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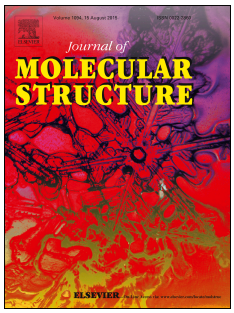

Synthesis, spectroscopic, crystal structure and *in vitro* cytotoxicity studies of *N*-thiophenoyl-*N*′-substituted phenyl thiocarbamide derivatives

Sunil K. Pandey, Seema Pratap, Gaetano Marverti, Manpreet Kaur, Jerry P. Jasinski

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Graphical Abstract

Synthesis, spectroscopic, crystal structure and *in vitro* cytotoxicity studies of N-thiophenoyl-N'-substituted phenyl thiocarbamide derivatives

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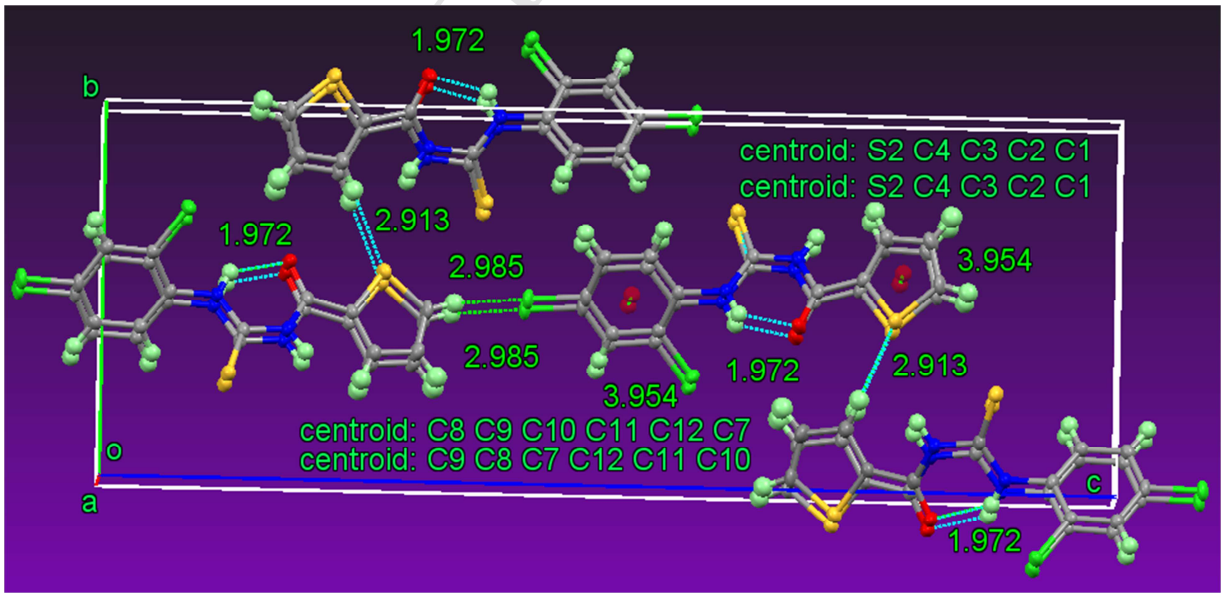
In the present study, a series of biologically active N, N'–disubstituted thiocarbamide compounds (**1−8**) have been synthesized and characterized by some spectroscopic (FT-IR, ^{1}H and ^{13}C NMR, UV-Visible) techniques. The molecular structure of compound **1** was determined by single crystal X-ray diffraction analysis. All the synthesized compounds (**1−8)** and five more **(9−13)** were screened for their *in vitro* cytotoxicity activities.



Synthesis, spectroscopic, crystal structure and *in vitro* cytotoxicity studies of N-thiophenoyl-N'-substituted phenyl thiocarbamide derivatives

Sunil K. Pandey [a], Seema Pratap [a*], Gaetano Marverti [b], Manpreet Kaur,[c] Jerry P. Jasinski [c]

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Abstract

A series of eight biologically active N, N'-disubstituted thiocarbamide compounds **(1–8)** have been prepared from thiophene-2-carbonyl isothiocyanate and various substituted aromatic primary amines (2,4-dichlorophenyl aniline, 4-chloro-3-nitrophenyl aniline, 4-methoxycarbonylphenyl aniline, 3-methoxycarbonylphenyl aniline, 2-methoxycarbonylphenyl aniline, 4-methoxyphenyl aniline, 2-methoxyphenyl aniline and 2-nitrophenyl aniline). Their structures were confirmed by elemental analyses, various spectroscopic techniques ((FT–IR, ^{1}H and ^{13}C NMR) and single crystal X-ray analysis of compound (**1**). In the molecular structure of compound (**1**) twisted confirmation of the carbonyl and thiocarbonyl group across C–N bond of thiocarbamide moiety and an offset face-to-face π–π stacking between two thiophene and two benzene ring of two molecules is observed. *In vitro* cytotoxicity assay of all the above compounds and five more (**9**−**13**) were carried out using seven human cancer cell lines; cervical (2008 and C13*), colorectal (HT29 and HCT116) and ovarian carcinoma (A2780, A2780/CP and IGROV-1). The results revealed that compounds **1**, **11**, **12** and **13** displayed promising inhibitory activity against all the cell lines tested.

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Keywords: Thiocarbamide; X-ray crystal structure; *In vitro* Cytotoxicity; Spectroscopic techniques; π–π stacking.

1. Introduction

N, N'-disubstituted thiocarbamides represent one of the most useful class of compounds with a wide range of applications in medicinal, analytical and other fields [1−5]. The structural motif of these compounds [-C(O)NHC(S)NH-] is composed of nitrogen, oxygen and sulfur heteroatoms which facilitate their hydrogen bonding abilities with different molecules and multitude of coordination behaviour with a variety of heavy trantion metal ions[6−9].The presence of thiocarbonyl moiety influences the lipophilicity/hydrophilicity and the electronic properties of the compounds [10, 11]. The medicinal applications of these compounds include their antibacterial, -fungal, anti-tubercular, anti-thyroid, anti-helmintic, rodenticidal, insecticidal, and herbicidal and plant growth regulator activities [12−17]. They also exhibit significant anticancer properties against various leukemia and solid tumors, however, the reports are very limited [18, 19]. Their inhibitory activity against protein tyrosine kinases (PTKs) [20, 21], human sirtuin type proteins, topoisomerase II and DNA repair synthesis has been reported [22].

These compounds have also been successfully used in liquid-liquid extraction of precious metals (Pt, Pd, Au Ag.), as anion and cation sensors [23, 24], liquid crystal materials [25, 26], as catalyst in chemical reactions [6, 27], as precursors or intermediates towards the synthesis of a variety of heterocyclic compounds. Due to non-linear optical properties single crystal of substituted thiocarbamides are being extensively employed in the electronic industry as polarization filters, electronic light shutters, electronic modulators, and as components in electrooptic and electro-acoustic devices, etc [28, 29].

We have reported anticancer properties of few N, N'-substituted thiocarbamide derivatives in our previous publications [12, 13, 30, 31]. Since, optimizing compound structure is a usual strategy to design and construct new antitumor agents, in the present study; we report the synthesis and anticancer activity of a series of novel thiophene containing substituted thiocarbamides.

2. Experimental Details

2.1. Chemicals, Instruments and Methods

Thiophene-2-carbonyl chloride, substituted aromatic primary amines (2, 4 – dichlorophenyl aniline, 4-chloro-3-nitrophenyl aniline, 4-methoxycarbonyl) phenyl aniline, 3-methoxycarbonyl phenyl aniline, 2-methoxycarbonyl phenyl aniline, 4-methoxyphenyl aniline, 2-methoxyphenyl aniline and 2-nitrophenyl aniline) and ammonium thiocyanate were purchased from Merck (Germany). Analytical grade acetone, acetonitrile, and dichloromethane were purchased from Rankem. The acetone was dried and freshly distilled prior to use. Melting points was measured on a X–4 digital melting-point apparatus and were uncorrected. Elemental analyses were performed on a CE-440 Exeter Analytical CHN analyzer. The infrared spectra of the title compounds as KBr pellets (4000−400 cm^{-1}) were recorded on a Varian 3100 FT-IR Excalibur series spectrophotometer.^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ by using TMS as an internal standard on a JEOL FT-NMR AL 500 spectrometer. The splitting of proton resonances in the reported ^{1}H NMR spectra were remarked as s = singlet, d = doublet, t = triplet, dd = doublet of doublets, and m = multiplet; coupling constants are reported in Hz. UV-Visible spectra were recorded on a Shimadzu (-UV-) 1700 Pharma Spec spectrophotometer, USA. A rectangular quartz cell with optical path length of 1cm was used.

2.2. Crystal structure determination

Data collection was performed using CrysAlisPRO on an Oxford Diffraction Xcaliber Ruby Gemini CCD differactometer using graphite- monochromatic CuKa (k = 1.54178 Å) at 123 K. The structure was solved by direct methods and refined by full-matrix least-square on F^2 using SHELXL-97 [32]. The non-hydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms were geometrically fixed and allowed to refine using the riding model. The details of the single crystal X-ray data collection, structure solution and structure refinement parameters are given in **Table 1**. Selected bond lenghths and bond angles are listed in **Table 2**.

2.3. General procedure for synthesis of compounds (1−8)

A solution of thiophene -2-carbonyl chloride (1.06 mmol, 10 mmol) in dry acetone (30 mL) was mixed to a suspension of ammonium thiocyanate (0.78g, 10 mmol) in dry acetone (30 mL) and was heated (60 °C) under reflux for 1 h. The resultant thiophene-2-carbonyl isothiocyanate solution was treated with equimolar quantity of appropriate substituted aromatic primary amine in acetone (Scheme 1) and further refluxed for 2 h at 27 °C [30, 31, 33−35]. The precipitated

ammonium chloride was filtered off and the yellow colour solution was evaporated in vacuum to dryness to obtain crude crystalline product. Yellow crystals of the compound 1 were grown by the slow evaporation of its solution in acetone/dichloromethane (1:1) in one week at 20°C.

2.3.1. N-(2, 4 – dichlorophenyl) - N'- (thiophene-2-carbonyl)-thiocarbamide (1)

Light yellow solid; (2, 4-dichloroaniline, 1.630g, 10 mmol); Yield: 80%, m.p. 175−176°C. FT−IR (KBr, cm^{-1}): 3411, 3117, ν (N−H); 3029, ν (Ar, C−H); 1667, ν (C═O); 1273, ν (C═S); 1157, ν(NCN); 776,(C−S); 661, ν_{as}(C−Cl); 478, ν_s(C−Cl). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ (ppm) 12.54 (s, 1H, −CSNH), 9.10 (s, 1H, −CONH), 8.37 (d, 1H, J = 8.5 Hz, Thiophene-H), 7.76 (dd, 1H, J_1 = 8.5 Hz, J_2 = 3.2 Hz, Thiophene-H), 7.74 (d, 1H, J = 5.1 Hz, Thiophene-H), 7.47-7.30 (m, 2H, Ar-H), 7.29-7.18 (m, 2H, Ar-H). ^{13}C NMR (125 MHz $CDCl_3$, 25°C): δ(ppm) 178.5 (C═S), 161.1 (C═O), 135.7, 134.7, 133.8, 132.5(Thiophene, Cs), 131.1, 129.5, 128.7, 128.6, 127.2, 126.9 (Ar, Cs). Anal. Calcd. for $C_{12}H_8N_2OS_2Cl_2$ (331.00): C, 43.51; H, 2.43; N, 8.46, Found: C, 43.47; H, 2.39; N, 8.38 %.

2.2.2. N-(4-chloro-3-nitrophenyl) - N'- (thiophene-2-carbonyl)-thiocarbamide (2)

Yellow solid; (4-chloro-3-nitroaniline, 1.731g, 10 mmol); Yield: 91%, m.p. 100−101°C. FT−IR (KBr, cm^{-1}): 3263, 3105, ν(N−H); 3072, ν(Ar, C−H); 1659, ν(C═O); 1556, ν_{as}(−NO_2); 1356,ν_s(−NO_2); 1272, ν(C═S); 1163,ν(NCN); 766, ν(C−S); 724, ν_{as}(C−Cl); 486, ν_s(C−Cl). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ (ppm) 12.71 (s, 1H, −CSNH), 9.03 (s, 1H, −CONH), 8.51 (d, 1H, J = 7.5 Hz, Thiophene-H), 7.85 (dd, 1H, J_1 = 6.0 Hz, J_2 = 3.5 Hz, Thiophene-H), 7.76 (d, 1H, J = 5.0 Hz, Thiophene-H), 7.70-7.54 (m, 2H, Ar-H), 7.24-7.15 (m, 2H, Ar-H). ^{13}C NMR (125 MHz $CDCl_3$, 25°C): δ (ppm) 182.1 (C═S), 161.5 (C═O), 137.1, 135.1, 134.5, 132.1(Thiophene, Cs), 131.3, 128.8, 128.5, 128.1, 124.4, 120.6 (Ar, Cs). Anal. Calcd. for $C_{12}H_8N_3O_3S_2Cl$ (341.50): C, 42.17; H, 2.36; N, 12.29, Found: C, 42.09; H, 2.33; N, 12.19 %.

2.2.3. N-(4-methoxycarbonyl) phenyl)-N'-(thiophene-2-carbonyl)-thiocarbamide (3)

Light yellow solid; (4-methoxycarbonylphenyl aniline, 1.520g, 10 mmol); Yield: 88%, m.p. 170−171°C. FT−IR (KBr, cm^{-1}): 3296, 3147, ν(N−H); 3097, ν(Ar, C−H); 1721, ν(−COOR); 1698, ν(C═O); 1270, ν(C═S); 1143, ν(NCN); 769,ν(C−S). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ (ppm) 12.65 (s, 1H, −CSNH), 8.94 (s, 1H, −CONH), 8.08 (d, 1H, J = 8.5 Hz, Thiophene-H), 7.87 (dd, 1H, J_1 = 8.5 Hz, J_2 = 3.2 Hz, Thiophene-H), 7.74 (d, 1H, J = 5.1 Hz, Thiophene-H), 7.24−7.21 (m, 2H, Ar-H), 7.20−7.19(m, 2H, Ar-H), 3.91(s, 3H, −$COOCH_3$). ^{13}C NMR (125 MHz $CDCl_3$, 25°C): δ (ppm) 177.4 (C═S), 161.1 (C═O), 139.1, 137.1, 134.5, 132.1(Thiophene,

Cs), 131.3, 130.7, 130.2, 128.3, 122.6, 121.4 (Ar, Cs), 58.1 (C, –$COOCH_3$). Anal. Calcd. for $C_{14}H_{12}N_2O_3S_2$ (320.38): C, 52.48; H, 3.78; N, 8.74, Found: C, 52.27; H, 3.72; N, 8.68 %.

2.2.4. N-(3-methoxycarbonyl) phenyl)-N'-(thiophene-2-carbonyl)-thiocarbamide (4)

Light yellow solid; (3-methoxycarbonylphenyl aniline, 1.520g, 10 mmol); Yield: 92%, m.p. 95−96°C. FT−IR (KBr, cm^{-1}): 3332, 3140, ν(N−H); 3094, ν(Ar, C−H); 1709,ν(−COOR); 1666, ν(C═O); 1263, ν(C═S); 1137,ν(NCN); 736,ν(C−S). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ (ppm) 13.27 (s, 1H, −CSNH), 10.01 (s, 1H, −CONH), 8.37 (d, 1H, J = 8.0 Hz, Thiophene-H), 8.04 (dd, 1H, J_1 = 8.0 Hz, J_2 = 3.4 Hz, Thiophene-H), 7.99 (d, 1H, J = 4.1 Hz, Thiophene-H), 7.37−7.34 (m, 2H, Ar-H), 7.33−7.20(m, 2H, Ar-H), 3.94(s, 3H, −$COOCH_3$). ^{13}C NMR (125 MHz $CDCl_3$, 25°C): δ (ppm) 177.9 (C═S), 166.4 (C═O), 138.4, 136.1, 134.2, 132.3(Thiophene, Cs), 131.4, 130.7, 130.8, 128.4, 127.0, 126.2 (Ar, Cs), 57.8 (C, –$COOCH_3$). Anal. Calcd. for $C_{14}H_{12}N_2O_3S_2$ (320.38): C, 52.48; H, 3.78; N, 8.74, Found: C, 52.39; H, 3.76; N, 8.66 %.

2.2.5. N-(2-methoxycarbonyl) phenyl)-N'-(thiophene-2-carbonyl)-thiocarbamide (5)

Light yellow solid; (2-methoxycarbonylphenyl aniline, 1.520g, 10 mmol); Yield: 85%, m.p. 97−98°C. FT−IR (KBr, cm^{-1}): 3332, 3300, ν(N−H); 3088,ν(Ar, C−H); 1715,ν(−COOR); 1690, ν(C═O);1262,ν(C═S); 1163,ν(NCN); 758,ν(C−S).^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ (ppm) 11.93 (s, 1H, −CSNH), 9.80 (s, 1H, −CONH), 8.71 (d, 1H, J = 9.0 Hz, Thiophene-H), 8.28 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.4 Hz, Thiophene-H), 7.99 (d, 1H, J = 4.1 Hz, Thiophene-H), 7.97−7.92 (m, 2H, Ar-H), 7.87−7.86(m, 2H, Ar-H), 3.87 (s, 3H, −$COOCH_3$). ^{13}C NMR (125 MHz $CDCl_3$, 25°C): δ (ppm) 178.8 (C═S), 160.1 (C═O), 139.9, 138.2, 134.6, 132.1(Thiophene, Cs), 131.1, 130.7, 130.7, 128.4, 127.8, 126.7 (Ar, Cs), 57.6 (C, –$COOCH_3$). Anal. Calcd. for $C_{14}H_{12}N_2O_3S_2$ (320.38): C, 52.48; H, 3.78; N, 8.74, Found: C, 52.38; H, 3.75; N, 8.67 %.

2.2.6. N-(4-methoxyphenyl) - N'- (thiophene-2-carbonyl)-thiocarbamide (6)

Yellow solid; (4-methoxyphenyl aniline, 1.231g, 10 mmol); Yield: 78%, m.p. 120−121°C. FT−IR (KBr, cm^{-1}): 3458, 3207, ν (N−H); 3086, ν (Ar, C−H); 1658, ν(C═O); 1285,ν(C═S); 1180, ν(NCN); 776, ν(C−S). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ(ppm) 13.27 (s, 1H, −CSNH), 8.97 (s, 1H, −CONH), 8.48 (d, 1H, J = 7.5 Hz, Thiophene-H), 8.05 (dd, 1H, J_1 = 6.5 Hz, J_2 = 2.1 Hz, Thiophene-H), 7.99 (d, 1H, J = 4.0 Hz, Thiophene-H), 7.59−7.57 (m, 2H, Ar-H), 7.33−7.31 (m, 2H, Ar-H), 3.95 (s, 3H, −OCH_3). ^{13}C NMR (125 MHz $CDCl_3$, 25°C): δ (ppm) 178.4 (C═S), 160.0 (C═O), 138.6, 136.2, 134.1, 132.5(Thiophene, Cs), 131.1, 130.9, 128.4, 126.5, 126.2,

122.6 (Ar, Cs), 52.6 (C, –OCH_3). Anal. Calcd. for $C_{13}H_{12}N_2O_2S_2$ (292.37): C, 53.40; H, 4.14; N, 9.58, Found: C, 53.24; H, 4.09; N, 9.47 %.

2.2.7. N-(2-methoxyphenyl) - N'- (thiophene-2-carbonyl)-thiocarbamide (7)

Yellow solid; (2-methoxyphenyl aniline, 1.231g, 10 mmol); Yield: 75%, m.p. 115−116°C. FT−IR (KBr, cm^{-1}): 3468, 3262, ν(N−H); 3105,ν(Ar, C−H); 1673, ν(C═O); 1270, ν(C═S); 1119,ν(NCN); 762,ν(C−S). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ (ppm) 12.40 (s, 1H, −CSNH), 9.03 (s, 1H, −CONH), 7.74 (d, 1H, J = 10.0 Hz, Thiophene-H), 7.65 (dd, 1H, J_1 = 9.5 Hz, J_2 = 4.1 Hz, Thiophene-H), 7.99 (d, 1H, J = 4.0 Hz, Thiophene-H), 7.59−7.57 (m, 2H, Ar-H), 7.33−7.31 (m, 2H, Ar-H), 3.95 (s, 3H, −OCH_3). ^{13}C NMR (125 MHz $CDCl_3$, 25°C): δ (ppm) 178.2 (C═S), 161.3 (C═O), 138.3, 136.1, 135.8, 134.6(Thiophene, Cs), 132.3, 131.0, 130.1, 129.1, 128.6, 125.4 (Ar, Cs), 58.5 (C, –OCH_3). Anal. Calcd. for Calc. for $C_{13}H_{12}N_2O_2S_2$ (292.73): C, 53.40; H, 4.14; N, 9.58, Found: C, 53.27; H, 4.11; N, 9.51 %

2.2.8. N-(2-nitrophenyl) - N'- (thiophene-2-carbonyl)-thiocarbamide (8)

Dark yellow solid; (2-nitroaniline aniline, 1.381g, 10 mmol); Yield: 91%, m.p. 155−156°C. FT−IR (KBr, cm^{-1}): 3318, 3101, ν(N−H); 3005,ν(Ar, C−H); 1661,ν(C═O); 1564, ν_{as}(−NO_2), 1319, ν_s(−NO_2); 1264, ν(C═S); 1154,ν(NCN); 743,ν(C−S). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ (ppm) 12.88 (s, 1H, −CSNH), 9.01 (s, 1H, −CONH), 8.28 (d, 1H, J = 8.5 Hz, Thiophene-H), 7.78 (dd, 1H, J_1 = 6.0 Hz, J_2 = 3.1 Hz, Thiophene-H), 7.75 (d, 1H, J = 5.5 Hz, Thiophene-H), 7.24−7.21 (m, 2H, Ar-H), 7.20−7.19(m, 2H, Ar-H). ^{13}C NMR (125 MHz $CDCl_3$, 25°C): δ (ppm) 178.1 (C═S), 161.5 (C═O), 145.2, 143.3, 135.5, 135.0(Thiophene, Cs), 131.1, 128.8, 124.6, 123.2, 121.1 (Ar, Cs).Anal. Calcd. For $C_{12}H_9N_3O_3S_2$ (307.40): C, 46.89; H, 2.95; N, 13.67, Found: C, 46.78; H, 2.92; N, 13.58 %

3. Biological assays

3.1. Cell lines

Seven human cancer cell lines namely, cervical (2008 and C13*), colorectal (HT29 and HCT116) and ovarian carcinoma (IGROV-1, A2780 and A2780/CP) were used. Among these, C13* and A2780/CP are cisplatin (ccDDP)-resistant cells [36, 37]. Cells were grown as monolayers in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum and 50 µg/ml gentamycin sulfate. All cell media and serum were purchased from Lonza (Verviers, Belgium). Cultures were equilibrated with humidified 5% CO_2 in air at 38 ºC. All studies were

performed in Mycoplasma negative cells, as routinely determined with the MycoAlert Mycoplasma detection kit (Lonza, Walkersville, MD, USA).

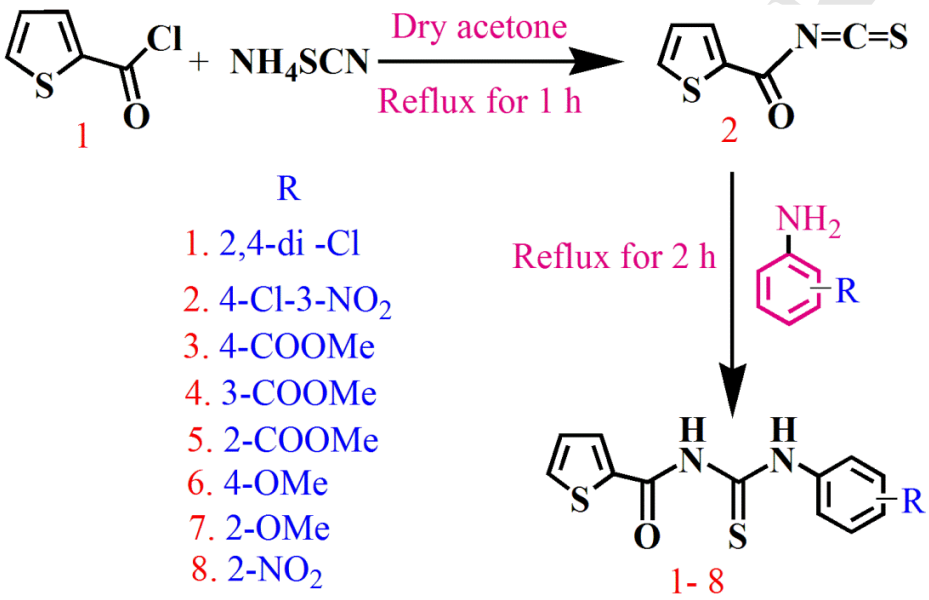
3.2 Cytotoxicity screening

In vitro cytotoxicity of compounds used in the present study was determined by MTT assay [38, 39]. The cells were seeded into 96-well plates and cultured overnight .Various concentrations of the test compounds dissolved in DMSO solvents were then added and incubated for 72 h. After incubation, the medium was removed and added fresh culture medium 100 µl containing 0.5 mg mL^{-1} MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide; Sigma) and then incubated at 38ºC for 4 h. The medium was removed and 100 µL DMSO solvent was added to dissolve the dark blue crystals. After incubation for 30 min at room temperature, to ensure that all crystals were dissolved, absorbance was measured using an ELISA plate reader at 570 nm with reference wavelength of 650 nm.

4. Results and Discussion

4.1 Synthesis and Characterization

Two step synthesis of compounds **1−8** were accomplished by the reaction of thiophene-2-carbonyl chloride with ammonium thiocyanate and refluxing the resultant thiophene-2-carbonyl isothiocyanate with appropriate substituted aromatic primary amines (**Scheme 1**). All the compounds were obtained in good yield. The slow evaporation at room temperature of acetone solution of compound **(1)** afforded single crystals suitable for X-ray diffraction.



Scheme 1 Synthetic route to new thiophene-2-carbonyl-thiocarbamides **(1 – 8)**

4.2. FT-IR study

In the IR spectra of compounds a sharp and a broad band observed at ~3400 cm^{-1} and ~3140 cm^{-1} could be assigned to N1−H and N2···H stretching modes, respectively [40, 41]. The appearance of N2−H stretching vibration at a lower wave number is due to the presence of intramolecular hydrogen bonding N2−H···O═C whereas the first band may be attributed to free ν(N1−H) [13]. Characteristic C−H stretching vibrations of the aromatic groups appear as medium intensity band at ~3050 cm^{-1}. A strong band at ~1660 cm^{-1} corresponds to ν(C=O) mode

which resembles in frequency to the hydrogen bonded C=O group (N−H···O═C) observed in many similar compounds. A strong and a medium intensity band at ~1265 cm^{-1} and ~760 cm^{-1} could be assigned to C═S and C−S stretching modes, respectively [12, 13].

4.3. NMR study

^{1}H NMR spectra of the compounds (**1−8**) in $CDCl_3$ exhibited two singlets at 9.00–10.00 and 12.00–13.31 ppm which could be assigned to free N1−H and hydrogen bonded N2−H, respectively [12, 13]. Aromatic protons appeared as multiplets between 7.20–8.50 ppm. The chemical shift value of free N−H suffers a downfield shift of ~3.0 ppm in DMSO-d_6. Such shifts could be ascribed to the probable hydrogen bonding between the NH and sulfoxide (S═O) moiety [14].

The ^{13}C NMR spectra of compounds **(1–8**) depicted all the signals due to magnetically distinct carbons present in them. The peaks related to the carbons in CONH and CSNH groups of the thiocarbamide resonate around 160–170 and 172–182 ppm, respectively [12-14]. The downfield resonance of C═S in comparison to C=O carbon may be related to steric and electronic factors [42].

4.4 Electronic study

Electronic spectra of compounds **1–8** recorded in dichloromethane over the scan range of 200–800 nm show identical trends (**Figure S1**). A representative UV-Vis spectrum of compound **2** is shown in Figure **1**. A weak band centered at ~261 nm and a strong band at ~296 nm in the absorption spectra of compounds can be ascribed to $\pi \rightarrow \pi^*$ transitions of thiocarbamide moiety [43].

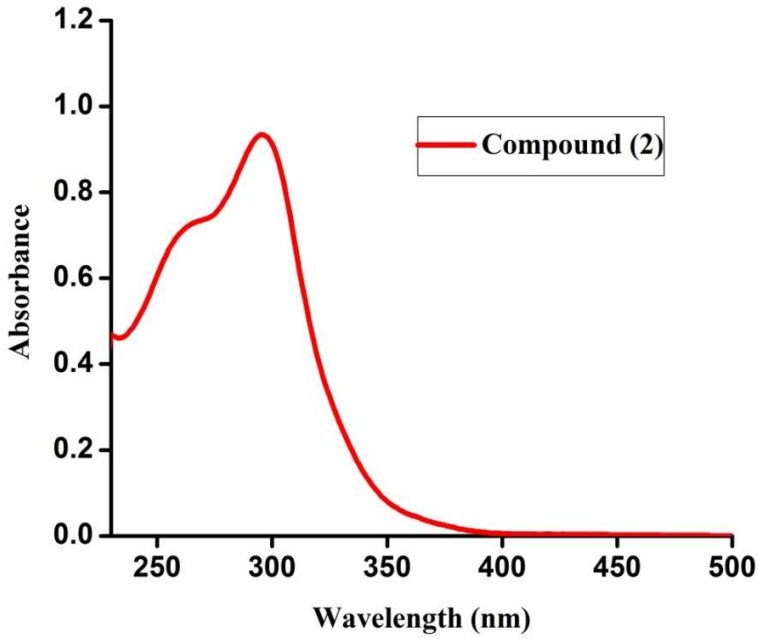
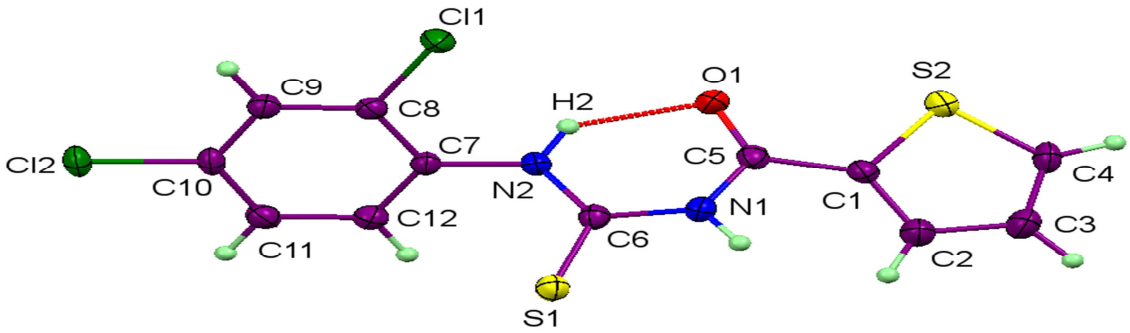


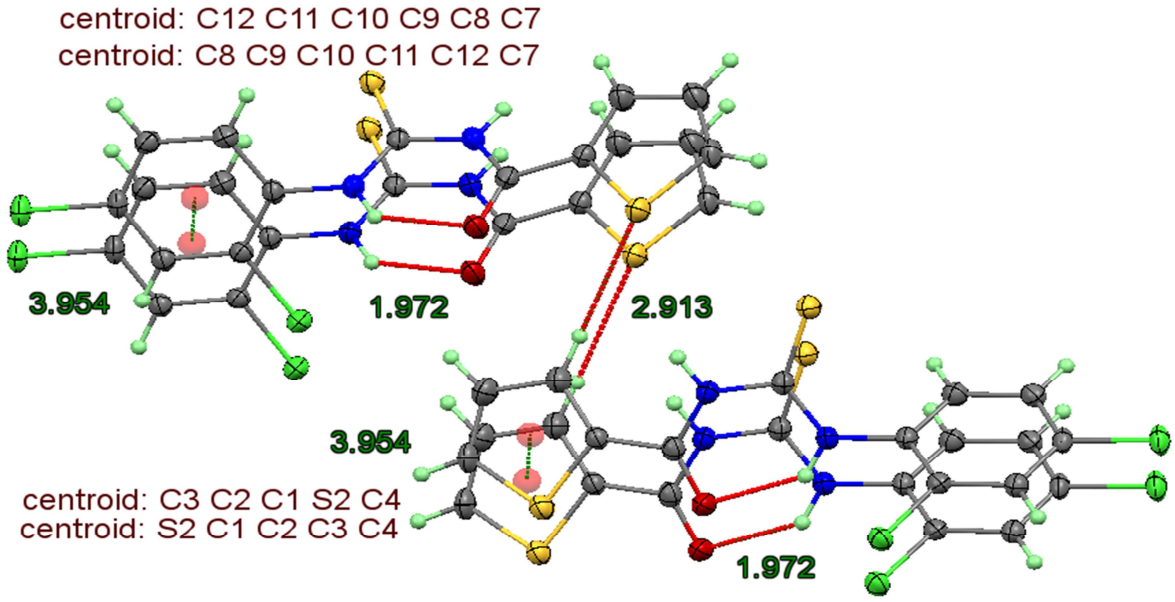
Fig. 1 UV−Vis spectrum of compound **2** in dichloromethane

4.5 Description of crystal structure

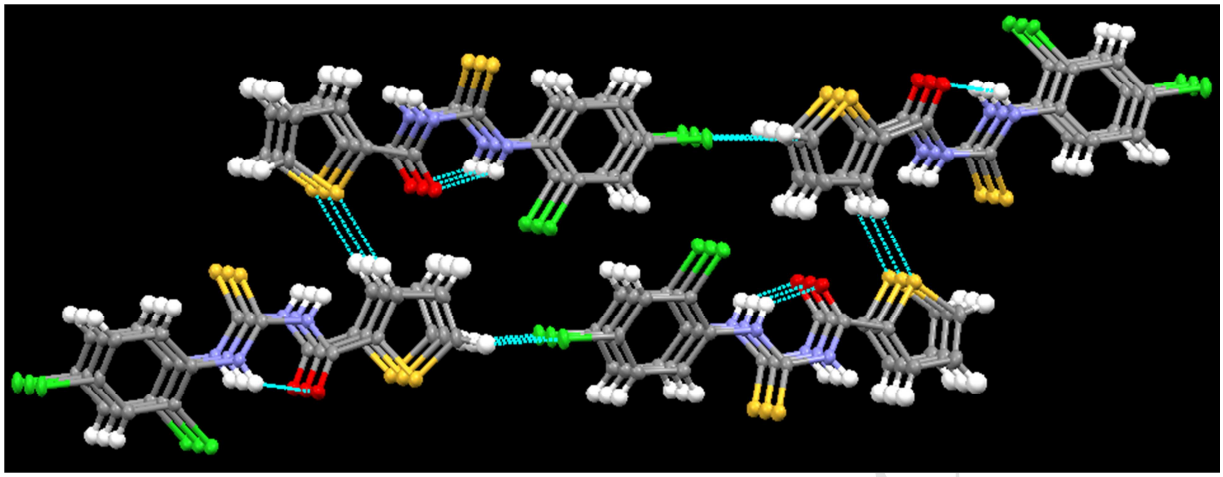
The ORTEP view, unit cell diagram and packing pattern of compound (**1**) are depicted in Figures **2(a), 2(b)** and **2(c)**, respectively**.** The crystallographic and refinement details are shown in **Table 1**. Selected bond lengths, bond angles and hydrogen bond interaction are listed in **Table 2** and **Table S1**. In molecular structure thiocarbonyl (C═S) and carbonyl (C═O) groups are opposite to each other and are almost coplanar with torsion angles S(1)−C(6)−N(1)−C(5) = 177.6(3)º and N(2)–C(6)−N(1)−C(5) = -3.3(5)°, respectively. The phenyl ring, thiophene and thiocarbamide moiety lie in a plane, which is evidenced by the torsion angle values N(1)−C(6)−N(2)−C(7) =173.8(3)º, C(6)–N(1)−C(5)−C(1)= -169.6(3)°, respectively [14,15]. The molecular structure shows an intramolecular N2–H···O=C hydrogen bond stabilizing the (–C(O)NHC(S)NH–) geometry, forming a pseudo six membered ring (**Table S1**). The C−N, C═S and other bond distances (**Table 2**) exhibit conventional bond parameters similar to other substituted thiocarbamides [44, 45]. Additionally, an offset face to face π−π stacking interaction is also observed in compound (**1**) between thiophene rings and substituted benzene rings of two molecules [13, 46]. Very weak intermolecular interactions C−H···Cl, C−S···H and Cπ−Cπ stabilize the crystal packing and lead to an infinite chain length along the b-axis Figure **2(c)**


Cl1
C9
C8
H2
O1
S2
Cl2
C7
C5
C4
C10
N2
N1
C1
C12
C6
C3
C11
C2
S1

(a)


centroid: C12 C11 C10 C9 C8 C7
centroid: C8 C9 C10 C11 C12 C7
3.954
1.972
2.913
3.954
centroid: C3 C2 C1 S2 C4
centroid: S2 C1 C2 C3 C4
1.972

(b)



(c)

Fig. 2 (a) ORTEP diagram of **1** showing the thermal ellipsoids at 50% probability. The intramolecular hydrogen bonds as shown by dashed lines. **(b)** Unit cell diagram viewed down the b axis for compound **1**. Intermolecular face-to-face $\pi-\pi$ stacking interaction is also observed in the compound **1**. **(c)** The packing diagram of compound **1**. Weak intermolecular interactions (C−H···Cl, C−S···H and Cπ−Cπ) lead to an infinite chain length along the b-axis.

Table 1. Crystal data and structure refinement for compound **1**

Compound	**1**
CCDC No	1561945
Empirical formula	$C_{12}H_8Cl_2N_2OS_2$
Formula weight	331.22
Temperature/K	173(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a (Å)	3.9537(2)
b (Å)	10.9953(6)
c (Å)	30.0239(16)
α,β, γ (°)	90
Volume/Å^3	1305.20(12)
Z	4
ρcalcg/cm^3	1.686
m/mm^{-1}	7.402
F(000)	672.0
Crystal size/mm^3	0.48 × 0.14 × 0.08
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	8.564 to 142.506
Index ranges	$-4 \le h \le 4$, $-12 \le k \le 13$, $-26 \le l \le 36$
Reflections collected	8346
Independent reflections	2501 [Rint = 0.0366, Rsigma = 0.0316]
Data/restraints/parameters	2501/0/172
Goodness-of-fit on F^2	1.029
Final R indexes [I>=2σ (I)]	R_1 = 0.0340, wR_2 = 0.0867
Final R indexes [all data]	R_1 = 0.0351, wR_2 = 0.0877
Largest diff. peak/hole / eÅ^{-3}	0.33/-0.36
Flack parameter	-0.008(13)

[a]R = P||Fo| − |Fc||/P |Fo|

[b]Rw = [Pw(Fo^2− Fc^2)$_2$/Pw(Fo^4)]1/2.

Table 2. Selected bond lengths (Å) and bond angles (º) for compound **1**

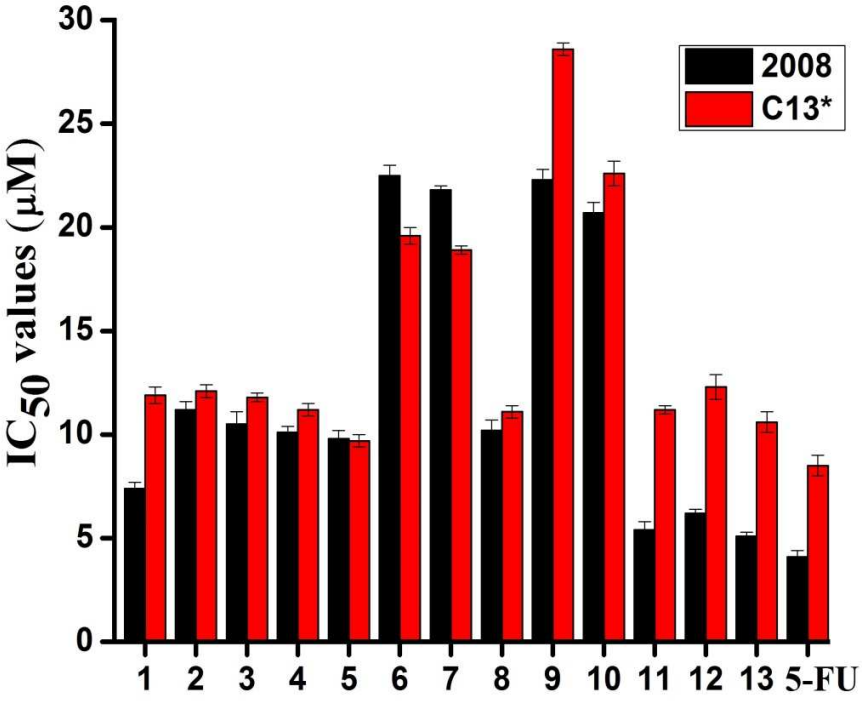
Bond Lengths (Å)			
N1−C5	1.372(4)	S2−C1	1.730(3)
N1−C6	1.394(4)	S2−C4	1.706(3)
N2−C6	1.346(4)	C10−C11	1.388(5)
N2−C7	1.419(4)	C12−C12	1.388(4)
O1−C5	1.229(4)	C8−Cl1	1.738(3)
C6−S1	1.660(3)	C10−Cl2	1.733(3)
Bond Angles (º)			
C4−S2−C1	91.58(16)	O1−C5−N1	123.4(3)
O1−C5−C1	122.2(3)	N1−C5−C1	114.4(3)
N1−C6−S1	118.3(2)	N2−C6−S1	126.6(2)
N2−C6−N1	115.1(3)	N2−C7−C8	120.3(2)
N2−C7−C12	118.9(3)	C5−C1−C2	130.6(3)

4.6 *In vitro* cytotoxicity screening

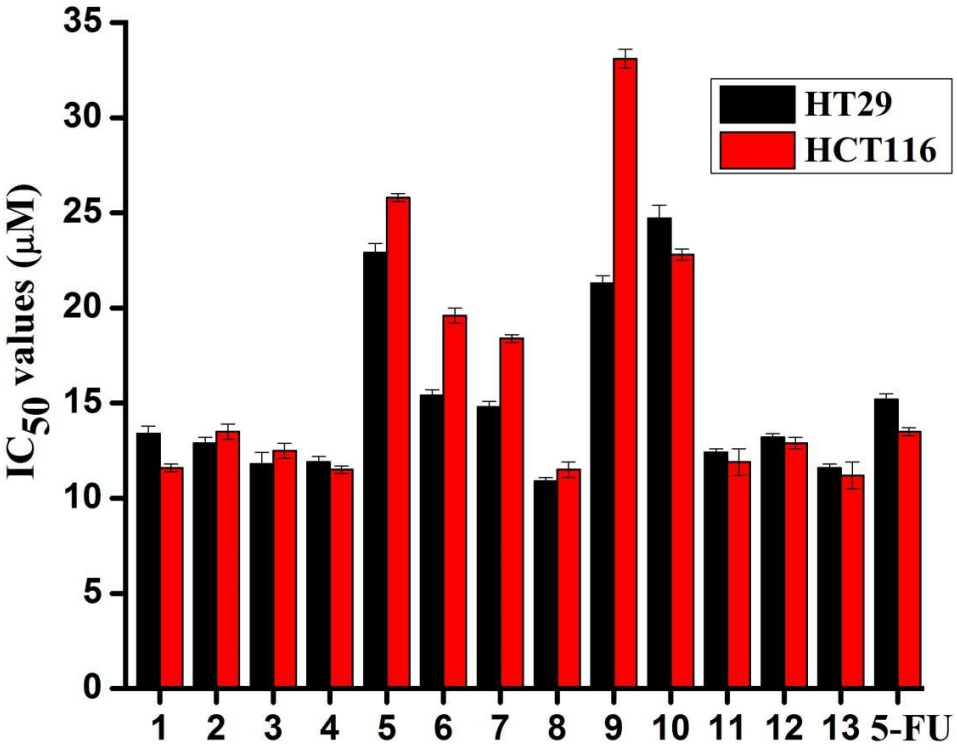
To evaluate the anti-cancer activity of synthesized substituted thiocarbamides (**1**−**8**) and five other (**9**−**13**), all the thirteen compounds (**1**−**13**) were tested for their in vitro cytotoxicity activities against a panel of seven human cancer cell lines, cervical (2008 and C13*), colorectal (HT29 and HCT116) and ovarian carcinoma (A2780, A2780/CP and IGROV-1) using 5-Fluorouracil (5-FU, a clinically used drug) as a positive control [47−49]. The result in the form of their IC_{50} values has been presented in **Table 3.** The comparative cytotoxicity of the compounds is depicted in Figure **3**. The structural analysis of compounds **9**−**13** (N−4−Sulfonamide phenyl-N'–2–thiophenoyl thiourea, N–3–Nitrophenyl–N'–2–thiophenoyl thiourea, N–2–Chloro–4–nitrophenyl–N'–2–thiophenoyl thiourea, N–2–Methoxy–4–nitrophenyl-N'–2–thiophenoyl thiourea and N–4–Chloro–2–nitrophenyl–N'–2–thiophenoyl thiourea) along with their other biological properties have already been published by us [50]. The tested compounds displayed potent inhibitory activity against all the cell lines having IC_{50} values in different ranges (**Table 3**). The strongest inhibitory activity was displayed by compounds **1**, **11**, **12** and **13** against all the cell lines**.** All the compounds except **5**, **9** and **10** displayed stronger inhibitory activity than the standard drug (5-FU) against HT29 cell line. In comparison to the standard drug (5-FU) better inhibitory properties were obtained for compounds **1**, **3**, **4**, **8**, **11**, **12** and **13** against HCT116 and for compounds **5** and **8** against A2780/CP cancer cell lines, respectively. The present study demonstrates that nature and number of functional groups attached to the phenyl ring of the thiocarbamide moiety exert a decisive influence on the cytotoxicity of this class of compounds. The most promising compounds **1**, **11**, **12** and **13** contained a nitro and/or chloro electron withdrawing group at ortho and para positions [51, 52] In general, all the above compounds exhibited better inhibitory properties than those reported in our previous papers [12, 13, 30, 31]. Previous studies have shown that the presence of electronegative atom/group at ortho and or/ para position of the aromatic ring increases the lipophilicity of molecules and is responsible for enhanced cytotoxicity in MTT model [53]. Based on the above results, potential anticancer candidates can be considered for structural modification and pharmacological evaluation.

Table 3. IC_{50} Values (µM) for the compounds **(1−13)** against seven human cancer cell lines; (**2008** and **C13*)** cervical, (**HT29** and **HCT116**) colorectal and (**A2780**, **A2780/CP** and **IGROV-1)** ovarian carcinoma.

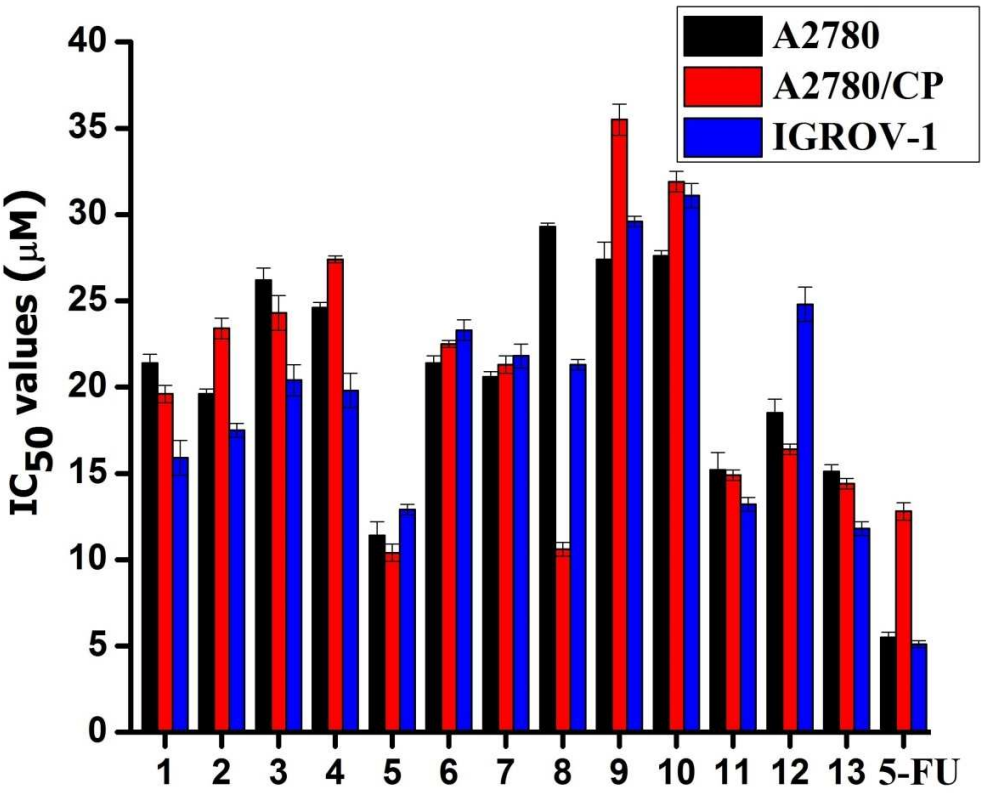
Compounds	**Cell lines**						
	2008	**C13***	**HT29**	**HCT116**	**A2780**	**A2780/CP**	**IGROV-1**
1.	7.4±0.3	11.9±0.4	13.4±0.1	11.6±2	21.4±5	19.6±5	15.9±1
2.	11.2±0.4	12.1±0.3	12.9±0.3	13.5±4	19.6±3	23.4±6	17.5±0.4
3.	10.5±0.6	11.8±0.2	11.9±0.6	12.5±0.4	26.2±0.7	24.3±1	20.4±0.9
4.	10.1±0.1	11.2±0.3	11.9±0.3	11.5±0.2	24.6±0.3	27.4±0.2	19.8±10
5.	9.8±0.4	9.75±0.3	22.9±0.5	25.8±0.2	11.4±0.8	10.4±0.5	12.9±1
6.	22.5±0.5	19.6±0.1	15.4±0.3	19.6±0.4	21.4±0.4	22.5±2	23.3±0.6
7.	21.8±0.2	18.9±0.2	14.8±0.6	18.4±0.2	20.6±0.3	21.3±5	21.8±0.7
8.	10.2±0.1	11.1±0.3	10.9±0.2	11.5±0.1	29.3±4	10.6±0.5	21.3±0.7
9.	22.3±0.5	28.6±0.8	21.3±0.6	33.1±0.1	27.4±0.8	35.5±0.9	29.6±0.3
10.	20.7±0.5	22.6±0.6	24.7±0.7	22.8±0.3	27.6±2	31.9±0.6	31.1±0.7
11.	5.4±0.1	11.2±0.2	12.4±0.2	11.9±0.7	15.2±1	14.9±3	13.2±0.4
12.	6.2±0.2	12.3±0.6	13.2±0.2	12.9±0.3	18.5±3	16.4±3	14.8±1
13.	5.1±0.2	10.6±0.5	11.6±0.2	11.2±0.7	15.1±1	14.4±2	11.8±1
5-FU	4.1±0.3	8.5±0.5	15.2±0.1	13.5±0.1	5.5±0.3	12.8±0.5	5.1±0.2


30
25
20
15
10
5
0
IC50 values (μM)
2008
C13*
1
2
3
4
5
6
7
8
9
10
11
12
13
5-FU

(A)



(B)



(C)

Fig. 3 Comparative *in vitro* cytotoxicity of compounds **1−13** for a panel of seven human cancer cell lines; **(A).** Cervical (2008 and C13*) **(B).** Colorectal (HT29 and HCT116) **(C).** Ovarian carcinoma (A2780, A2780/CP and IGROV-1).

5. Conclusions

A facile synthesis of a series of N, N'-disubstituted thiocarbamides were performed using the prescribed method in this paper. All these compounds were structurally characterized by using various spectroscopic and single crystal X-ray diffraction techniques. The crystal structure of compound **1** revealed the planar confirmation of thiocarbamide unit which may be due to the formation of intramolecular hydrogen bond N2−H···O═C. An offset face-to-face π –π stacking is also present between aromatic rings of compound **1.**The cytotoxic effects of these compounds were evaluated against seven human cancer cell lines using the standard MTT assay. The IC_{50} values indicated all of them to be potent anticancer agents against all the cell lines. Most of the tested compounds conferred better inhibitory activity than the standard drug (**5-FU**) against **HT29** and **HCT116** cell lines and are worthy of further structural modification and pharmacological evaluation.

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Supporting Information Summary

UV−Vis spectra of compounds **(1−8)** in dichloromethane **Figure S1**. Scan copies of ^{1}HNMR **Fig**.(**S2−S9**) and ^{13}CNMR **Fig.(S10−S17**). X-ray crystallographic hydrogen bonding Table **S1** of compound **(1)** have been provided in supporting information. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge

CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository numbers CCDC **1561945** for compound **(1)** (Fax: +44-1223-336-033; Email: deposit@ccdc.cam.ac.uk, http://www.cam.ac.uk)

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Highlights:

- ❖ Single crystal X-ray of compound **1** and various spectroscopic techniques (FT-IR, UV-Vis, ^{1}H and ^{13}C NMR) were used to establish the structure of the compounds.
- ❖ The presence of intramolecular hydrogen bond (N─H···O═C) compels planarity of the molecule and trans orientation of C=O and C=S group.
- ❖ The compounds were screened for their *In vitro* cytotoxic activity against seven human cancer cell lines.
- ❖ The compounds screened for their cytotoxic activity in seven human cell line carcinomas exhibited promising anticancer activity
- ❖ All the compounds except **5**, **9** and **10** displayed stronger inhibitory activity than the standard drug (5-FU) against HT29 cell line.