis, Spectroscopic Characterization, Crystal structure, Antimicrobial and In Vitro Hemolytic Studies of Some Novel Substituted Thiourea Derivatives

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Abstract A series of *N*,*N*′-disubstituted thioureas, [R–CONHCSNH–R′] where (R = thiophenyl, furonyl, phenyl and R′ = 4-sulphonamido phenyl, pyrimidine-2yl, thiazole-2yl, 3-nitro phenyl, 2-nitro-4-chloro phenyl, 2-chloro-4-nitro phenyl, 2-methoxy-4-nitro phenyl, and 6-phenyl-1,3,5-triazinyl were synthesized, characterized and screened for their antimicrobial activities. The structures of synthesized compounds were established by elemental analysis and spectroscopic techniques (FT-IR, ^{1}H NMR, and ^{13}C NMR). Single crystal study on compounds **1a** and **1c** have been done. The compound **1a** crystallizes in monoclinic space group Cc, with a = 15.2974(5) Å, b = 11.7766(4) Å, c = 8.1059(3) Å, α = 90°, β = 106.31(3)°, γ = 90° and Z = 4 molecules per unit cell, where as compound **1c** crystallizes in orthorhombic space group Pbca, with a = 7.6307(6) Å, b = 11.3895(9) Å, c = 24.121(2) Å, α = β = γ = 90° and Z = 8 molecules per unit cell. All the compounds were tested for their inhibitory activities against four human pathogen bacteria and three fungal strains. The screening data revealed that five compounds showed moderate to good activity whereas one of the compound **1k** displayed excellent activity. In vitro hemolytic activity of the compounds has shown them to be nontoxic in nature.

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Introduction

There has always been a great interest in the synthesis of new substituted thiourea derivatives owing to their diverse applicability in the pharmaceutical industry, material science and analytical chemistry. The coordination chemistry of substituted thioureas is well documented in literature as they form complexes with many metal ions which facilitate them to be used in solvent extraction, pre-concentration, highly efficient chromatographic separations and fluorimetric determinations [1, 2]. The hydrogen-bonding ability of the thiourea moiety has extensively been used in construction of anion receptors [3–5] and in the organocatalysts [6]. In particular, thioureas have been successfully used in environmental control, as ionophores in ion selective electrodes [7–9]. Thiourea and its derivatives have also been used as effective corrosion inhibitors because sulphur atom is easily protonated in acidic solution [10–12]. Nowadays, these derivatives have been employed successfully as catalysts in the palladium-catalyzed Suzuki and Heck reactions because thiourea ligands are thermally stable and insensitive to air and moisture [13–15]. The pharmaceutical importance of substituted thioureas is proved by the wide variety of biological activities exhibited by them such as antibacterial, antifungal [16], anticancer [17, 18], anti-tubercular [19] and antiviral [20]. A number of *N*,*N*′-disubstituted carbonyl thioureas have been used extensively as commercial fungicides, herbicides [21] and insecticides [22]. The inhibitory activity of some pyridazine derivatives carrying thiourea moiety against

Staphylococcus aureus, *Escherichia coli*, and *Candida albicans* and *C. parapsilosis* have been reported [23]. The insecticidal properties of *N*-aryl-*N*′-2,6 dichloro benzoyl thioureas have been reported and it has also been reported that these compounds are well tolerated by plants and have low mammalian toxicity [24]. Measuring hemolytic activities of compounds is becoming a new area of research nowadays, as it is being used as an indicator of their cytotoxicities. In the light of the above facts we present here the synthesis, characterization and antimicrobial screening of a series of substituted thiourea compounds.

Experimental Details

Materials

Thiopphene-2-carbonyl chloride, furoyl chloride, benzoyl chloride, ammonium thiocynate, 2-amino thiazole, 2-amino pyrimidine, sulphanilamide, m-nitro aniline, 2-chloro-4-nitro aniline, 2-nitro-4-chloro aniline, 2-methoxy-4-nitro aniline and 2,4-diamino-6-phenyl-1,3,5-triazine were purchased from Sigma Aldrich chemical company (USA), S.D. Fine (Mumbai), Hi-media (Mumbai) and used without further purification. Solvents acetone, ethyl acetate, petroleum ether and ethanol were obtained from Merck and Ranchem.

Techniques

Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart-SMP10 melting point apparatus. IR absorption spectra were recorded on a Varian 3100 FT–IR Excalibur series spectrophotometer using KBr pellets in the range of 4,000–400 cm^{-1}, ^{1}H and ^{13}C NMR spectra were obtained on a JEOL FT-NMR AL 300 MHz spectrometer using tetramethylsilane as the internal standard. The ^{1}H NMR and ^{13}C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me4Si). The splitting patterns are designated as follows; s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses were performed on a CE-440 Exeter Analytical CHN analyzer. All the new compounds gave C, H and N analysis within 0.03 % of the theoretical values. Purity of the compounds was checked by thin layer chromatography (TLC), 5 × 20 cm plates coated with silica gel GF254 type 60 (25–250 mesh) using an ethyl acetate-petroleum ether mixture (1:2) as solvent.

X-ray Crystal Structure Analysis

Crystal data and details of data collection and refinement for **1a** and **1c** are summarized in Table 1. Diffraction data of both **1a** and **1d** at 123 K were measured on an Xcalibur, Ruby, Gemini X-ray diffractometer equipment (Cu $K\alpha$ radiation, $\lambda = 1.54184$ Å). The structure were solved by direct methods [25] and refined anisotropically by full-matrix least-squares using SHELXL-97 against F^2 using all data [25]. The non-hydrogen atoms are refined anisotropically. Hydrogen atoms are refined using the riding model. All calculations were performed by using the winGX system, ver. 1.0.05.

Experimental Procedure for Antimicrobial Activity

Disc Diffusion Method

Antimicrobial activities of newly synthesized compounds were evaluated on different gram-positive and gram-negative human pathogens viz. *E. coli* ATCC 25323, *Pseudomonas aeruginosa* ATCC 27893, *Enterococcus faecalis*, *S. aureus* ATCC 25323 and on different strains of *candida* viz. *C. albicans* ATCC 90028, *C. tropicalis* ATCC 750, *C. krusie* ATCC 6258 according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS) [26] using the agar disc diffusion method. Briefly, a 24/48 h-old culture of selected bacteria/fungi were mixed with sterile physiological saline (0.85 %) and the turbidity was adjusted to the standard inoculums of Mac-Farland scale 0.5 [$\sim 10^6$ colony forming units (CFU) per millilitre]. Petri plates containing 20 ml of Mueller–Hinton agar (MHA, Hi-Media) were used for all the bacteria tested. Fungi were cultured in Sabouraud's dextrose agar (SDA)/potato dextrose agar (PDA) (Hi-Media) and were purified by single spore isolation technique [27]. The inoculums was spread on the surface of the solidified media and Whatman no. 1 filter paper discs (6 mm in diameter) impregnated with the test compound (20 µl/disc) were placed on the plates. Ciprofloxacin (5 µg/disc, Hi-Media) was used as positive control for bacteria. Fluconazole (10 µg/disc, Hi-Media), was used as positive control for fungi. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24 h at 37 °C and the fungal culture was incubated for 72 h at 25 °C. The inhibition zone diameters were measured in millimeters. All the tests were performed in triplicate.

Determination of MIC

Minimum inhibitory concentration (MIC) of any compound is defined as the lowest concentration which completely inhibits visible growth (turbidity on liquid media). MIC values were determined by broth micro dilution method, according to NCCLS guidelines document M27-A [26]. Equal volume of tested compounds with different

Table 1 Summary of crystallographic data and structural parameters of the compound **1a** and **1c**

Compound	**1a**	**1c**
Empirical formula	$C_{12}H_{11}N_3O_3S_3$	$C_{12}H_9N_9O_3S_2$
Formula weight	341.42	253.31
Temperature	123(2) K	122.95(10) K
Wavelength	1.54184 Å	1.54184 Å
Crystal system	Monoclinic	Orthorhombic
Space group	C c	P b c a
Crystal size (mm^3)	0.50 × 0.14 × 0.06	0.5 × 0.16 × 0.03
Unit cell dimensions	a = 15.2974(5) Å, α = 90°	a = 7.6307(6) Å, α = 90°
	b = 11.7766(4) Å, β = 106.31(3)°	b = 11.3895(9) Å, β = 90°
	c = 8.1059(3) Å, γ = 90°	c = 24.121(2) Å, γ = 90°
Volume ($Å^3$)	1,401.50(8)	2,096.3(3)
Z	4	8
Density (mg/m^3)	1.618	1.605
Absorption coefficient (mm^{-3})	4.974	4.534
F(000)	704	1,040
Theta range for data collection	4.81–75.44°	3.66–72.55°
Index ranges	$-15 \leq h \leq 19$	$-9 \leq h \leq 5$
	$-14 \leq k \leq 13$	$-10 \leq k \leq 13$
	$-9 \leq l \leq 4$	$-27 \leq l \leq 29$
Reflections collected	2,523	4,707
Independent reflections	1535 [R(int) = 0.0260]	2018 [R(int) = 0.0,761]
Absorption correction	Analytical	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	1535/4/198	2018/0/154
Goodness-of-fit on F^2	1.024	1.069
Final R indices [I > 2 sigma(I)]	R1 = 0.0261, wR2 = 0.0690	R1 = 0.0768, wR2 = 0.1997
R indices (all data)	R1 = 0.0261, wR2 = 0.0692	R1 = 0.0935, wR2 = 0.2221
Largest diff. peak and hole	0.229 and −0.224 e $Å^{-3}$	0.662 and −0.516 e $Å^{-3}$

dilutions and nutrient broth were mixed in wells of micro titer plate, which were diluted two fold in serial to determine the MIC. Specifically 0.1 ml with approximately 5×10^5 CFU/ml of actively dividing bacterial/fungal cells was inoculated in each well. The culture plates were inoculated for 24 h at 37 °C for bacterial growth and 25 °C for fungal growth. The standard antibiotic, ciprofloxacin (5 μg/ml) for bacteria and fluconazole (10 μg/ml) for fungi were used as positive controls and 100 μl of DMSO used as a negative control. At the end of the incubation period, the MIC values were determined.

Determination of Hemolytic Activity of Compounds on Human Red Blood Cells (hRBC)

Hemolytic assay of Compounds **1a**–**1k** were carried out and tested for hemolytic activities on human hRBC at a fixed concentration of 100 μgm/ml. Freshly collected human red blood cells were taken and washed three times by sterile phosphate buffered saline (PBS) solution at pH 7.4. Each washing step was carried out by centrifuging the cells at 3,000 rpm, for ~7 min at room temperature, discarding the supernatant after each wash. The hRBC were resuspended to give a concentration of 5×10^8cells/ml PBS. Cell suspension was used throughout in the preparation of experimental and control tubes. The method reported by [28, 29] was followed. Compounds solution containing 100 μM test compounds were mixed with 200 μl of hRBC suspension and final volume was made up to 1 ml with buffer. The reaction mixture was then incubated at 37 °C for 1 h with continuous shaking. The controls were 1 % v/v DMSO in PBS and sterile water, after which the reaction mixture was, centrifuged (1,300 rpm, 5 min) and absorbance read at 540 nm. Turbidity was measured by reading absorbance at 540 nm using a multi-well plate Bio-Rad ELISA reader and observing a decrease in optical density associated with cRBCs lyses.

General Procedure for Synthesis

A solution of thiophenoyl chloride (1.065 ml, 0.01 mol (1.465 g) or furoyl chloride (0.984 ml, 0.01 mol (1.305 g) in acetone (50 ml) was added drop-wise to a suspension of ammonium thiocyanate (0.76 g, 0.01 mol) in acetone (30 ml) and the reaction mixture was refluxed for 45 min. After cooling to room temperature, a solution of corresponding amine (0.01 mol) in acetone (25 ml) was added and the resulting mixture refluxed for 1.5 h. The reaction mixture was poured into five time its volume of cold water. The desired thioureas precipitated as solid were washed with water and recrystallized in ethanol. The light yellow color crystals of two compounds (**1a**, **1c**) suitable for single crystal study were obtained by slow evaporation of ethanolic solution of compounds at ~30 °C.

*N-[4-Sulfonamide phenyl]-N′-[2-thiophenoyl] thiourea (**1a**)*

Yield: 81 % (2.765 g), m.p.; 210–211 °C. Elemental analyses: calculated for $C_{12}H_{11}N_3O_3S_3$ (%), C, 32.78; H, 2.56; N, 9.56; Found, C, 32.72; H, 2.50; N, 9.54; IR (KBr, cm^{-1}): 3,337, 3,295, 3,255 ν_s (N–H), 3,093, 3,014 (aromatic C–H), 1,654 (amide-I, C=O), 1,590 (Ph), 1,535 (thioamide-I), 1,342 (thioamide-II), 1,158 (thioamide-III), 728 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 13.13 (s, 1H, hydrogen bonded-N*H*), 9.21 (s, 1H, –N*H*), 8.79 (d, J(H, H) = 8.1, 1H, thiophene-H), 8.35 (d, J(H, H) = 7.2 Hz, 1H, thiophene-H), 7.72 (t, J(H,H) = 7.6 Hz, 1H, thiophene-H), 7.49 (d, J(H, H) = 6.9 Hz, 2H, Ar–H), 6.91(s, 2H, $-SO_2NH_2$), 6.65 (d, J(H, H) = 6.9 Hz, 2H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d6, 25 °C): δ_c 179.30(C=S), 170.12(C=O), 151.86, 145.13, 132.23, 131.68, 131.28, 128.34, 128.95, 127.86.

*N-[Pyrimidine-2-yl]-N′-[2-thiophenoyl] thiourea (**1b**)*

Yield: 76 % (2.006), m.p.; 182–183 °C. Elemental analyses: calculated for $C_{10}H_8N_4OS_2$ (MW = 264.32) in wt%, C, 45.43; H, 3.05; N, 21.19; Found C, 45.49; H, 3.11; N, 21.29; IR (KBr, cm^{-1}): 3,373, 3,097 ν_s (N–H), 3,020 (Ar–H), 1,658 (amide-I, C=O), 1,600, 1,564 (Ph), 1,504 (thioamide-I), 1,321 (thioamide-II), 1,155 (thioamide-III), 745 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, DMSO-d6, 25 °C): δ 13.58 (s, 1H, hydrogen bonded-N*H*), 12.10 (s, 1H, free-N*H*), 8.79 (d, J(H,H) = 7.5 Hz, 2H, pyrimidine-H), 8.05 (d, J(H, H) = 7.2 Hz, 1H thiophene-H), 7.47 (d, J(H, H) = 7.4, 1H, thiophene-H), 7.28 (t, J_1(H, H) = 7.8 Hz, J_2(H,H) = 7.6 Hz, 1H, thiophene-H), 7.10 (t, J_1(H, H) = 7.2 Hz, J_2(H, H) = 7.4 Hz, 1H, PYrimidine-H); ^{13}C NMR (75 MHz, DMSO-d6, 25 °C): δ_c 176.45(C=S), 158.24(C=O), 156.16, 154.23, 146.57, 143.55, 116.87, 115.42, 112.02.

*N-[3-(Thiazole-2-yl)]-N′-[2-furoyl] thiourea (**1c**)*

Yield: 82 %(2.07 g), m.p.; 172–173 °C. Elemental analyses: calculated for $C_9H_7N_3O_2S_2$ (%), C, 42.67; H, 2.78; N, 16.58; Found, C, 42.61; H, 2.78; N, 15.53. IR (KBr, cm^{-1}): 3,244, 3,109 ν_s (N–H), 3,013 (aromatic C–H), 1,656 (amide-I, C=O), 1,582, (Ph, C=C), 1,521 (thioamide-I), 1,312 (thioamide-II), 1,189 (thioamide-III), 753, 718 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, DMSO-d6, 25 °C): δ 13.15 (s, 1H, hydrogen bonded-N*H*), 9.01 (s, 1H, free-N*H*), 7.56 (d, J(H, H) = 8.1, 2H, furan-H), 7.41 (t, J_1(H, H) = 7.2 Hz, J_2(H, H) = 7.6 Hz, 1H, furan-H), 7.11 (d, J(H, H) = 9.3, 1H, thiazole-H), 6.68 (d, J(H, H) = 9.3 Hz, 1H, thiazole-H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ_c 175.46(C=S), 161.65(C=O), 158.36, 138.42, 134.88, 133.54, 131.26, 130.84, 127.44.

*N-[3-(Thiazole-2-yl)]-N′-[2-thiophenoyl] thiourea (**1d**)*

Yield: 83 %(2.23 g), m.p.; 143–144 °C. Elemental analyses: calculated for $C_9H_7N_3OS_3$ (%), C, 40.12; H, 2.61; N, 15.51; Found, C, 40.15; H, 2.66; N, 15.54; IR (KBr, cm^{-1}): 3,219, 3,088 ν_s (N–H), 3,008 (aromatic C–H), 1,654 (amide-I, C=O), 1,588, (Ph, C=C), 1,542 (thioamide-I), 1,308 (thioamide-II), 1,197 (thioamide-III), 736 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 13.60 (s, 1H, hydrogen bonded-N*H*), 9.26 (s, 1H, free-N*H*), 7.62 (d, J = 8.3, 2H, thiophene-H), 7.44 (t, J_1(H, H) = 7.2 Hz, J_2(H, H) = 7.8 Hz, 1H, thiophene-H), 7.03 (d, J(H, H) = 9.3, 1H, thiazole-H), 6.66 (d, J(H,H) = 9.0 Hz, 1H, thiazole-H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ_c 174.52(C = S), 160.81(C=O), 159.46, 138.50, 134.78, 132.54, 131.21, 130.04, 128.27.

*N-[m-Nitro phenyl]-N′-[2-thiophenoyl] thiourea (**1e**)*

Yield: 81 %(2.37 g), m.p.; 204–205 °C. Elemental analyses: calculated for $C_{12}H_9N_2O_3S_2$ (%), C, 46.85; H, 2.92; N, 13.66; Found, C, 46.79; H, 2.96; N, 13.69; IR (KBr, cm^{-1}): 3,373, 3,097 ν_s (N–H), 3,012 (aromatic C–H), 1,658 (amide-I, C=O), 1,600, 1,562 (Ph, C=C), 1,519 (thioamide-I), 1,345 (thioamide-II), 1,145 (thioamide-III), 716 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, DMSO-d6, 25 °C): δ 12.55 (s, 1H, hydrogen bonded-N*H*), 11.78 (s, 1H, free-N*H*), 8.75 (d, J(H, H) = 7.6, 1H, thiophene-H), 8.38 (d, J(H, H) = 7.8 Hz, 1H, thiophene-H), 8.05 (t, J(H, H) = 6.8 Hz, 1H, thiophene-H), 7.69 (s, J(H, H) = 7.2 Hz, 1H, Ar–H), 7.26 (m, 3H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ_c 179.45 (C=S), 161.84 (C=O), 147.50, 139.23, 136.57, 135.55, 132.87, 131.10, 129.97, 128.80, 120.86, 119.02.

*N-[2-cl-4-Nitro phenyl]-N′-[2-furoyl] thiourea (**1f**)*

Yield: 81 %(2.63 g), m.p.; 173–174 °C. 42 Elemental analyses: calculated for $C_{12}H_8N_3O_4S_1Cl$ (%), C, 44.24; H, 2.48; N, 12.91; Found, C, 44.18; H, 2.42; N, 12.87; IR (KBr, cm^{-1}): 3,373, 3,097 ν_s (N–H), 3,020 (aromatic C–H), 1,658 (amide-I, C=O), 1,600, 1,564 (Ph), 1,504 (thioamide-I), 1,321 (thioamide-II), 1,155 (thioamide-III), 745 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 13.10 (s, 1H, hydrogen bonded-N*H*), 9.11 (s, 1H, free-N*H*), 8.72 (d, J = 9.1 Hz, 1H, furon-H), 8.36 (s, 1H, Ar CH), 8.12 (d, J(H, H) = 8.2 Hz, 1H, thiophene-H), 7.67 (t, J_1(H, H) = 7.1 Hz, J_2(H, H) = 7.8 Hz, 1H, thiophene-H), 7.74 (d, J(H,H) = 8.2 Hz, 1H, Ar–H), 6.70 (d, J(H, H) = 7.6 Hz, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ_c 179.67 (C=S), 170.04 (C=O), 146.45, 139.24, 137.53, 134.64, 131.87, 130.69, 129.46, 128.80, 124.25, 122.42.

*N-[Pyridine-2-yl]-N′-[2-thiophenoyl] thiourea (**1g**)*

Yield: 78 %(2.05 g), m.p.; 242–243 °C. Elemental analyses: calculated for $C_{11}H_9N_3O_1S_2$ (%), C, 50.17; H, 3.44; N, 15.95; Found, C, 50.24; H, 3.48; N, 15.98; IR (KBr, cm^{-1}): 3,373, 3,097 ν_s (N–H), 3,020 (aromatic-H), 1,658 (amide-I, C=O), 1,600, 1,564 (Ph), 1,504 (thioamide-I), 1,321 (thioamide-II), 1,155 (thioamide-III), 745 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 12.90 (s, 1H, hydrogen bonded-N*H*), 8.88 (s, 1H, free-N*H*), 8.78 (d, J(H, H) = 8.1 Hz, 1H, thiophene-H), 8.44 (t, J_1(H, H) = 7.7 Hz, J_2(H, H) = 7.2 Hz, 1H thiophene-H), 7.77 (d, J(H, H) = 7.8 Hz, 1H thiophene-H), 7.23 (m, 4H, Py-H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ_c 177.65 (C=S), 169.12 (C=O), 154.04, 148.52, 138.23, 137.87, 132.58, 131.27, 129.14, 118.07, 113.68.

*N-[2-Methoxy-4-nitro phenyl]-N′-[2-furoyl] thiourea (**1h**)*

Yield: 78 %(2.63 g), m.p.; 183–184 °C. Elemental analyses: calculated for $C_{13}H_{11}N_3O_4S_2$ (%), C, 46.28; H, 3.28; N, 12.45; Found, C, 46.36; H, 3.32; N, 12.49; IR (KBr, cm^{-1}): 3,373, 3,097 ν_s (N–H), 3,020 (aromatic C–H), 1,658 (amide-I, C=O), 1,600, 1,564 (Ph), 1,504 (thioureido-I), 1,321 (thioureido-II), 1,155 (thioureido-III), 745 (thioureido-IV); The main signals in ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 13.01 (s, 1H, hydrogen bonded-N*H*), 9.26 (s, 1H, free-N*H*), 9.21 (d, J(H, H) = 5.7, 1H, thiophene-H), 7.92 (d, J(H, H) = 9.0 Hz, 1H, thiophene-H), 7.79 (s, 1H, Ar–H), 7.63 (d, J(H, H) = 7.2 Hz, 1H, Ar–H), 7.35(t, J_1(H, H) = 7.6 Hz, J_2(H, H) = 7.2 Hz, 1H thiophene-H) 6.86 (d, J(H, H) = 9.0, 1H, Ar–H) 3.98(s, 3H, O*C*H_3); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ_c 179.48 (C=S), 169.64 (C=O), 159.88, 145.50, 138.23, 132.57, 131.55, 130.87, 129.10, 120.37, 116.80, 105.86, 55.98.

*N-[2-cl-4-Nitro phenyl]-N′-[2-thiophenoyl] thiourea (**1i**)*

Yield: 76 %(2.59 g), m.p.; 173–174 °C. Elemental analyses: calculated for $C_{12}H_8N_3O_3S_2Cl$ (%), C, 42.16; H, 2.36; N, 12.29; Found, C, 42.24; H, 2.38; N, 12.35; IR (KBr, cm^{-1}): 3,373, 3,097 ν_s (N–H), 3,020 (aromatic C–H), 1,658 (amide-I, C=O), 1,600, 1,564 (Ph), 1,504 (thioureido-I), 1,321 (thioureido-II), 1,155 (thioureido-III), 745 (thioureido-IV); The main signals in ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 13.07 (s, 1H, hydrogen bonded-N*H*), 8.98 (s, 1H, free-N*H*), 9.05 (d, J(H, H) = 9.0 Hz, 1H, thiophene-H), 8.35 (s, 1H, Ar CH), 8.18 (d, J(H, H) = 8.4 Hz, 1H, thiophene-H), 7.97 (t, J_1(H, H) = 7.2 Hz, J_2(H, H) = 7.8 Hz, 1H, thiophene-H), 7.76 (d, J(H, H) = 8.1, 1H, Ar–H), 6.72 (d, J(H, H) = 7.2, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ_c 179.45(C=S), 169.74(C=O), 146.50, 142.23, 137.57, 136.54, 131.87, 130.39, 129.67, 128.80, 124.50, 122.32.

*N-[4-Chloro-2-nitro phenyl]-N′-[2-thiophenoyl] thiourea (**1j**)*

Yield: 75 %(2.56 g), m.p.; 145–147 °C. Elemental analyses: calculated for $C_{12}H_8N_3O_3S_2Cl$ (%),C, 42.16; H, 2.36; N, 12.29; Found, C, 42.22; H, 2.39; N, 12.34; IR (KBr, cm^{-1}): 3,373, 3,097 ν_s (N–H), 3,012 (aromatic C–H), 1,658 (amide-I, C=O), 1,600, 1,562 (Ph, C=C), 1,519 (thioamide-I), 1,345 (thioamide-II), 1,145 (thioamide-III), 716 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, DMSO-d6, 25 °C): δ 12.39 (s, 1H, hydrogen bonded-N*H*), 11.77 (s, 1H, free-N*H*), 8.37 (d, J = 7.8 Hz, 1H, thiophene-H), 8.15 (d, J(H, H) = 7.8 Hz, 1H thiophene-H), 8.09 (s, 1H, Ar–H), 7.65 (d, J(H, H) = 7.2, 1H, Ar–H), 7.28 (d, J(H, H) = 7.8, 1H, thiophene-H), 6.85 (d, J_1(H, H) = 7.2, Hz, J_2(H, H) = 6.9 Hz, 1H Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ_c 179.64 (C=S), 169.80 (C=O), 149.56, 137.62, 135.26, 134.64, 132.55, 130.81, 129.6, 128.94, 128.80, 126.86.

*N-[6-Phenyl-1,3,5-triazinyl]-N′-benzoyl thiourea (**1k**)*

Yield: 70 %(3.59 g), m.p.; 162–164 °C. Elemental analyses: calculated for $C_{25}H_{19}N_7O_2S_2$ (%), C, 58.46; H, 3.72; N, 19.09; Found, C, 58.58; H, 3.76; N, 19.18; IR (KBr, cm^{-1}): 3,325, 3,135 ν_s (N–H), 3,052 (aromatic C–H), 1,656 (amide-I, C=O), 1,610, 1,568 (Ph, C=C), 1,526 (thioamide-I), 1,348 (thioamide-II), 1,141 (thioamide-III), 726 (thioamide-IV); The main signals in ^{1}H NMR (300 MHz, DMSO-d6, 25 °C): δ 13.10 (s, 2H, hydrogen bonded-N*H*), 11.66 (s, 2H, free-N*H*), 8.24 (d, J(H, H) = 7.2 Hz, 2H, aromatic-H), 8.09(d, J(H, H) = 7.6 Hz, 4H aromatic-H), 7.98–6.96 (m, 9H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d6, 25 °C): δ_c 178.14 (C=S), 167.52

(C=O), 152.86, 144.72, 135.54, 133.89, 132.45, 131.60, 130.65, 129.46, 128.64, 127.24, 121.23.

Note: [thioamide-I; δ (N_2–H), thioamide-II; ν_{as} (N–C–N), thioamide-III; ν_s (N–C–N), thioamide-IV; ν (C=S).

Result and Discussion

Synthesis and Characterization

The general synthetic route for the target compounds is outlined in scheme 1. The target compounds were synthesized by slight modification of the reported literature procedure [30]. On the basis of elemental analysis and spectral studies the structures of the compounds have been proposed. In the ^{1}H NMR spectra of all the compounds in $CDCl_3$ the signal appearing at ~9.00 ppm is due to O=C–N_1H proton. These peaks suffer a downfield shift of ~3 ppm when the spectra are recorded in DMSO-d6. This is due to polarity effect of solvent. Since DMSO is highly polar solvent hence upon addition of DMSO-d6, a dipole–dipole interaction occurs between the oxygen atom of DMSO-d6 and H-atom of O=C–N_1H group. The S=C–N_2H peak in all the above compounds appeared in range 12.53–14.09 ppm. The high δ value of this peak in comparison to N_1H proton is due to strong intramolecular (C=O···N_2H) hydrogen bonding resulting in formation of pseudo six-member ring as shown in Fig. 1. Since intramolecular hydrogen bonding is unaffected by polarity of solvent, so this peak appears nearly in same region in DMSO. In the ^{13}C NMR spectra of the compounds the peaks at ~179 and ~165 ppm may be attributed to C=S and C=O group carbons respectively. The rest of the peaks are due to ring carbons. Absorption bands appearing at ~3,300 and ~3,100 cm^{-1} in the IR spectra of compounds are due to free –NH and associated –NH group respectively. The band appearing at ~1,655 cm^{-1} due to amide group (C=O–NH), is lower than normal amide group absorption (1,680 cm^{-1}) which may be attributed to the formation of hydrogen bonding explained above.

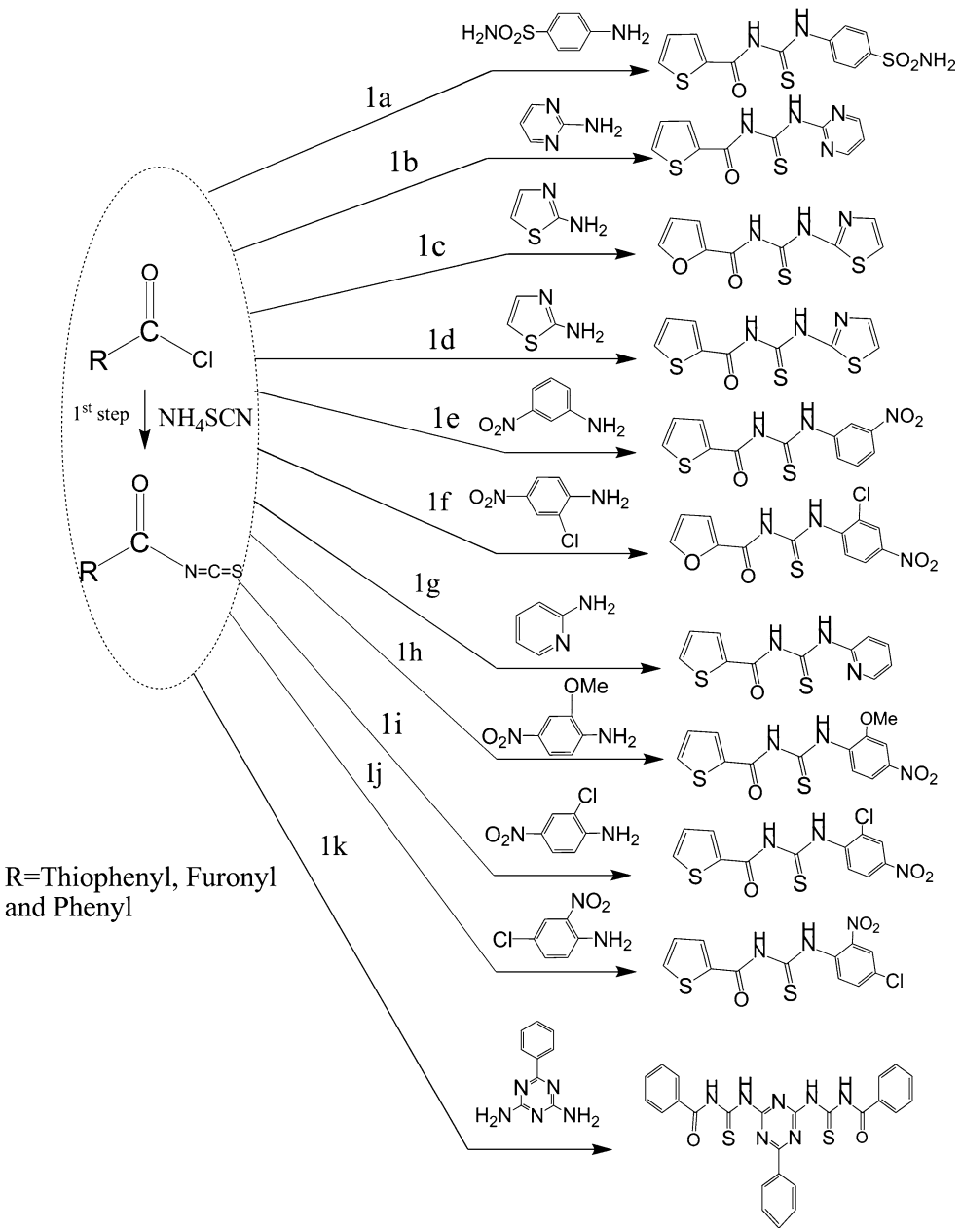
The Crystal Structure

The molecular structure of compounds **1a** and **1c** have been depicted in Figs. 1 and 2 respectively. Selected bond lengths and bond angles are listed in Table 2. The main bond lengths are within the range obtained for similar compounds [31, 32]. The bond lengths S2–C6, 1.656 Å; S1–C6, 1.648 Å; O1–C5, 1.234 Å and O2–C5, 1.231 Å show usual double bond character. However, for the C–N bonds, bond lengths N1–C5, N1–C6 and N2–C6 in both compound **1a** and **1c** are shorter than the normal C–N single-bond length of about 1.48 Å. The above can be explained on the basis of existence of resonance in this part of the molecule. In **1a** the thiophene ring (C4/C3/C2/C1/S1) is inclined 6.91(0.16)° with respect to the plane formed by the thiourea moiety (N1–C6–S2–N2), while the phenyl ring (C7/C8/C9/C10/C11/C12) is inclined 13.58(0.14)° with thiourea moiety. The thiophene ring (C4/C3/C2/C1/S1) is inclined 10.53(0.14)° with respect to the plane formed by the phenyl ring (C7/C8/C9/C10/C11/C12). In **1c** the furan ring (O1/C1/C2/C3/C4/C5) are inclined 4.37(0.28)° with respect to the plane formed by the thiourea moiety (N1–C6–S1–N2), while the thiazole ring (S2/C7/N3/C8/C9) is inclined 3.69(0.25)° with respect to the plane formed by thiourea moiety. The angle between plane formed by furan ring (O1/C1/C2/C3/C4/C5) and thiazole ring (S2/C7/N3/C8/C9) is 1.20(0.29). The *trans–cis* geometry in the thiourea moiety is stabilized by the N2–H2···O1(O2) intramolecular hydrogen bonding which also locks the –CONHCSNHR– unit into a stable planar six-membered ring structure (Figs. 1, 2; Tables 4, 5). It has been reported that such strong interactions are maintained in solutions also [33]. In S-shaped conformation between the C=O and C=S groups (two donors sites rich in electron density), the O–S distance is maximum, contributing to a minimum conformational energy of the molecule as a whole [34]. In the crystal structure of **1a,** symmetry related molecules are linked by three different N–H···O interactions and one C–H···O interaction to form one dimensional supramolecular chain along b-axis (Fig. 3a, b), while in **1c** symmetry related molecules are linked by N1–H1···N3 interaction to form one dimensional supramolecular chain along a-axis (Fig. 4a, b; Tables 3, 4, 5).

Antimicrobial Activity

All the synthesized thiourea derivatives (**1a**–**1k**) were checked for their antibacterial and antifungal activities, quantitatively and qualitatively. The inhibitory activity was quantified by the occurrence of a growth inhibition zone and MIC values (Tables 6, 7, 8, 9). The results of the antibacterial screening revealed that six of the tested compounds inhibited the growth of bacterial colonies. Compound **1b** inhibited the growth of *S. aureus* (MIC ~300 μg/ml) whereas compound **1d** inhibited the growth of *E. coli* only with MIC values 300 and 150 μg/ml respectively. Compounds **1d** and **1e** inhibited the growth of both *S. aureus* and *E. coli* with MIC values 75 and 300 μg/ml respectively. Compound **1k** exhibited excellent activity against all the bacterial strains used, with the MIC values even lower than the control drug except for *P. aeruginosa* (Table 7). The results of the antifungal screening showed

Scheme 1 Synthetic rout of compounds from **1a** to **1k**



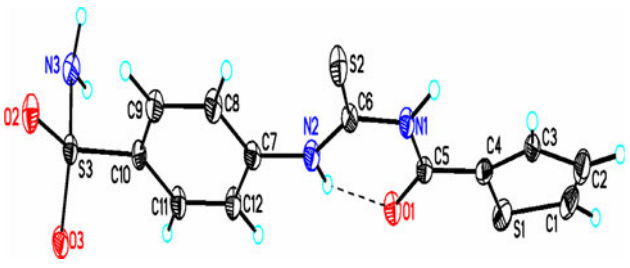


Fig. 1 Single-crystal X-ray structure of *N*-[4-sulphonamide phenyl]-*N*′-[2-thiophenoyl] thiourea (**1a**) with the atom labeling scheme and 50 % displacement ellipsoids. The *dashed line* shows intermolecular and intramolecular hydrogen bonds

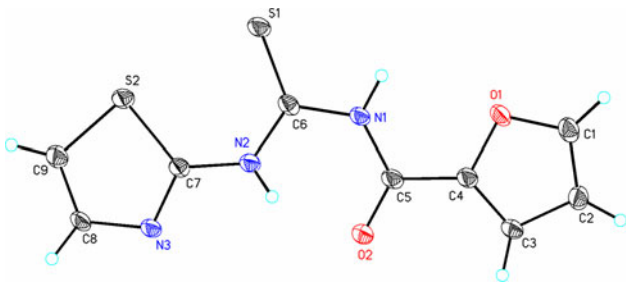


Fig. 2 Single-crystal X-ray structure of *N*-[3-(thiazole-2-yl]-*N*′-[2-furoyl] thiourea (**1c**), with the atom labeling scheme and 50 % displacement ellipsoids

Table 2 Bond length and bond angles of **1a** and **1c**

1a		**1c**	
Bond length (Å)			
S(2)–C(6)	1.656(2)	S(1)–C(6)	1.648(4)
O(1)–C(5)	1.234(3)	O(2)–C(5)	1.231(5)
N(1)–C(5)	1.372(3)	N(1)–C(5)	1.374(5)
N(1)–C(6)	1.410(3)	N(1)–C(6)	1.410(5)
N(2)–C(6)	1.344(3)	N(2)–C(6)	1.357(5)
N(2)–C(7)	1.404(3)	N(2)–C(7)	1.384(5)
C(4)–C(5)	1.466(3)	C(4)–C(5)	1.463(6)
Bond angles (°)			
C(5)–N(1)–C(6)	128.0(2)	C(5)–N(1)–C(6)	126.8(4)
C(6)–N(2)–C(7)	132.2(2)	C(6)–N(2)–C(7)	127.4(4)
C(5)–C(4)–S(1)	117.32(19)	C(3)–C(4)–C(5)	130.2(4)
O(1)–C(5)–N(1)	122.9(2)	O(2)–C(5)–N(1)	124.0(4)
O(1)–C(5)–C(4)	120.5(2)	O(2)–C(5)–C(4)	119.3(4)
N(1)–C(5)–C(4)	116.6(2)	N(1)–C(5)–C(4)	116.8(4)
N(2)–C(6)–N(1)	113.3(2)	N(2)–C(6)–N(1)	114.8(4)
N(2)–C(6)–S(2)	129.00(19)	N(2)–C(6)–S(1)	125.4(3)
N(1)–C(6)–S(2)	117.66(18)	N(1)–C(6)–S(1)	119.8(3)
C(8)–C(7)–N(2)	126.6(2)	N(2)–C(7)–S(2)	125.6(3)
C(12)–C(7)–N(2)	113.5(2)	N(3)–C(7)–N(2)	118.6(4)

Table 3 Torsion angles [°] of **1a** and **1c**

Torsion angles (°)	**1a**	Torsion angles (°)	**1c**
C(6)–N(1)–C(5)–O(1)	5.4(4)	C(6)–N(1)–C(5)–O(2)	0.5(7)
C(6)–N(1)–C(5)–C(4)	−173.9(2)	C(6)–N(1)–C(5)–C(4)	−178.7(4)
C(7)–N(2)–C(6)–N(1)	−171.9(2)	C(7)–N(2)–C(6)–N(1)	−176.5(4)
C(7)–N(2)–C(6)–S(2)	8.9(4)	C(7)–N(2)–C(6)–S(1)	4.9(7)
C(5)–N(1)–C(6)–N(2)	−8.8(3)	O(1)–C(4)–C(5)–O(2)	−179.9(4)
C(5)–N(1)–C(6)–S(2)	170.5(2)	C(3)–C(4)–C(5)–N(1)	−179.2(4)
C(6)–N(2)–C(7)–C(8)	5.4(5)	C(6)–N(2)–C(7)–N(3)	175.1(4)
C(6)–N(2)–C(7)–C(12)	−178.4(3)	C(6)–N(2)–C(7)–S(2)	−6.6(7)

Table 4 Hydrogen bonds for 1a [Å and °]

D–H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
N(1)–H(1B)···O(3)#1	0.78(4)	2.14(4)	2.883(3)	160(4)
N(2)–H(2B)···O(1)	0.82(4)	1.87(4)	2.602(3)	147(4)
N(3)–H(3N1)···O(1)#2	0.859(19)	2.12(2)	2.958(3)	165(4)
N(3)–H(3N2)···O(2)#3	0.84(2)	2.14(2)	2.962(3)	166(5)

Symmetry transformations used to generate equivalent atoms: #1 x − 1/2, y − 1/2, z − 1 #2 x + 1/2, −y + 3/2, z + 1/2 #3 x, −y + 1, z − 1/2

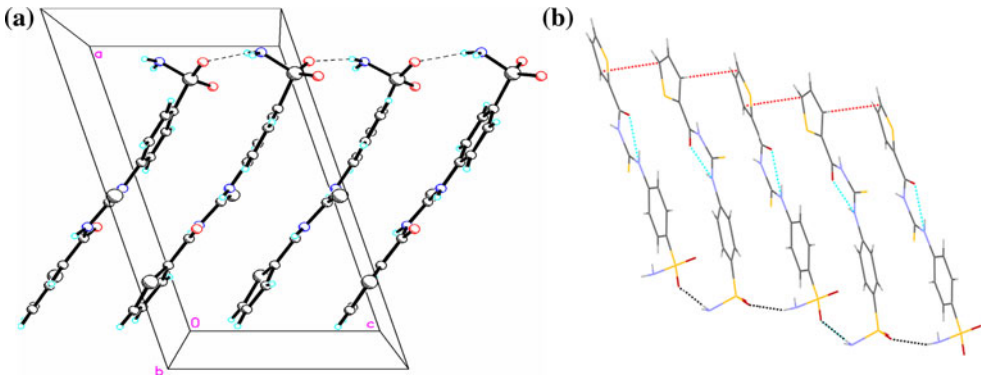


Fig. 3 **a**, **b** Illustrates the packing pattern of *N*-[4-sulphonamide phenyl]-*N*′-[2-thiophenoyl] thiourea (**1a**) along *b* axis, the *dashed lines* represent the intermolecular and intramolecular hydrogen bonds

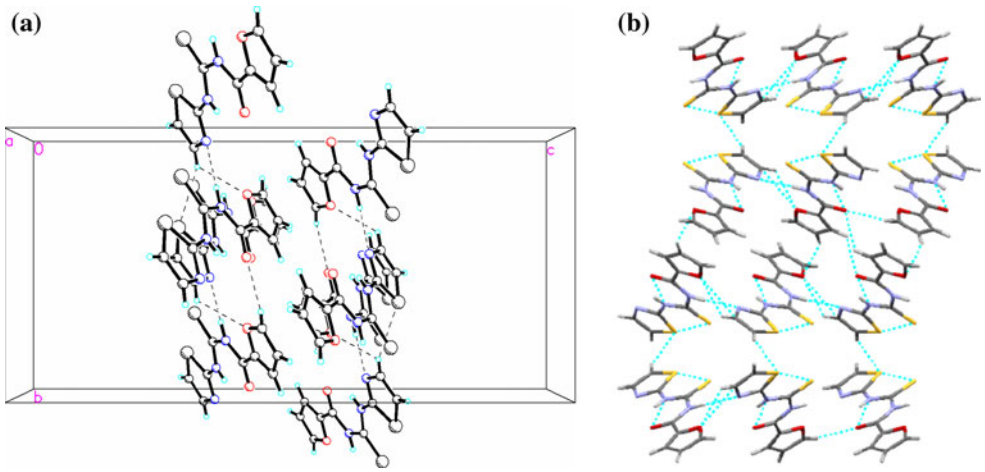


Fig. 4 **a**, **b** Illustrates the packing pattern of *N*-[3-(thiazole-2-yl]-*N*′-[2-furoyl] thiourea (**1c**) along *a* axis, the *dashed lines* represent the intermolecular and intramolecular hydrogen bonds

that the compounds **1c** and **1g** displayed activity against *C. allicans* and *C. tropicalis* only whereas compounds **1d** and **1k** inhibited the growth of all the tested fungal strains.

Table 5 Hydrogen bonds for **1c** [Å and °]

D–H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
N(1)–H(1N1)···N(3)#1	0.91(5)	2.18(6)	3.092(5)	178(6)
N(2)–H(2N2)···O(2)	0.79(5)	1.93(5)	2.612(5)	144(5)

Symmetry transformations used to generate equivalent atoms: #1 −x + 3/2, y − 1/2, z

Compound **1j** inhibited the growth of *C. albicans* and **1e** of *C. krusie* only with MIC value 150 μg/ml (Tables 8, 9). These results are depicted well for both bacteria and fungus in Figs. 5, 6, 7, 8, 9. The difference in the bioactivity of the compounds can be correlated to the lipophilic behavior of the compounds. It has been observed that the compounds having high lipophilic tendency show good inhibitory activity [35, 36]. The presence of a heterocyclic ring together with the thioamide group in a compound probably enhances its lipophillic nature, which results in a higher capability to penetrate the micro-organisms through the lipid layer of the cell membrane [35, 36].

Table 6 Antibacterial activity of compounds against the various human pathogens

Zone of inhibition (in mm)				
Compounds	Gram (+ve) bacteria		Gram (−ve) bacteria	
	S. aureus (ATCC 25323)	*P. aeruginosa* (ATCC 27853)	*E. faecalis* (Clinical isolate)	*E. coli* (ATCC 35218)
1a	–	–	–	–
1b	9.70 ± 0.40	–	–	–
1c	–	–	–	10.60 ± 0.55
1d	9.76 ± 0.66	–	–	12.80 ± 0.36
1e	10.86 ± 0.35	–	–	12.60 ± 0.55
1f	–	–	–	–
1g	–	–	–	–
1h	–	–	–	–
1i	–	–	–	–
1j	–	–	–	–
1k	22.36 ± 0.30	16.50 ± 0.40	15.80 ± 0.36	11.46 ± 0.28
Ciprofloxacin	29.87 ± 0.49	23.34 ± 0.80	28.88 ± 0.46	33.45 ± 0.73
DMSO	–	–	–	–

Table 7 Antibacterial activity of compounds against the various human pathogens

MIC (μg/ml)				
Compounds	Gram (+ve) bacteria		Gram (−ve) bacteria	
	S. aureus (ATCC 25323)	*P. aeruginosa* (ATCC 27853)	*E. faecalis* (Clinical isolate)	*E. coli* (ATCC 35218)
1a	–	–	–	–
1b	300	–	–	–
1c	–	–	–	150
1d	300	–	–	75
1e	300	–	–	75
1f	–	–	–	–
1g	–	–	–	–
1h	–	–	–	–
1i	–	–	–	–
1j	–	–	–	–
1k	1.20	9.7	2.4	4.8
Ciprofloxacin	6.25	3.12	3.2	6.25
DMSO	–	–	–	–

Table 8 Antifungal activity of compounds against the various human pathogens

Zone of inhibition (in mm)			
Compounds	*Candida albicans* (ATCC 90028)	*Candida tropicalis* (ATCC 750)	*Candida krusie* (ATCC 6258)
1a	–	–	–
1b	–	–	–
1c	9.60 ± 0.50	10.36 ± 0.25	–
1d	11.60 ± 0.26	10.53 ± 0.51	13.7 ± 0.45
1e	–	–	11.60 ± 0.26
1f	–	–	–
1g	10.46 ± 0.40	9.85 ± 0.35	–
1h	–	–	–
1i	–	–	–
1j	10.63 ± 0.25	–	–
1k	14.50 ± 0.51	12.70 ± 0.43	15.60 ± 0.43
Fluconazole	21.80 ± 0.30	16.66 ± 0.65	20.70 ± 0.43
DMSO	–	–	–

Table 9 Antifungal activity of compounds against the various human pathogens

MIC (μg/ml)			
Compounds	*Candida albicans* (ATCC 90028)	*Candida tropicalis* (ATCC 750)	*Candida krusie* (ATCC 6258)
1a	–	–	–
1b	–	–	–
1c	300	300	–
1d	150	300	150
1e	–	–	300
1f	–	–	–
1g	150	150	–
1h	–	–	–
1i	–	–	–
1j	150	–	–
1k	75	75	37.5
Fluconazole	6.25	3.12	25
DMSO	–	–	–

Hemolytic Activity

The results of the in vitro hemolytic study of the compounds have been shown in Fig. 10 and represent as bar graph in Table 10. Almost all the tested compounds exhibited moderate to low hemolysis, which indicated their low toxicity. Especially the compounds with very good antimicrobial activity showed negligible level of toxicity.

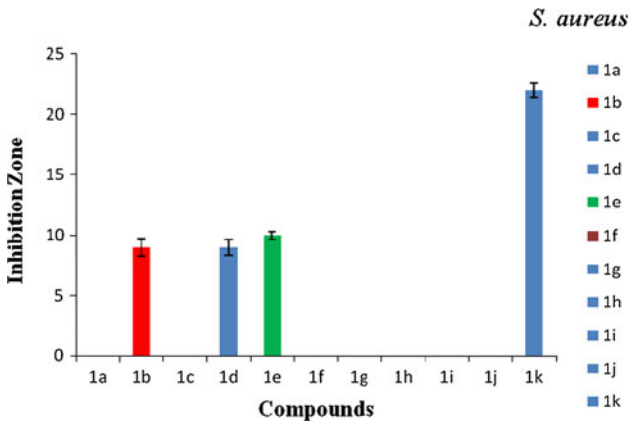


Fig. 5 Zone of inhibition of compounds against *S. aureus*

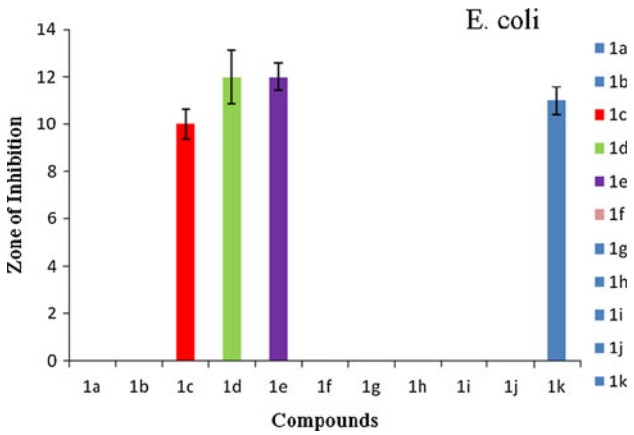


Fig. 6 Zone of inhibition of compounds against *E. coli*

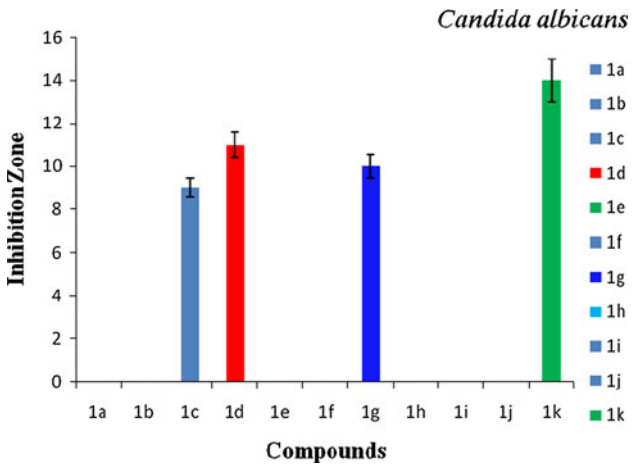


Fig. 7 Zone of inhibition of compounds against *Candida albicans*

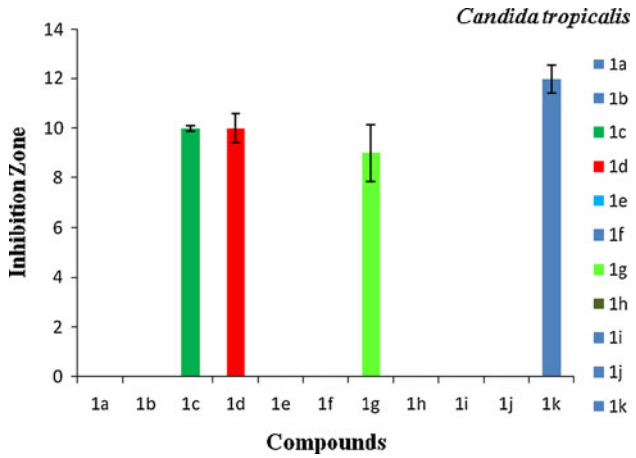


Fig. 8 Zone of inhibition of compounds against *Candida tropicalis*

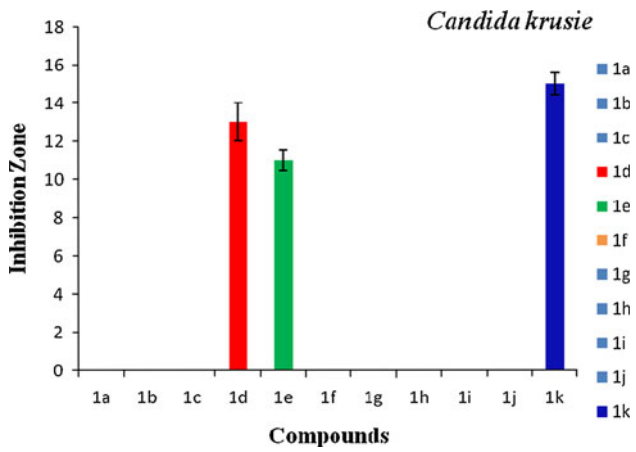


Fig. 9 Zone of inhibition of compounds against *Candida krusie*

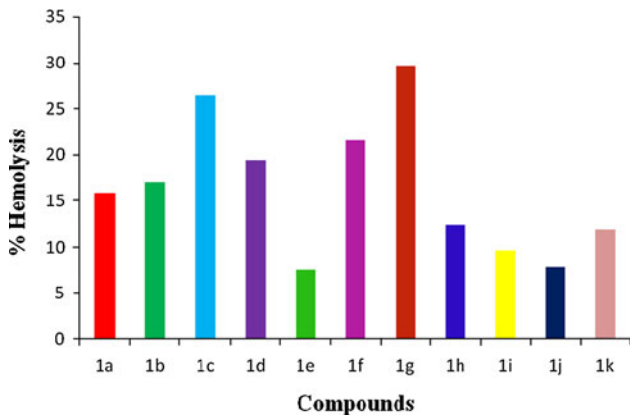


Fig. 10 Hemolysis of compounds (**1a**–**1k**)

Table 10 Hemolytic activity of compounds (**1a**–**1k**)

Compounds	% Hemolysis
1a	15.85
1b	17.04
1c	26.52
1d	19.48
1e	7.54
1f	21.56
1g	29.67
1h	12.45
1i	9.67
1j	7.83
1k	11.87

Conclusion

Twelve novel *N*,*N*′-disubstituted thiourea compounds have been synthesized by the reaction of aroyl chloride with substituted aromatic and hetrocyclic amines. These compounds have been characterized by elemental analysis, FT–IR, ^{1}H and ^{13}C NMR spectroscopy and single crystal study for **1a** and **1c**. The single crystal structures of the compounds revealed S-shaped conformation between C=O and C=S groups and presence of intramolecular hydrogen bonding between N_2H proton and oxygen of C=O group.

The results of antibacterial screening revealed that among all the compounds screened, compound **1b**–**1e** showed moderate to good anti-bacterial activity when compared with ciprofloxacin used as standard while the compound **1k** exhibited the highest activity against all the bacterial strains tested (zone of inhibition up to 11–22 mm at concentrations of 4.8–1.20 μg/ml). Other compounds having electron withdrawing group on phenyl ring did not show any inhibition activity. The results of antifungal screening showed that compound **1c**–**1g** have moderate to good activity while compound **1k** exhibited very promising activity especially against *C. krusie* with MIC value 37.5 μg/ml and zone of inhibition 15 mm when compared with the standard drug fluconazole.

On the basis of screening result obtained for compounds, the antimicrobial screening results reveal that the compounds containing a heterocyclic ring with two or three hetero atoms exhibited good to excellent activity. The low cytotoxicity of the compounds can be inferred on the basis of their moderate to low hemolytic activity. The findings of our study suggest that few of the tested compounds are worth of further investigations.

Supplementary Material

CCDC 880462, 880463 contains the supplementary crystallographic data for compound **1a** and **1d** respectively.

These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; fax (+44) 1223 336 033; or email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk

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