

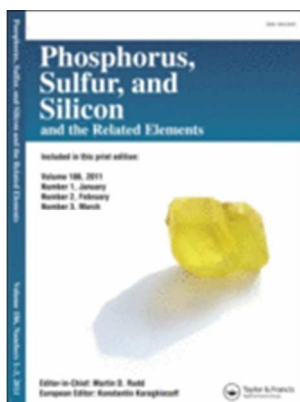
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Synthesis, molecular structure exploration and in vitro cytotoxicity screening of five novel N, N'- disubstituted thiocarbamide derivatives

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Keywords:	Thiocarbamide, X-ray crystallography, Cytotoxicity, Spectroscopic techniques, π - π stacking

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Synthesis, molecular structure exploration and *in vitro* cytotoxicity screening of five novel N, N'- disubstituted thiocarbamide derivatives

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ABSTRACT

The synthesis of five N,N'-substituted thiocarbamides, namely N-(naphthyl)-N'-(pentoxycarbonyl) thiocarbamide (**H₂L1**), N-(2-Chloro-4-nitrophenyl)-N'-(pentoxycarbonyl) thiocarbamide (**H₂L2**), N-(2-methoxy-4-nitrophenyl)-N'-(pentoxycarbonyl) thiocarbamide (**H₂L3**), N-(3-nitrophenyl)-N'-(pentoxycarbonyl) thiocarbamide (**H₂L4**) and N-(naphthyl)-N'-(2, 2, 2-trichloroethoxycarbonyl) thiocarbamide (**H₂L5**) was performed by the reaction of pentoxycarbonyl chloroformate with naphthyl amine, 2-chloro-4-nitroaniline, 2-methoxy-4-nitroaniline, 3-nitroaniline, respectively, for the first four and by the reaction of 2, 2, 2-trichloroethoxycarbonyl chloroformate with naphthyl amine for the last compound. These compounds were fully characterized by using various spectroscopic (FT-IR, ¹H and ¹³C NMR) and single crystal X-ray studies of **H₂L1** and **H₂L5**. In the crystal structure of both the compounds the (C=S) and (C=O) groups are trans to each other across the C–N bond. The crystal packing of **H₂L1** shows that the molecules form centrosymmetric dimers connected by N2–H····S hydrogen bonds. In **H₂L5** an offset face-to-face π – π stacking is observed between two naphthalene rings of two molecules. *In vitro* cytotoxicity of synthesized compounds was evaluated using five human carcinoma cell lines 2008, C13* (cervical carcinoma), A2780, A2780/CP and IGROV-1 (ovarian carcinoma). The IC₅₀ values of compounds **H₂L2** – **H₂L4** demonstrated them to be very promising anticancer agents.

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Keywords: Thiocarbamide; X-ray crystallography; Cytotoxicity; Spectroscopic techniques; π – π stacking

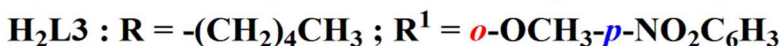
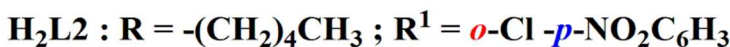
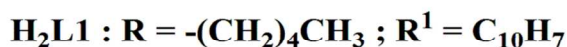
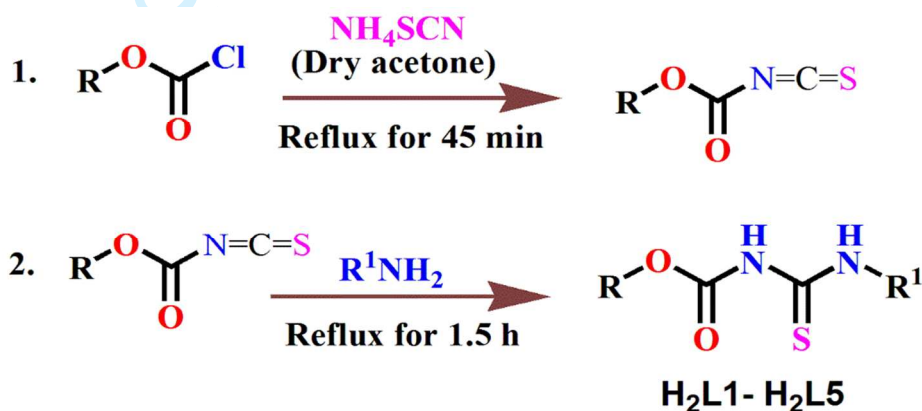
Introduction

The structural studies of N,N'-disubstituted thiocarbamides have been the subject of extensive investigation on the ground of their potential medicinal, environmental¹⁻³ and analytical applications.^{4,5} The vivid usage of these compounds may be credited to the presence of C=S and N-H groups in their structural motif. The presence of these groups facilitate the interaction of thiocarbamides with the variety of metal ions through coordinate bonds and also with many anions via the formation of intermolecular hydrogen bonds.⁶⁻¹¹ In addition, the presence of intramolecular hydrogen bond (C=O·····H-N) causes monodentate coordination through thione sulfur forming trigonal planar copper (I) complexes which act as catalysts in many organic synthesis.¹²⁻¹⁵ The anticancer properties of substituted thiocarbamides have not been explored much though some of them have displayed excellent cytotoxic behavior against various human cancer cell lines.¹⁶⁻¹⁹ It has been reported that structural changes in these molecules, their modes of bonding to the DNA have considerable impact on their anticancer properties.²⁰⁻²² Some additional therapeutic values of substituted thiocarbamides can be reflected by their use as antimicrobial, antiviral, antimalarial, antitubercular, antiallergic, antithyroid, rodenticidal, herbicidal agents.²³⁻²⁷ These compounds have also been proven valuable in liquid-liquid extraction of precious metals (Pt, Pd, Au Ag.), as anion and cation sensors^{28, 29}, liquid crystal materials^{30, 31}, non-linear optical materials^{32, 33}, as catalyst in oxidation reaction of alcohols.^{34, 35} In view of the above facts and as part of our continuing work, we present here the "Synthesis, molecular structure exploration and *in vitro* cytotoxicity screening of five novel N, N'-disubstituted thiocarbamide derivatives".¹²⁻¹⁴

Results and discussion

Synthesis and Characterization

The synthesis of compounds **H₂L1–H₂L5** was achieved by the reaction of n-pentoxycarbonyl/2, 2, 2-trichloroethoxycarbonyl isothiocyanate and suitable substituted primary amines. All the compounds were obtained in good yield. The slow evaporation at room temperature of acetone solution of compounds **H₂L1** and **H₂L5** (Scheme 1) afforded single crystals suitable for X-ray diffraction.



Scheme 1. Synthesis of compounds **H₂L1 – H₂L5**

FT-IR Spectra

The FT-IR spectra of the compounds displayed two N–H stretching vibrational bands in 3418–3443 and 3160–3180 cm^{−1} regions, respectively. The high energy strong band (3418–3443 cm^{−1}) may be assigned to free N–H and the latter weak band (3160–3180 cm^{−1}) to the intramolecularly hydrogen bonded N1–H (N1–H⋯O=C).^{12–14} The bands appearing at ~1670, ~1440 and ~778 cm^{−1} region may be assigned to ν(C=O), ν(C–N) and ν(C=S) vibrations, respectively. The appearance of ν(C=O) at a lower wave number region in comparison to free ν(C=O) vibrations (1710 cm^{−1}) may be attributed to the formation of intramolecular hydrogen bond between carbonyl oxygen and –N1H.³⁶

NMR Spectra

The ^1H NMR spectra of the compounds exhibited two singlets at 8.58–8.65 ppm and 11.63 – 11.35 ppm corresponding to N1H and N2H protons, respectively. The presence of intramolecular hydrogen bonding interaction between N1H and carbonyl oxygen is responsible for its high chemical shift value.^{6, 37} In all the compounds the signals for aliphatic protons appeared between 1.01–4.19 ppm and those for aromatic protons between 7.52–8.21 ppm, respectively, in their usual regions. The ^{13}C NMR spectra of compounds exhibits signals due to all the carbon present in them. The resonances at 178.6–179.5 and 151.2–153.1 ppm correspond to thiocarbonyl and carbonyl carbons, respectively.^{12–14} Due to steric and electronic factors carbon in C=S is more exposed than C=O hence resonates downfield at ~179 ppm.³⁸

Crystal structure description

The important crystallographic data refinement parameters, selected bond lengths, bond angles and hydrogen bonds for the compounds **H₂L1** and **H₂L5** are gathered in **Tables 1, 2** and **S 1 (Supplemental Materials)**, respectively. The crystal structure, unit cell diagram and packing pattern of **H₂L1** and **H₂L5** are depicted in **Figures 1(a), 1(b), S1**, and **2(a), 2(b), S2**, respectively. The molecular structures of the compounds show them to be almost coplanar. The C=O and C=S bonds lengths are of typical double bond nature whereas all the C–N bonds indicate a partial double bond character.^{12–13} The shortening of C–N bonds confirm the presence of resonance in this part of molecule (**Table 2**).^{12–15} All the bond distances and angles for these compounds exhibit conventional nature and show no significant differences from those reported earlier.³⁹ The strong intramolecular hydrogen bond between N1–H·····O=C is responsible for trans orientation of the carbonyl and thiocarbonyl moieties across the C–N bond. An additional intermolecular hydrogen bond viz, N2–H·····S=C in **H₂L1** joins two molecules together to be a dimer. Beside strong intramolecular hydrogen bond an offset face-face π – π stacking interaction is also observed in **H₂L5** between naphthalene rings. Weak intermolecular interactions C–H·····O, C–S·····H, C–H·····N and C π –C π stabilize the crystal packing and lead to an infinite chain length along the a-axis (**Figure 2b** and **S 2**).⁴⁰

[Insert Figure 1]

[Insert Figure 2]

[Insert Table 1]

[Insert Table 2]

***In vitro* cytotoxicity screening**

The inhibition of cancer cell proliferation by compounds **H₂L1–H₂L5** has been examined by using the methods describe in experimental section. Naphthyl amine and three other aromatic amines having one or two electron withdrawing groups have been chosen purposely as to see their effect on cytotoxic property of the compounds since it has been observed that electron withdrawing groups attached to the aromatic ring of the compounds enhance their cytotoxicity considerably.^{17–19} The results in the form of IC₅₀ values (the concentration which produces 50% growth inhibition of cell lines) are shown in **Table S 1 (Supplemental Materials)**. The comparative cytotoxicity of the compounds is depicted in **Figure S 3**. The results indicated that the compounds **H₂L2–H₂L4** were more potent inhibitors than **H₂L1** (IC₅₀ ~100 μM) and **H₂L5** (IC₅₀ ~75 μM) against all the cell lines tested. The best result was obtained for 2008 cell line. The significant inhibitory activity for compounds **H₂L2–H₂L4** can be related to the presence of electronegative group as substituents on the aromatic ring attached to N1.¹⁹ The best result is obtained for **H₂L2** having two electronegative groups attached to the aromatic ring. These compounds exhibited more improved results than the compounds reported in our previous papers.^{12, 13} The most promising compound **H₂L2** is worthy of further investigation.

Experimental Procedure

Chemicals, Instruments and Methods

n-pentyl chloroformate and 2, 2, 2-trichloroethyl chloroformate, primary amine derivatives 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.¹H and ¹³C NMR spectra were obtained on a JEOL FT-NMR AL 300 and 500 MHz spectrometer in CDCl₃ and DMSO-d₆, 25°C with chemical shifts relative to SiMe₄. The splitting of proton resonances

in the reported ^1H NMR spectra were remarked as s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet triplet and m = multiplet; coupling constants are reported in Hz.

Crystal structure determination

Data collection was performed using CrysAlisPRO on an Oxford Diffraction Xcaliber Ruby Gemini CCD diffractometer using graphite- monochromatic CuK α ($k=1.54178\text{ \AA}$) at 123 K. The structures were solved by direct methods and refined by full-matrix least-square on F^2 using SHELXL-97.⁴¹ 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 refinement converged to a final $R1 = 0.0399$, $wR2 = 0.0996$ for **H₂L1** and $R1 = 0.0524$, $wR2 = 0.0987$ for **H₂L5**. Drawings were made using ORTEP-III⁴² and MERCURY.⁴³

Synthesis of compounds

All the compounds were prepared by in two stages as shown in **Scheme 1**.⁴⁰ An acetone solution of n-pentyl chloroformate (1.44 mmol, 30 mL) for **H₂L1–H₂L4** /2, 2, 2-trichloroethyl chloroformate (1.37 mmol, 30 mL) for **H₂L5** was mixed to the ammonium thiocyanate (10 mmol, 0.76 g) solution in acetone (30 mL) and the resulting mixture was refluxed for 45 min at 75°C. After cooling to room temperature, a solution of suitable aromatic primary amine (10 mmol) in acetone (15 mL) was added slowly with stirring and the mixture was refluxed for 90 minute to complete the reaction. The precipitated ammonium chloride was filtered off and the pale yellow solution was evaporated in vacuum to dryness to get a crude product. Slow evaporation of acetone solution of the ligands **H₂L1** and **H₂L5** at 20–25°C yielded bright yellow single crystals suitable for X-ray diffraction.

N-(naphthyl)-N'-(pentoxycarbonyl) thiocarbamide (**H₂L1**)

Yellow solid; m.p 100-101°C; (Naphthylamine, 1.432g, 10 mmol) Yield: 80%; FT–IR (KBr, cm^{-1}) 3418, 3162 $\nu(\text{N–H})$, 3050 $\nu(\text{aromatic C–H})$; 2955, $\nu(\text{aliphatic C–H})$; 1715, $\nu(\text{ester –COOR})$; 1598, $\nu(\text{C=O})$; 1537, $\nu(\text{aromatic, C=C})$; 1531, [Thioamide band I, $\nu(\text{C–N}) + \nu(\text{N–H})$]; 1379, [Thioamide band II, $\nu(\text{C–N}) + \nu(\text{C=S})$]. ^1H NMR(300 MHz, CDCl_3 , δ , ppm): 11.63 (s, 1H, –CSNH), 8.59 (s, 1H, –CONH), 7.97 (m, 1H, C_{10}H_7), 7.87 (m, 2H, C_{10}H_7), 7.69 (m, 1H, C_{10}H_7), 7.55 (m, 3H, C_{10}H_7), 4.20 (t, 2H, $J = 6.5\text{ Hz}$, – OCH_2), 2.49 (m, 2H, –(CH_2)–), 1.67 (m, 4H, 2x(– CH_2), 0.93 (t, 3H, – CH_3 , $J = 5.7\text{ Hz}$, – CH_3). ^{13}C NMR (75 MHz, CDCl_3 , δ , ppm): 179.5

(C=S), 153.1(C=O), 134.1, 133.4, 128.7, 127.8, 125.1, 121.6 (Ar-C), 67.1 (–OCH₂), 28.3–22.1 (–CH₂)₃–; 13.8 (–CH₃). Elemental analyses for C₁₇H₂₀N₂O₂S (316.31) (%) Calcd: C, 64.55; H, 6.37; N, 8.85. Found: C, 64.43; H, 6.30; N, 8.79.

N-(2-Chloro-4-nitrophenyl)-N'-(pentoxycarbonyl) thiocarbamide (H₂L2)

Yellow solid; m.p 85-86°C; (2-Chloro-4-nitroaniline, 1.731g, 10 mmol) Yield: 76%; FT-IR (KBr, cm⁻¹): 3494, 3176 ν(N-H), 3128 ν(aromatic C-H); 2958, ν(aliphatic C-H); 1733, ν(ester –COOR); 1588, ν(C=O); 1518, ν(aromatic, C=C); 1501, [Thioamide band I, ν(C-N) + ν(N-H)]; 1342, [Thioamide band II, ν(C-N) + ν(C=S)]. ¹H NMR (500 MHz, DMSO-d₆, δ, ppm): 10.74 (s, 1H, CSNH), 9.01 (s, 1H, CONH), 8.04 (d, 1H, J = 3.1 Hz, –C₆H₃), 7.88 (dd, 1H, J = 8.5, 3.1 Hz, –C₆H₃), 6.78 (d, 1H, J = 9.0 Hz, –C₆H₃), 4.12 (t, 2H, J = 7.0 Hz, –OCH₂), 2.46 (m, 2H, –(CH₂)–), 1.50 (m, 4H, 2x(–CH₂)), 0.79 (t, 3H, J = 4.1 Hz, –CH₃). ¹³C NMR (125 MHz, DMSO-d₆, δ, ppm): 181.7 (C=S), 153.7 (C=O), 136.5, 133.4, 126.7, 126.1, 125.1, 124.9 (Ar-C), 66.2 (–OCH₂), 28.1–22.1 (–CH₂)₃–; 14.2 (–CH₃). Elemental analyses for C₁₃H₁₆N₃O₄SCl (345.80) (%) Calcd: C, 45.15; H, 4.66; N, 12.15. Found: C, 44.73; H, 4.63; N, 12.11.

N-(2-methoxy-4-nitrophenyl)-N'-(pentoxycarbonyl) thiocarbamide (H₂L3)

Dark yellow solid m.p 90-91°C; (2-Methoxy-4-nitroaniline, 1.682g, 10 mmol) Yield: 88%; FT-IR (KBr, cm⁻¹): 3407, 3179 ν(N-H), 3031 ν(aromatic C-H); 2958, ν(aliphatic C-H); 1731, ν(ester –COOR); 1598, ν(C=O); 1537, ν(aromatic, C=C); 1417, [Thioamide band I, ν(C-N) + ν(N-H)]; 1367, [Thioamide band II, ν(C-N) + ν(C=S)]. ¹H NMR (500 MHz, DMSO-d₆, δ, ppm): 12.31 (s, 1H, –CSNH), 11.53 (s, 1H, –CONH), 9.03 (d, 1H, J = 9.5 Hz, –C₆H₃), 7.90 (dd, 1H, J = 9.5, 3.0 Hz, –C₆H₃), 7.83 (d, 1H, J = 2.5 Hz, –C₆H₃), 4.15 (s, 3H, –OCH₃), 4.13 (t, 2H, J = 6.5 Hz, –OCH₂), 2.49 (m, 2H, –(CH₂)–), 1.60 (m, 4H, 2x(–CH₂)), 0.85 (t, 3H, J = 7.0 Hz, –CH₃). ¹³C NMR (125 MHz, DMSO-d₆, δ, ppm): 178.2 (C=S), 150.6 (C=O), 134.1, 133.4, 128.7, 127.8, 125.1, 122.6 (Ar-C), 57.2 (OCH₃), 66.8 (–OCH₂), 28.0–22.1 (–CH₂)₃–; 14.26 (–CH₃). Elemental analyses for C₁₄H₁₉N₃O₅S (341.38) (%) Calcd: C, 49.26; H, 5.61; N, 12.31. Found: C, 49.23; H, 5.58; N, 12.29.

N-(3-nitrophenyl)-N'-(pentoxycarbonyl) thiocarbamide (H₂L4)

Dark yellow solid; m.p 78-79°C; (3-nitroaniline, 1.381g, 10 mmol) Yield: 78%; FT-IR (KBr, cm⁻¹): 3430, 3326 ν(N-H), 3098 ν(aromatic C-H); 2923, ν(aliphatic C-H); 1736, ν(ester –COOR); 1627, ν(C=O); 1580, ν(aromatic, C=C); 1526, [Thioamide band I, ν(C-N) + ν(N-H)];

1338, [Thioamide band II, $\nu(\text{C-N}) + \nu(\text{C=S})$]. ^1H NMR (500 MHz, DMSO-d_6 , δ , ppm): 11.46 (s, 1H, $-\text{CSNH}$), 8.34 (s, 1H, $-\text{CONH}$), 7.58 (d, 1H, $J = 6.1$ Hz, $-\text{C}_6\text{H}_4$), 7.57 (dd, 1H, $J = 6.1$, 2.5 Hz, $-\text{C}_6\text{H}_4$), 7.49 (dt, 1H, $J = 6.1$, 2.5, 2.0 Hz, $-\text{C}_6\text{H}_4$), 6.95 (d, 1H, $J = 2.5$ Hz, $-\text{C}_6\text{H}_4$), 4.19 (t, 2H, $J = 7.0$ Hz, $-\text{OCH}_2$), 2.17 (m, 2H, $-(\text{CH}_2)-$), 1.66 (m, 2H, $2\times(-\text{CH}_2)$), 0.92 (t, 3H, $J = 5.0$ Hz, $-\text{CH}_3$). ^{13}C NMR (125 MHz, DMSO-d_6 , δ , ppm): 181.7 (C=S), 166.9 (C=O), 149.3, 147.4, 142.8, 136.4, 129.9, 120.6 (Ar-C), 67.2 ($-\text{OCH}_2$), 28.6–22.2 ($-(\text{CH}_2)_3-$), 13.9 ($-\text{CH}_3$). Elemental analyses for $\text{C}_{13}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$ (311.35) (%) Calcd: C, 50.15; H, 5.50; N, 13.50. Found: C, 50.11; H, 5.47; N, 13.47.

N-(naphthyl)-N'-(2, 2, 2-trichloroethoxycarbonyl) thiocarbamide ($\text{H}_2\text{L5}$)

Yellow solid; m.p 80–81°C; (Naphthylamine, 1.432g, 10 mmol) Yield: 85%; FT-IR (KBr, cm^{-1}): 3443, 3235, $\nu(\text{N-H})$, 3010 $\nu(\text{aromatic C-H})$; 2961, $\nu(\text{aliphatic C-H})$; 1732, $\nu(\text{ester } -\text{COOR})$; 1505, $\nu(\text{C=O})$; 1597, $\nu(\text{aromatic, C=C})$; 1519, [Thioamide band I, $\nu(\text{C-N}) + \nu(\text{N-H})$]; 1383, [Thioamide band II, $\nu(\text{C-N}) + \nu(\text{C=S})$]. ^1H NMR (300 MHz, CDCl_3 , δ , ppm): 11.34 (s, 1H, $-\text{CSNH}$), 8.61 (s, 1H, $-\text{CONH}$), 7.92 (m, 1H, C_{10}H_7), 7.90 (m, 2H, C_{10}H_7), 7.86 (m, 1H, C_{10}H_7), 7.56 (m, 3H, C_{10}H_7), 4.88 (s, 2H, $-\text{OCH}_2$). ^{13}C NMR (75 MHz, CDCl_3 , δ , ppm): 178.6 (C=S), 151.2 (C=O), 134.1, 133.4, 128.7, 127.8, 125.1, 121.6 (Ar-C), 77.4 ($-\text{OCH}_2$), 75.1 ($-\text{CCl}_3$). Elemental analyses for $\text{C}_{14}\text{H}_{11}\text{N}_2\text{O}_2\text{SCl}_3$ (377.57) (%) Calcd: C, 44.54; H, 2.94; N, 7.42. Found: C, 44.42; H, 2.90; N, 7.40.

Biological assays

Cell lines

Two human cervical cancer cell lines (2008 and C13*) and three ovarian cancer cell lines (IGROV-1, A2780 and A2780/CP) were used. Among these, C13* and A2780/CP are cisplatin (ccDDP)-resistant cells.^{44, 45} Cells were grown as monolayers in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum and 50 $\mu\text{g/ml}$ 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 °C. All studies were performed in Mycoplasma negative cells, as routinely determined with the MycoAlert Mycoplasma detection kit (Lonza, Walkersville, MD, USA).

Cytotoxicity screening

In vitro cytotoxicity of compounds used in the present study was determined by MTT assay.⁴⁶ 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 μ l containing 0.5 mg mL⁻¹ MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma) and then incubated at 38°C for 4 h. The medium was removed and 100 μ L 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.

Conclusion

In our present work, five N, N'-disubstituted thiocarbamide derivatives were prepared using the mentioned method in this paper and all of them were characterized by various spectroscopic and single crystal X-ray diffraction techniques. The crystal structure of **H₂L1** and **H₂L5** revealed the planar confirmation of thiocarbamide unit which may be due to the formation of intramolecular hydrogen bond N1–H·····O=C. An intermolecular hydrogen bond N2–H·····S=C links two thiocarbamide molecules as a dimer in case of **H₂L1**. An offset face-to-face π – π stacking is present in **H₂L5**. *In vitro* cytotoxicity study of the synthesized compounds against five human cancer cell lines indicated significant inhibitory activity for compounds **H₂L2–H₂L4** in which **H₂L2** is most promising.

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Supplementary Information

X-ray crystallographic hydrogen bonding Table S 1 of compounds (**H₂L1**) and (**H₂L5**), Cytotoxicity Table S 2 of compounds (**H₂L1–H₂L5**) and Figures (S 1, S 2 and S 3) have been provided in Supporting Information.

CCDC 999080 and 999081 contain the supplementary crystallographic data for compounds (**H₂L1**) and (**H₂L5**). These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing deposit@ccdc.cam.ac.uk or by contacting the Cambridge Crystallographic Data.

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Table 1: Summary of crystal data and structure refinement of **H₂L1** and **H₂L5**

Compounds	H ₂ L1	H ₂ L5
Empirical Formula	C ₁₇ H ₂₀ N ₂ O ₂ S	C ₁₄ H ₁₁ Cl ₃ N ₂ O ₂ S
Formula Weight	316.41	377.66
Crystal System / Space Group	Triclinic / P-1	Triclinic / P-1
a / Å	7.139(5)	8.115(5)
b / Å	10.751(5)	8.899(5)
c / Å	12.442(5)	12.238(5)
α / °	109.738(5)	75.345(5)
β / °	99.872(5)	87.734(5)
γ / °	106.335(5)	64.477(5)
V / Å ³	824.2(8)	769.1(7)
Z	2	2
D _{calc} (g/cm ³)	1.275	1.631
μ (mm ⁻¹)	1.812	0.738
Crystal size (mm)		0.5481 x 0.2877 x 0.1066
Color / Shape	Bright yellow single crystal	Bright yellow single crystal
Temp (K)	120(2)	120(2)
Theta range for collection	3.950 to 76.019°	2.790 to 41.226°
Reflections collected	5337	20621
Independent reflections	3279 [R(int) = 0.0200]	9968 [R(int) = 0.0257]
Data/restraints/parameters	3279 / 7 / 231	9968 / 0 / 199
Goodness of fit on F ²	1.067	1.040
Final R indices [I > 2σ(I)]	R1 = 0.0375, wR2 = 0.0984	R1 = 0.0376, wR2 = 0.0884
R indices (all data)	R1 = 0.0399, wR2 = 0.0996	R1 = 0.0524, wR2 = 0.0987
Largest difference peak/hole	0.497 and -0.244 e. Å ⁻³	1.283 and -0.592 e. Å ⁻³

Table 2: Selected bond lengths (Å), and bond angles (°) for **H₂L1** and **H₂L5**

Compound H₂L1		Compound H₂L5	
Bond lengths (Å)		Bond lengths (Å)	
C(11)–S(1)	1.674(16)	C(1)–N(1)	1.429(12)
C(11)–N(1)	1.331(18)	N(1)–C(11)	1.335(11)
C(1)–N(1)	1.431(18)	C(11)–S(1)	1.667(12)
O(1)–C(12)	1.327(18)	N(2)–C(12)	1.364(13)
O(2)–C(13)	1.455(16)	O(1)–C(12)	1.213(11)
N(2)–C(11)	1.381(18)	O(2)–C(12)	1.347(11)
N(2)–C(12)	1.378(18)	O(2)–C(13)	1.424(12)
C(16)–C(17)	1.491(4)	C(13)–C(14)	1.769(11)
C(15)–C(16)	1.520(3)	C(14)–Cl(1)	1.764(11)
C(15)–C(16)	1.520(3)	C(14)–Cl(2)	1.771(11)
C(13)–C(14)	1.502(6)	C(14)–Cl(3)	1.769(11)
Bond angles (°)		Bond angles (°)	
C(12)–O(2)–C(13)	116.50(3)	C(11)–N(1)–C(1)	124.25(8)
C(11)–N(1)–C(1)	123.60(10)	C(12)–O(2)–C(13)	117.13(7)
C(12)–N(2)–C(11)	127.52(12)	C(12)–N(2)–C(11)	127.38(7)
C(2)–C(1)–C(10)	121.79(12)	C(2)–C(1)–N(1)	120.13(8)
C(2)–C(1)–N(1)	119.97(13)	C(10)–C(1)–N(1)	118.14(9)
C(10)–C(1)–N(1)	118.24(12)	N(1)–C(11)–N(2)	116.10(8)
N(1)–C(11)–N(2)	116.90(12)	N(1)–C(11)–S(1)	125.55(7)
N(1)–C(11)–S(1)	124.48(11)	N(2)–C(11)–S(1)	118.35(6)
N(2)–C(11)–S(1)	118.61(10)	O(1)–C(12)–O(2)	124.84(8)
O(1)–C(12)–O(2)	125.94(12)	O(1)–C(12)–N(2)	126.88(8)
O(1)–C(12)–N(2)	125.60(13)	O(2)–C(12)–N(2)	108.26(7)
O(2)–C(12)–N(2)	108.46(11)	O(2)–C(13)–C(14)	110.00(8)
O(2)–C(13)–C(14)	107.40(8)	C(13)–C(14)–Cl(1)	111.32(6)
C(13)–C(14)–C(15)	115.70(5)	C(13)–C(14)–Cl(2)	106.93(6)
C(14)–C(15)–C(16)	111.66(19)	C(13)–C(14)–Cl(3)	110.27(7)

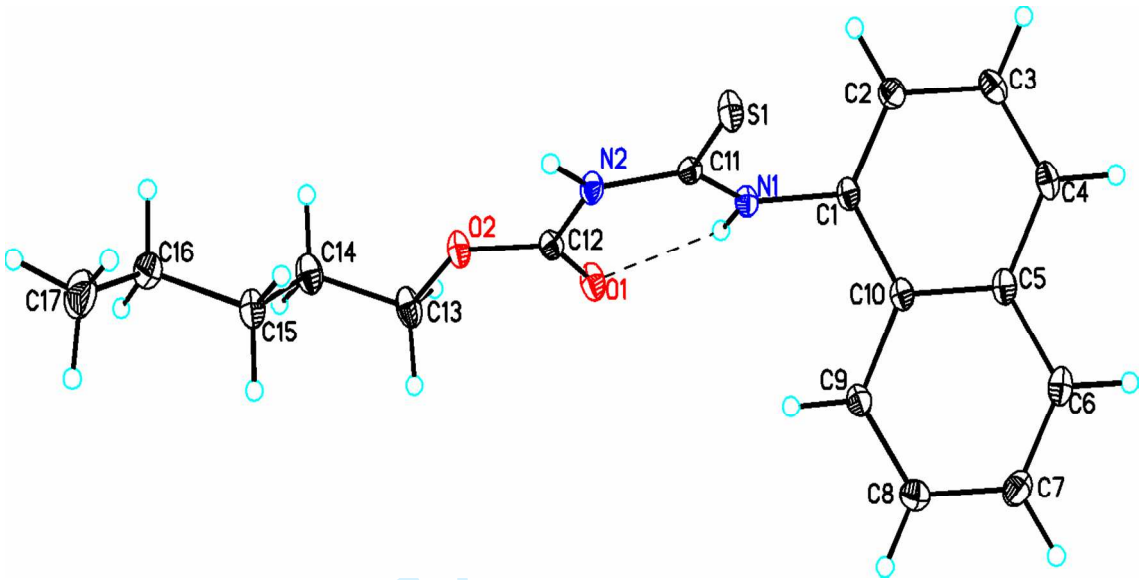


Figure 1(a): Molecular structure of **H₂L1**. The intramolecular hydrogen bonds as shown by dashed lines.

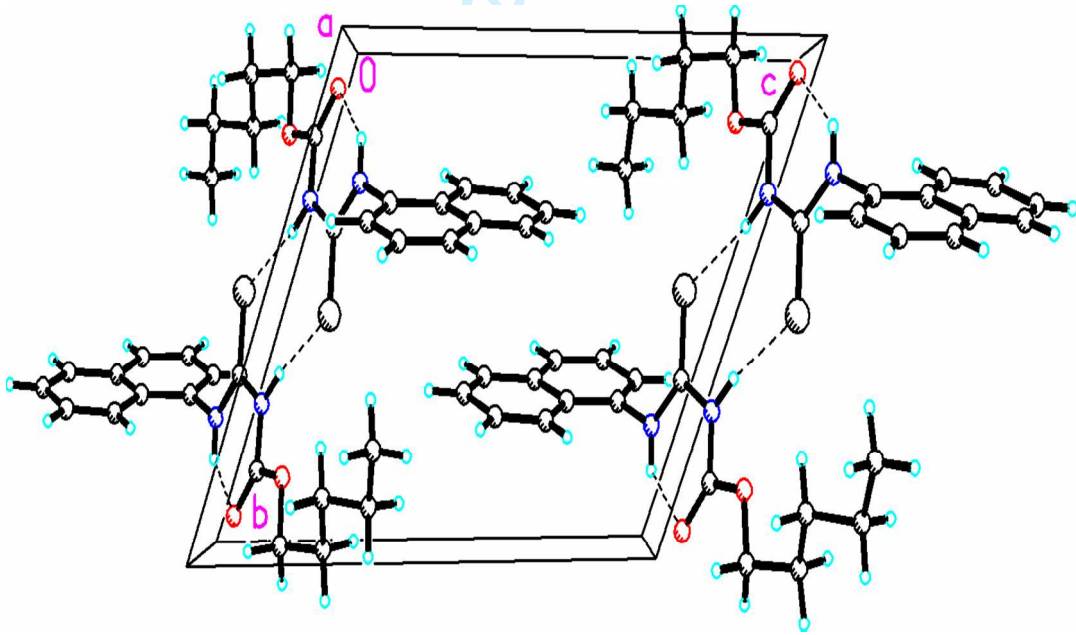


Figure 1(b): Unit cell diagram of **H₂L1**.

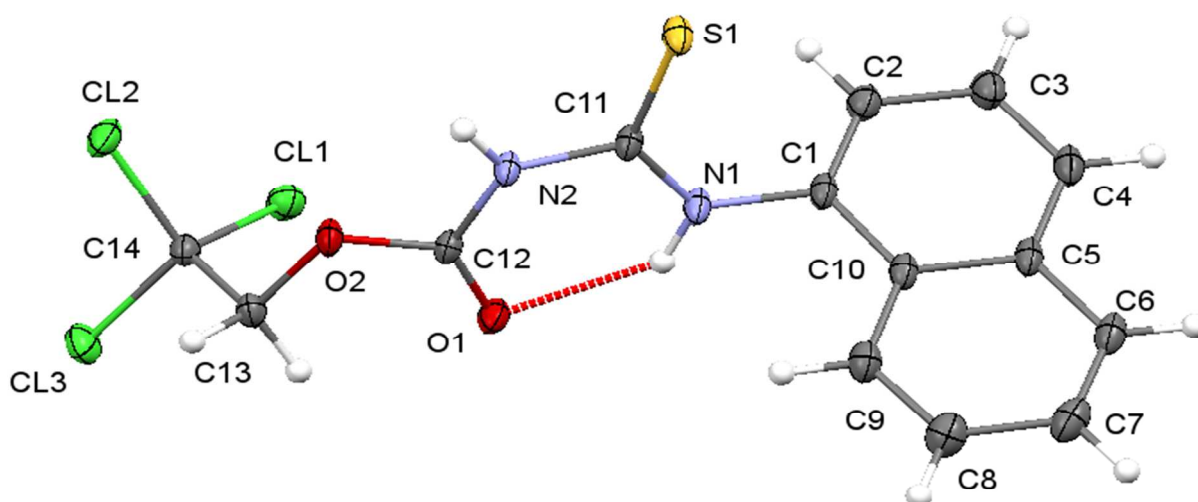


Figure 2(a): Molecular structure of $\text{H}_2\text{L5}$. The intramolecular hydrogen bonds as shown by dashed lines.

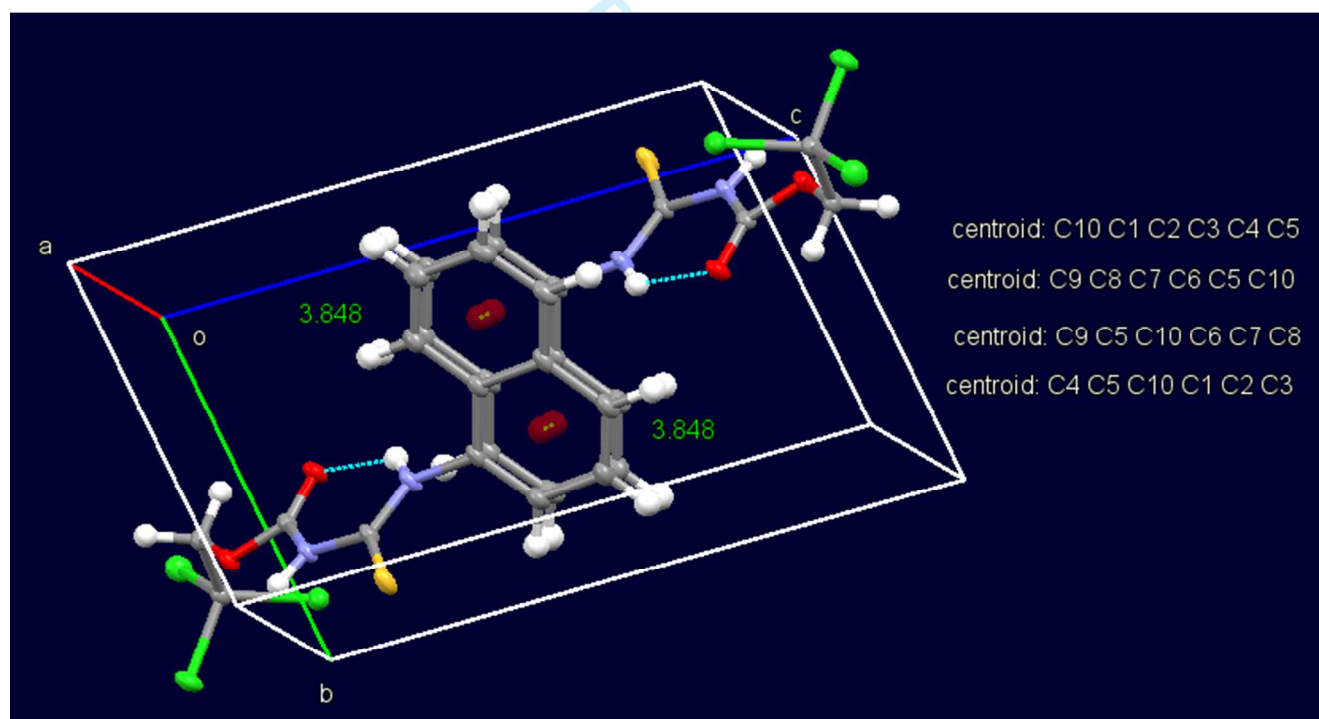


Figure 2(b): Unit cell diagram viewed down the b axis for $\text{H}_2\text{L5}$. Intermolecular face-to-face π - π stacking interaction is also observed in the compound.

Synthesis, molecular structure exploration and in vitro cytotoxicity screening of five novel N, N'- disubstituted thiocarbamide derivatives

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Supplemental Materials

Table S 1: Important hydrogen bonding interactions of **H₂L1** and **H₂L5** [Å and °].

D–H···A	D–H	H···A	D···A	< (DHA)
H₂L1				
N(1)–H(1N)···O(1)	0.83(2)	2.05(19)	2.701(2)	134.6(17)
N(1)–H(1N)···O(1)#1	0.83(2)	2.48(2)	3.160(2)	139.9(16)
N(2)–H(2N)···S(1)#2	0.89(2)	2.45(2)	3.327(16)	166.8(16)
H₂L5				
N(1)–H(1A)···O(1)	0.88	2.00	2.6995(13)	135.7
N(2)–H(2B)···S(1)#1	0.88	2.53	3.3811(14)	162.5

Symmetry transformations used to generate equivalent atoms:

H₂L1 .#1 -x+2, -y+1, -z+1 #2 -x+2, -y+2, -z+1 **H₂L5**. #1 -x+1, -y, -z+2

Table S 2: IC₅₀ Values (μM) for the compounds **H₂L1** – **H₂L5** against two human cervical Cell lines (**2008** and **C13***) and three human ovarian carcinoma cell lines (**A2780**, **A2780/CP** and **IGROV-1**).

Compounds	2008 cells	C13*cells	A2780 cells	A2780/CP cells	IGROV-1 cells
H₂L1	73.4±5	91.7±5	102.8±5	101.5±11	105.6±9
H₂L2	21.0±5	26.3±6	28.9±5	25.6±5	23.0±2
H₂L3	31.2±5	37.2±7	32.5±7	34.4±5	29.1±3
H₂L4	31.8±7	38.4±6	36.2±5	35.2±3	38.0±6
H₂L5	67.5±1	97.7±2	73.9±1	78.9±4	72.2±1
5-FU	4.1±0.3	8.5±0.5	5.5±0.3	12.8±0.5	5.1±0.2

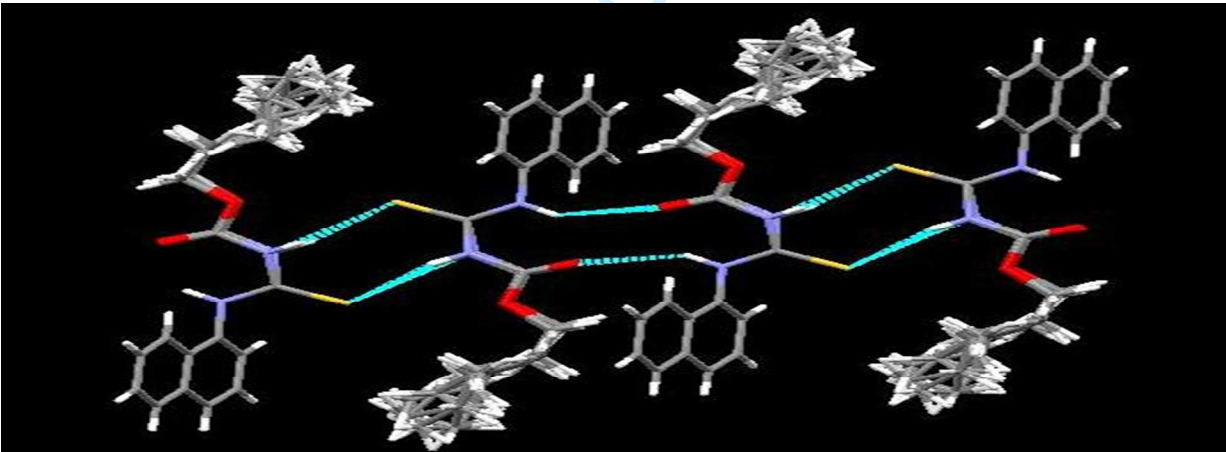


Figure S 1: Four molecules linked with H-bonds indicated by dashed lines. Weak intermolecular interactions (C–O···H–N and C–S···H–N) lead to an infinite chain length along the a-axis.

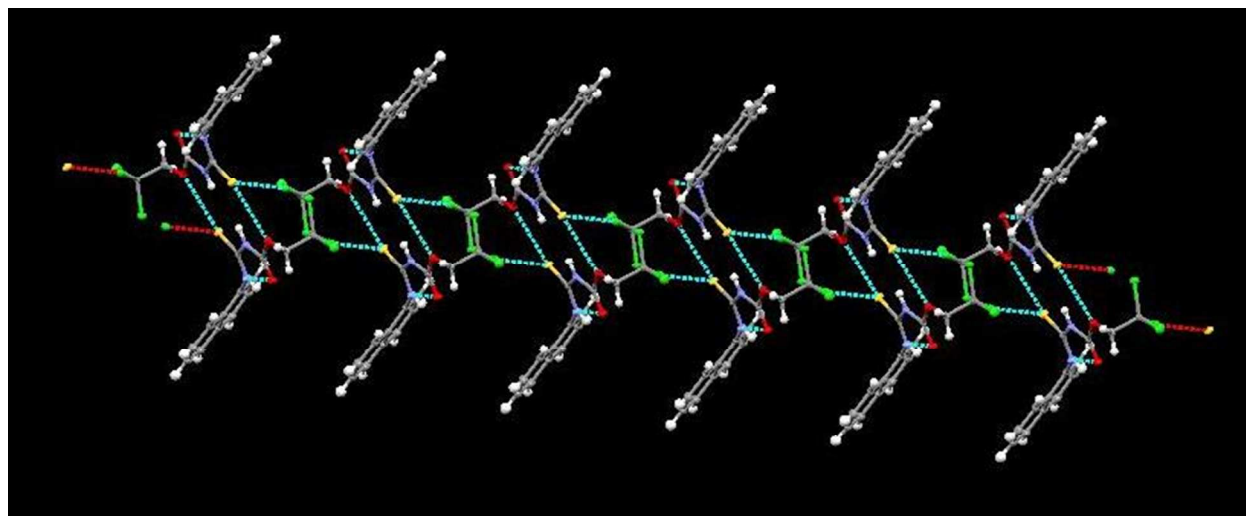


Figure S 2: The packing diagram of **H₂L5**. Weak intermolecular interactions (C–O····H, C–S····H, and C–Cl····H) lead to an infinite chain length along the a-axis.

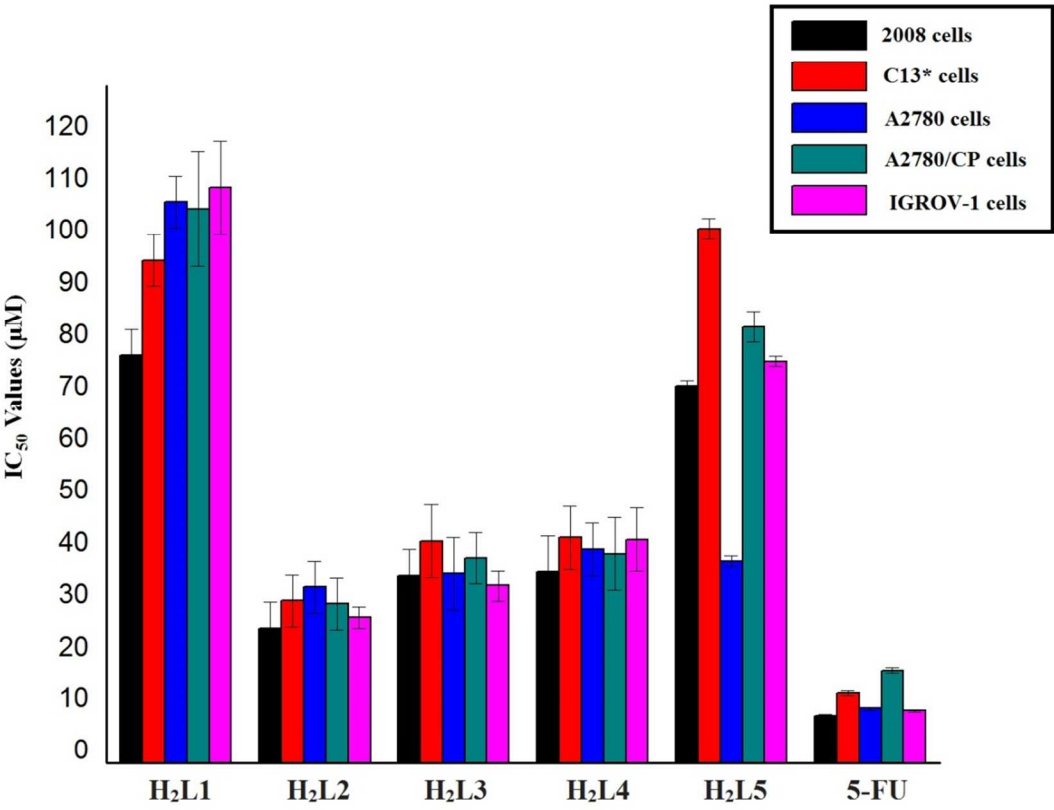


Figure S 3: Comparative cytotoxicity of compounds H₂L1 – H₂L5