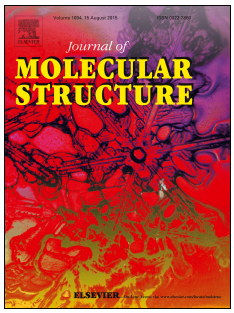

Synthesis, characterisation, Hirshfeld surface and *in vitro* cytotoxicity evaluation of new N-aryl-N′-Alkoxycarbonyl thiocarbamide derivatives

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Graphical Abstract

Synthesis, characterisation, Hirshfeld surface and *in vitro* cytotoxicity evaluation of new N-Aryl-N'-Alkoxycarbonyl thiocarbamide derivatives

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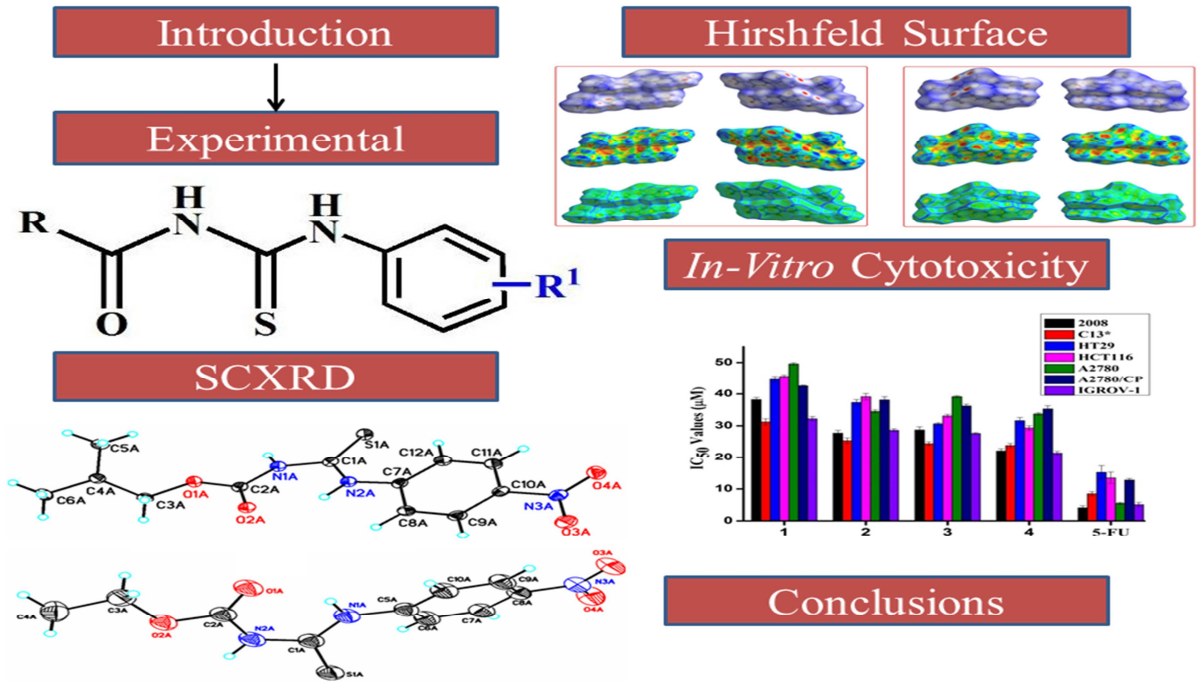

A series of four N, N'–disubstituted thiocarbamide derivatives **(1−4)** were synthesized for their in-vitro anticancer activity evaluation. Significant activity was found in compounds **2** and **4**. All compounds were characterized well through different spectroscopic techniques; however, structural evaluation of compounds **1** and **3** was done through single crystal X-ray diffraction data. Further quantitative analysis of noncovalent interactions for compounds **1** and **3** was done

by Hirshfeld surfaces analysis which showed that O···H and S···H interactions are playing a major role in stabilizing the crystal lattice.

Synthesis, characterisation, Hirshfeld surface and *in vitro* cytotoxicity evaluation of new N-Aryl-N'-Alkoxycarbonyl thiocarbamide derivatives

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Abstract

Four new compounds N-(4-nitrophenyl)-N'-(isobutoxycarbonyl) thiocarbamide (**1**), N-(2, 4-nitrophenyl)-N'-(isobutoxycarbonyl) thiocarbamide (**2**), N-(4-nitrophenyl)-N'-(ethoxycarbonyl) thiocarbamide (**3**) and N-(2-Chloro- 4-nitrophenyl)-N'-(ethoxycarbonyl) thiocarbamide (**4**) were prepared and their structures confirmed by using various spectroscopic (FT-IR, UV-Visible, ^{1}H and ^{13}C NMR) and single crystal X-ray studies of **1** and **3.** The presence of intramolecular (N−H···O═C) hydrogen bond in the crystal structure of both the compounds causes planarity of carbonyl thiocarbamide unit and trans orientation of C=O and C=S group. The intermolecular contacts (C−H···S, C−H···O and N−H···S) present in crystal structures have been examined by Hirshfeld surface analysis and their associated 2D fingerprint plots. All the compounds were assessed for their *in vitro* cytotoxic properties against a panel of seven human cancer cells such as cervical carcinoma (2008, C13*), colorectal (HT29 and HCT116) and ovarian carcinoma (A2780, A2780/CP and IGROV-1). Among them, compounds **2** and **4** exhibited better activity than **1** and **3** against all the cell lines tested.

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Keywords: Thiocarbamide; X-ray crystal structure; *In vitro* Cytotoxicity; Hirshfeld surface analysis.

1. Introduction

Since last few decades, cancer has been considered biggest life threatening health problem in the world. It is a chronic disease characterised by uncontrolled cell proliferation leading to tumor formation. In order to get good anticancer drug with less side-effects and toxicity a large number of compounds have been synthesized and tested over the year. N, N'-disubstituted thiocarbamide derivatives are one such class of compounds exhibiting promising anticancer properties against a variety of human cancer cell lines [1–5]. The alkoxy thiocarbamide derivatives are considered one of the popular members in the family which are gaining much attention due to their easy availability. The remarkable chelating ability of these compounds based on the presence of soft S and hard N donor atoms in their structural motif facilitate their use as analytical reagents in liquid–liquid extraction of precious metals (Pt, Pd, Au, Ag) and in extraction of toxic metals using a solid supported liquid membrane system [6−8]. The technological applications of these compounds include their use as effective corrosion inhibitors [9], non-linear optical materials in electronic industries [10], collectors in froth flotation processes [11, 12], precursors for the synthesis of heterocyclic and organosulfur compounds [1−3], catalyst in oxidation of alcohols [13, 14] and in the palladium-catalyzed Suzuki and Heck reactions [15−17] and as chemosensors for selective and sensitive naked-eye recognition for anions [18−20]. The presence of intramolecular hydrogen bond in the structural scaffold of thiocarbamides has a profound effect on its conformation and complexation behaviour [13, 14, 21, 22]. In addition, presence of different types of intermolecular interactions (C−H···S, C−H···O and N−H···S) contributes to the crystal packing. Hirshfeld surface (HS) analysis can be used to get information about these interactions [23−26].

Therapeutic importance of these compounds includes their use as herbicidal, fungicidal, bactericidal, insecticidal, antithyrodal and plant growth regulatory agents [27−31]. They have been patented as anti-diabetic, anti-arthritic, and anti-coagulant agents for the treatment of cognitive problem and prostate disorder [32]. Recently substituted thiocarbamide derivatives have been proved to be most promising anticancer agents because of their good inhibitory activity against a variety of human cancers [4, 33, 34]. It has also been reported that structural changes in thiocarbamide moiety greatly affects its anticancer activity [33−35]. In surge to get better anticancer compounds, our group has synthesized and studied the anticancer properties of various N, N'-disubstituted thiocarbamides [36−42].

In continuation of our previous work, the present paper describes the "Synthesis, characterisation, Hirshfeld surface and *in vitro* cytotoxicity evaluation of four new N-Aryl-N'-Alkoxycarbonyl thiocarbamide derivatives".

2. Experimental Procedure

2.1. Chemicals, Instruments and Methods

Analytical grade isobutoxy chloroformate, ethoxy chloroformate and other chemicals used in synthesis of compounds were purchased from Merck (Germany). The acetone was dried and freshly distilled prior to use. Melting points were measured on an 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.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.

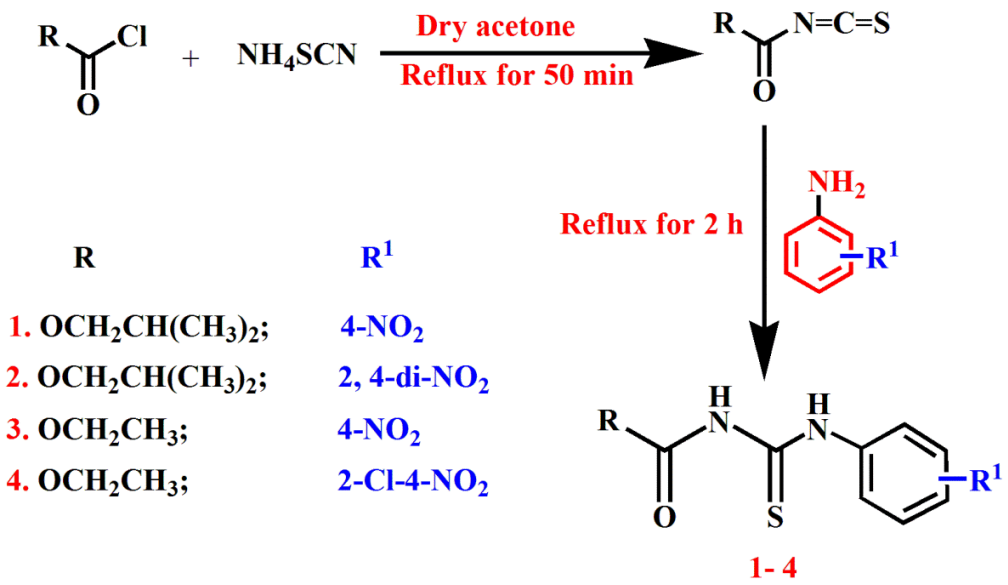
2.2. X-ray data collection and structure refinement

Data collection was performed using CrysAlisPro on an Oxford Diffraction Xcalibur diffractometer with a Ruby Gemini CCD detector with graphite - monochromatic MoKα (λ = 0.71073 Å) radiation for compound **1** at 173(2) K and CuKa (λ = 1.54178 Å) radiation for compound **3** at 293(2) K. The structure was solved by direct methods in the space group and refined by full-matrix least-square on F^2 using SHELXL-97 [43]. The non-hydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms were fixed geometrically and were allowed refined using a riding model.

2.3. Synthesis of compounds (1−4)

Procedure described in previous literatures was followed for the synthesis compounds after a slight alteration [3–5]. Isobutoxy chloroformate (1.31 mmol, 30 mL)/ Ethoxy chloroformate (1.04 mmol, 30 mL) dissolved in dry acetone was mixed to the ammonium thiocyanate solution (0.78 mmol, 30 mL) in ten minutes duration .The reaction mixture was refluxed for 50 min at 75 $^{\circ}C$. The resultant isobutoxy/ethoxy 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 30 °C. The by-product ammonium chloride was filtered off and the pale yellow solutions were evaporated in vacuum to get the desired products. The well-shaped crystals of compounds **1** and **3** were grown by the slow evaporation techniques using acetone as a solvent.



Scheme1: Synthetic route to new isobutoxy/ethoxy carbonyl thiocarbamides (**1−4).**

2. 4. Biological studies

2.4.1. 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 [44, 45]. 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).

2. 4. 2. *In vitro* cytotoxic screening

In vitro cytotoxicity of compounds was determined by MTT assay [46, 47]. 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.

3. Results and discussion

3.1. Synthesis and Characterization

The route adapted for the synthesis of the compounds **1−4** is outline in **Scheme 1**. The treatment of isobutoxycarbonyl isothiocyanate/ethoxycarbonyl isothiocyanate with appropriate substituted aromatic primary amines under reflux conditions afforded light yellow; air stable compounds **1−4** in good yield (79−85%).

3. 2. IR spectra

FT-IR spectral data of all compounds have been presented in SI (sections **2.3.1−2.3.4).** The compounds display two –NH stretching vibrations at ~3440 cm^{-1}(medium) and ~3160 cm^{-1}(broad) assignable to free ν(NH) and hydrogen bonded ν (NH), respectively**.** The formation of the intramolecular N–H···O═C hydrogen bond is responsible for the impressive red-shift and strong intensification of the low frequency NH band [39−42]. This interaction also affects the C═O stretching mode which appears at a lower wave number (~1665 cm^{-1}) than that of the ordinary carbonyl absorption (~1730 cm^{-1}) [41,42]. The medium-intensity absorption observed at ~778 cm^{-1} is assigned to the ν (C═S) stretching mode. All the assignments are in good agreement with previously studied N, N'-disubstituted thiocarbamide derivatives [36−42]. The asymmetric and symmetric $-NO_2$ stretching vibrations of compounds were observed as medium intensity bands at ~1566 cm^{-1} and ~1325 cm^{-1}, respectively [41]. In compound **4**, C−Cl stretching frequencies are observed as coupled modes at ~720 cm^{-1} and ~ 458 cm^{-1} corresponding to antisymmetric and symmetric vibrations, respectively [41,42].

3. 3. NMR spectra

^{1}H and ^{13}C spectral data of all compounds have been presented in SI (sections **2.3.1−2.3.4).** In the ^{1}H NMR spectra of the compounds, the protons of the two −NH groups are obtained as single peak at 12.22−11.76 ppm and 9.10−8.30 ppm, respectively. The presence of intramolecular hydrogen bonding interaction between CSNH and carbonyl oxygen (N–H···O═C) is responsible for its high chemical shift value [40−42]. All the aliphatic protons and aromatic protons of the compounds resonate between 1.01−4.19 ppm and 7.52−8.21 ppm, respectively, in their usual regions. In the ^{13}C NMR spectra of compounds, the chemical shifts of carbons pertaining to CONH and CSNH moieties resonate at 154.4−148.2 ppm and 178.1−176.0 ppm, respectively. Signals for aromatic carbons appeared in the region between 140−120 ppm [36−42].

3. 4. UV–Vis Studies

Electronic spectra of compounds **1–4** recorded in dichloromethane over the scan range of 200-800 nm show identical trends (Figure **S1**). All compounds present in their absorption spectra a weak band at around ~260 nm and a strong band at around ~295 nm which can be ascribed to intraligand charge transfer $\pi \rightarrow \pi^*$ transitions [41, 42, 48].

3. 5. Single crystal structure analyses

For compounds **1** and **3** the crystallographic data and refinement information, hydrogen bonds are gathered in **Tables 1** and **2** whereas selected bond lengths, bond angles, torsion angles are presented in **Tables S1** and **S2**, respectively. The crystal structure, unit cell diagram and packing pattern of both the compounds are depicted in Figures **1(a, b), S2 (a, b)** and **2(a, b),** respectively. In both the compounds the asymmetric unit contains two crystallographically independent but chemically equal molecules A and B. All the bond lengths and bond angles are almost equal for the molecules A and B in both the cases. There are only minor differences in the torsion angles of the thiocarbamide moiety with the aromatic ring (**Table S2**). C=O and C=S bond lengths correspond to a double bond whereas all the C−N bonds of thiocarbamide core are the shorter than the typical single C−N bond length due to delocalization in compounds [49−52]. The hydrogens at N1 and N2 are anti and syn, respectively, with respect to the C=S bond. Presence of intramolecular hydrogen bonds C═O···H−NC═S and C−H···S forming a planar six membered ring in each case stabilize the crystal structure of the compounds [51−53]. In both the crystals, the molecules were connected by N−H···O, N−H···S and C−H···O intermolecular hydrogen bonds with the chain running along b-axis in **1** and **3,** respectively, (Figure **2**) [51−53].

3. 6. Hirshfeld surface analysis

Hirshfeld surface is the region of a crystal space around the molecule that is defined by a weight function derived from the atomic electron densities. Therefore, it provides a new way of exploring packing modes as well as quantitative analysis of the intermolecular interactions in molecular crystals [54]. *Crystal Explorer* software is a tool used for the visualizing and exploring the intermolecular contacts (i.e. hydrogen bonding, vander Waals contacts, C-H···π, and π···π stacking) by enabling their 2D fingerprints. These 2D fingerprint plots provide a summary of the frequency of each combination of d_e and d_i across the surface of the molecule and so indicate not only which interactions are present, but also the relative area of the surface corresponding to each interaction [54, 55].

The 2D fingerprint plots, bar graph for percentage contributions of various interactions and Hirshfeld surfaces (d_{norm}, *Shapeindex* and *Curvedness*) are shown in Figures **3** and **4** (decompose fingerprint plots are shown in **Table S3**). The asymmetric unit of both the compounds possessed two independent molecules (i.e. Z'= 2), therefore, Hirshfeld surfaces were generated over both molecules (i.e. **1** and **3**) as well as over individual molecules (i.e. **1A**, **1B** and **3A**, **3B**). If we compare the percentage contribution of polar interactions (N···H, O···H and S···H) in unified form of both the molecules in the asymmetric unit, compound **1** showed 23.3 % for O···H, 13.9 % for S···H and 3.4 % for N···H while in compound 3 34.4 % for O···H, 10.9 % for S···H and 1.4 % for N···H. Similar order of percentage contribution in individual molecules were also observed (see the bar graph in Figure **3b**). The presence of sharp spikes for O···H and S···Hnteractions in 2D fingerprints revealed that these interactions are stronger than others, however no such spike was observed for N···H interaction in any of the mdecules. The D···A distance (i.e.d_i + d_e) for O···H interactions contributed by C–H···O interactions were ~2.45 Å for compound**1**, however, it was ~2.30 Å for compound **3**. This showed that C–H···O interactions are stronger in compound **3** than **1**. The D···A distances (i.e.d_i + d_e) for S···H interactions contributed by N–H···S and C–H···S, both in compound**1** were ~2.40 Å, however, it was ~2.30 Å for compound **3** and it was contributed by only N–H···S interactions. The percentage contribution due to aromatic interactions (i.e. C–H··π and π··π) were insignificant. Although H···H interactions were 10.9 % and 13.9 % in compounds **1** and **3**, respectively. The absence of side wings in the fingerprints indicated that there was no contribution of C–H··π interactions in C···H contacts.

However, the presence of bright bins in the fingerprint of compound **3** indicated the contribution of π···π interactions in C···C contacts [23−26].

In compound **1** d_{norm} showed six short contacts (i.e. red colour) over each molecule whereas **3** showed five short contacts (Figure **3a**). These prominent short contacts were belonging to the S···H and O···H interactions. But we cannot identify that underlying species is donor or acceptor. The nature of contacts (i.e. either donor or acceptor) on the Hirshfeld surfaces could easily be identified by mapping on a shape index. The area over the acceptor was red and donor was blue over shape index. Similarly intermolecular stacking can easily be identified by curvedness. The curvedness plots showed that flat surface patches were more prominent in molecule **1** than **3** (Figure **4a, b**). It indicates that diversion of intermolecular planes in crystal lattice of compound **1** was modest however in **3** it was significant [25, 26].

3. 7. In vitro cytotoxicity

The anticancer activity of the synthesized compounds were assayed against a panel of seven human cancer cell lines, including cervical (2008 and C13*), colorectal (HT29 and HCT116) and ovarian carcinoma (IGROV-1, A2780 and A2780/CP) and the results expressed as IC_{50} are summarized in **Table 3.** 5-Flourouracil was used as control [56−58]. The comparative cytotoxicity of the compounds is depicted in Figure **5**. In general, the compounds exhibited good inhibitory properties for all the cell lines tested. It can be inferred from **Table 3** that all the compounds showed comparatively better activity against cervical (2008 and C13*) and ovarian carcinoma (IGROV-1) cell lines in accordance with our previous results [36−42]. In comparison to **1** and **3**, Compound **2** and **4** exhibited strong inhibitory activity against all the cell lines which might be due to the presence of two nitro and/or chloro electron withdrawing group at ortho and para positions [59, 60]. 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 [4, 5]. In general, there is not much change in the inhibitory activity of these compounds in comparison to our previously reported ones [36−42]. Based on the above results, potential anticancer candidates can be considered for structural modification and pharmacological evaluation.

4. Conclusions

In the present work, four novel N, N'-disubstituted thiocarbamides have been synthesized and characterized using various spectroscopic and single crystal X-ray studies. From our experimental

results, it can be concluded that compounds adopt *syn-anti* planar conformation by virtue of the presence of strong intramolecular C=O···H–N hydrogen bond interaction in both solution and solid state. Also, we report detailed analysis of intermolecular interactions (C−H···O and N−H···S) present in compounds by Hirshfeld surfaces analysis and associated fingerprint plots of compounds **1** and **3**. *In vitro* cytotoxicity results of the synthesized compounds against a panel of seven human cancer cell lines revealed them to be potential inhibitors of cervical (**2008**, **C13***) and ovarian (**IGROV-1**) cancer cells. In addition, the better inhibitory activity of compounds **2** and **4** than **1** and **3** may be credited to the presence of two electron-withdrawing groups (Cl and NO_2) on the phenyl ring.

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Supporting Information Summary

Detailed experimental procedure and spectral data of all compounds (Sections **2.3.1−2.3.4).** UV−Vis spectra of compounds **(1−4)** in Figure **S1,** Unit cell diagram of compounds **1** and **3** have been provided in Figure **S2** and FT-IR spectra of compounds (**1−4**) in Figure **S3**. Selected bond lengths (Å) and bond angles (°) for compounds **1** and **3** in Table **S1,** Selected torsion angles (°) for compounds **1** and **3** in Table **S2** and Various weak interactions in compound **1** and **3** in **Table S3.** Scan copies of ^{1}**HNMR** Figures (**S4−S7**) and ^{13}**CNMR** Figures (**S8−S11**).

CCDC **1411613** and **1570186** contain the supplementary crystallographic data for compounds (**1)** and (**3)**. 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: Crystal data and structure refinement for compounds **1** and **3**.

Compounds	**1**	**3**
CCDC No.	**1411613**	**1570186**
Empirical Formula	**$C_{12}H_{15}N_3O_4S$**	**$C_{10}H_{11}N_3O_4S$**
Formula Weight	297.33	269.28
Crystal System / Space Group	Triclinic / P-1	Triclinic / P-1
a / Å	10.6343(6)	8.0975(5)
b / Å	11.2933(6)	11.7854(8)
c / Å	12.5340(6)	13.9092(9)
α / °	89.576(4)	73.746(6)
β / °	75.582(4)	74.419(6)
γ / °	73.192(5)	84.785(5)
V / $Å^3$	1392.21(13)	1227.32(15)
Z	4	4
D_{calc} (g/cm^3)	1.419	1.457
μ (mm^{-1})	0.249	2.481
F(000)	624.0	560.0
Crystal size (mm^3)	0.39 × 0.36 × 0.34	0.22 × 0.06 × 0.04
Radiation	MoKα (λ = 0.71073)	CuKα (λ = 1.54184)
Color / Shape	Bright yellow single crystal	Bright yellow single crystal
Temp (K)	173(2)	293(2)
Theta range for collection	5.972 to 65.64	6.844 to 142.586
Index ranges	$-16 \le h \le 8$, $-17 \le k \le 16$, $-19 \le l \le 18$	$-7 \le h \le 9$, $-14 \le k \le 13$, $-14 \le l \le 16$
Reflections collected	16672	8213
Independent reflections	9146 [Rint = 0.0308, Rsigma = 0.0476]	4626 [Rint = 0.0316, Rsigma = 0.0427]
Data/restraints/parameters	9146/0/365	4626/0/327
Goodness of fit on F^2	1.014	1.031
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0447$, $wR_2 = 0.1049$	$R_1 = 0.0478$, $wR_2 = 0.1259$
R indices (all data)	$R_1 = 0.0681$, $wR_2 = 0.1212$	$R_1 = 0.0698$, $wR_2 = 0.1418$
Largest difference peak/hole	0.34/-0.26 $Å^{-3}$	0.27/-0.24 $Å^{-3}$

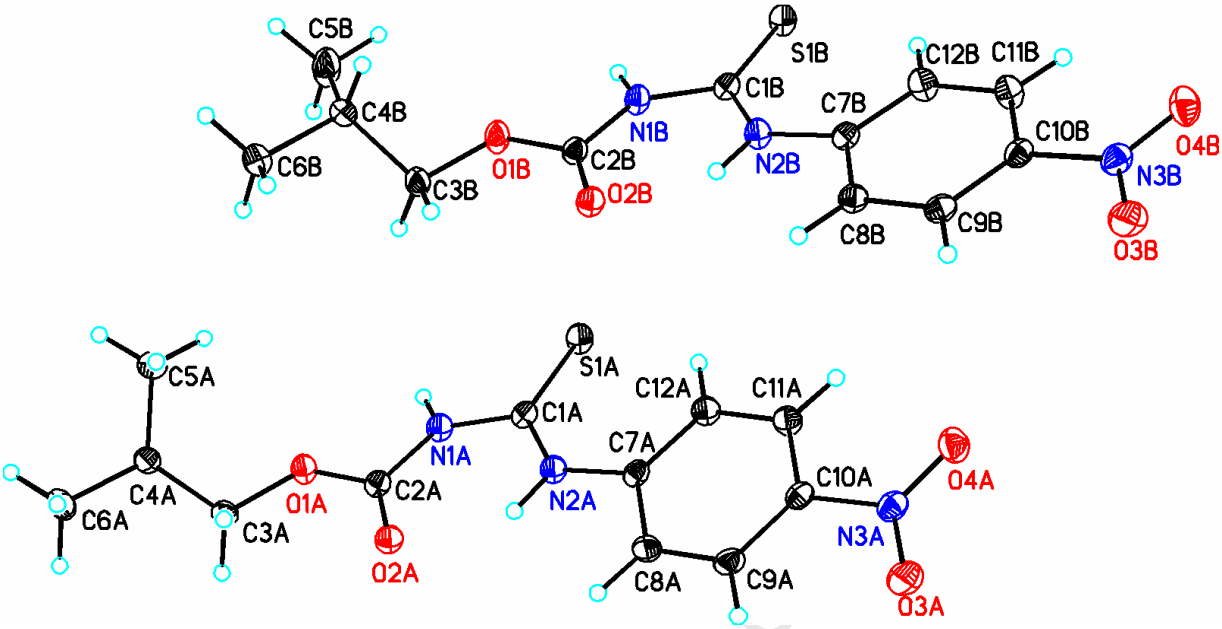
Table 2: Selected weak hydrogen bonding intermolecular interactions for compounds **1** and **3** (Å°).

D−H···A	D−H	H···A	D···A	<(D−H−A)
Compound 1				
N1B−H1B···S1A #1	0.88	2.53	3.3677(11)	159.3
N2B−H2B···O2B	0.88	1.89	2.6457(15)	142.9
C12B−H12B···S1B	0.95	2.55	3.2261(14)	128.5
N1A−H1A···S1B #1	0.88	2.62	3.4502(11)	157.6
N2A−H2A···O2A	0.88	1.93	2.6688(15)	140.7
C9A−H9A···O2A#2	0.95	2.57	3.4162(16)	148.9
C12A−H12A···S1A	0.95	2.51	3.1930(14)	128.5
Compound 3				
N1A−H1A···O1A	0.86	1.96	2.673(3)	140.1
N2A−H2A···S1B #1	0.86	2.47	3.299(2)	161.5
C3A−H3AB···O3B#2	0.97	2.61	3.216(3)	121.0
C9A−H9A···O1B#2	0.93	2.59	3.396(3)	146.0
N1B−H1B···O1B	0.86	1.95	2.678(3)	141.3
N2B−H2B···S1A#1	0.86	2.56	3.401(19)	167.7
C3B−H3BB···O3B #2	0.97	2.59	3.479(4)	151.8
C6B−H6B···S1B	0.93	2.47	3.147(2)	129.9
C7B−H7B···O4A #3	0.93	2.63	3.401(3)	141.0
C9B−H9B···O1A #2	0.93	2.47	3.366(3)	161.0

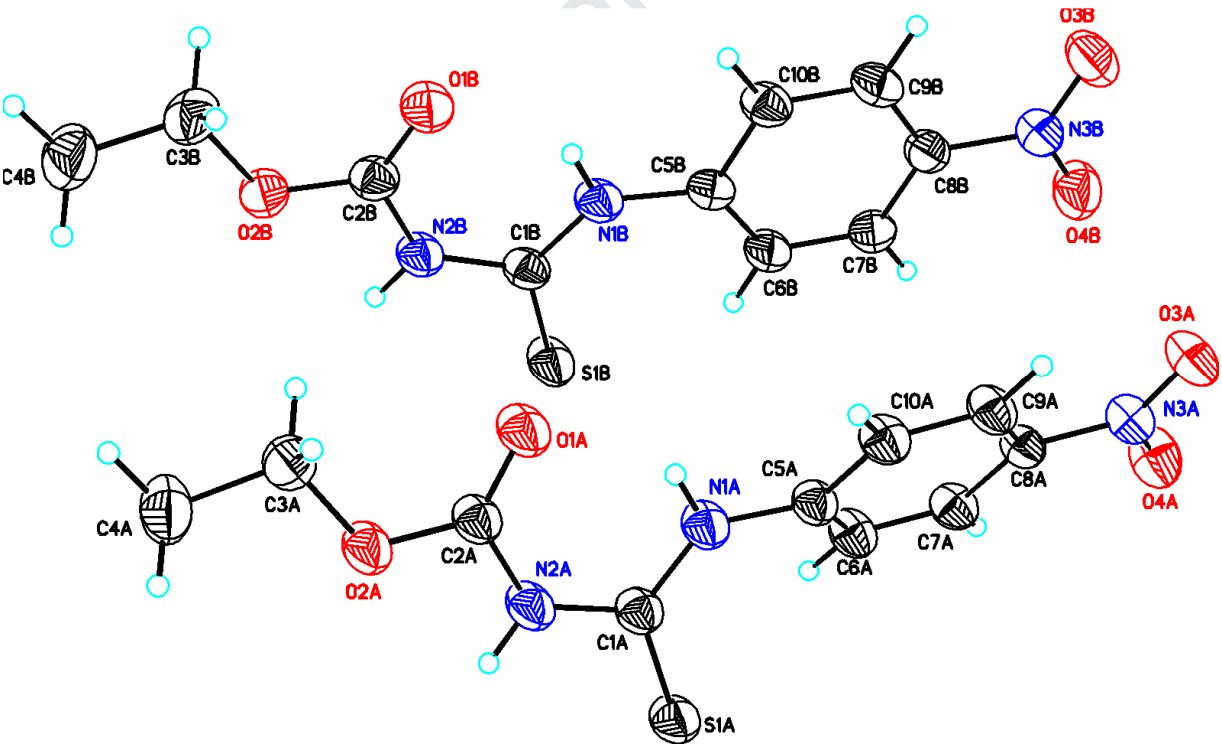
Symmetry transformations used to generate equivalent atoms:
1. #1. -X, 1-Y, -Z. #2. –X, -Y, 1-Z
3. #1.1-X, 2- Y, 1-Z; #2. 2-X, 1-Y, 1-Z. #3. 1-X, 1-Y, 2-Z

Table 3: IC_{50} values (µM) for the compounds **1−4** against seven human cancer cell lines; (cervical **2008** and **C13*),** (colorectal **HT29** and **HCT116**) and (ovarian carcinoma **A2780, A2780/CP** and **IGROV-1)**

Compounds	**2008**	**C13***	**HT29**	**HCT116**	**A2780**	**A2780/CP**	**IGROV-1**
1	38.0±0.2	31.2±0.1	44.6±1	45.6±0.5	49.6±0.4	42.5±0.3	32.2±0.2
2	27.7±0.3	25.3±0.9	37.3±0.9	39.1±0.1	34.6±0.5	38.1±1	28.6±5
3	28.7±1	24.4±2	30.6±4	33.7±0.5	39.1±0.3	36.2±0.6	27.6±0.3
4	22.1±0.5	23.4±0.7	31.6±2	29.1±0.8	33.1±0.8	35.5±1	21.6±0.7
5-FU	4.1±0.3	8.5±0.5	15.2±2.1	13.5±1.8	5.5±0.3	12.8±0.5	5.1±0.2

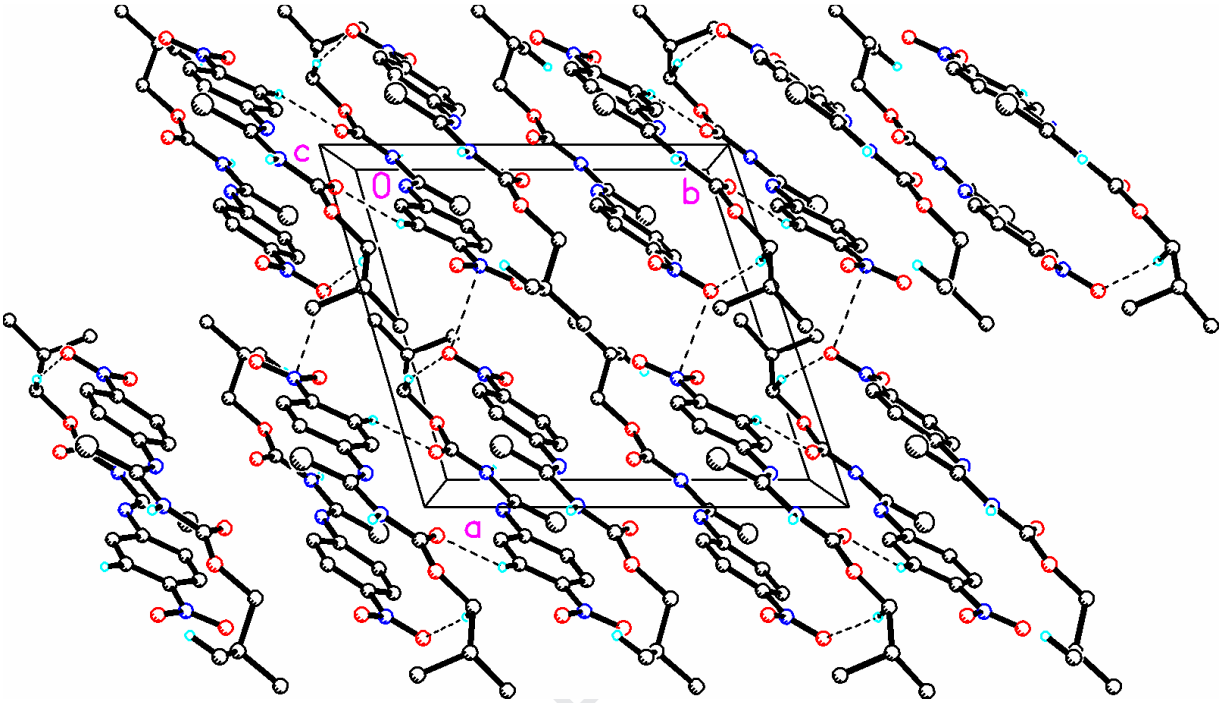


(a)

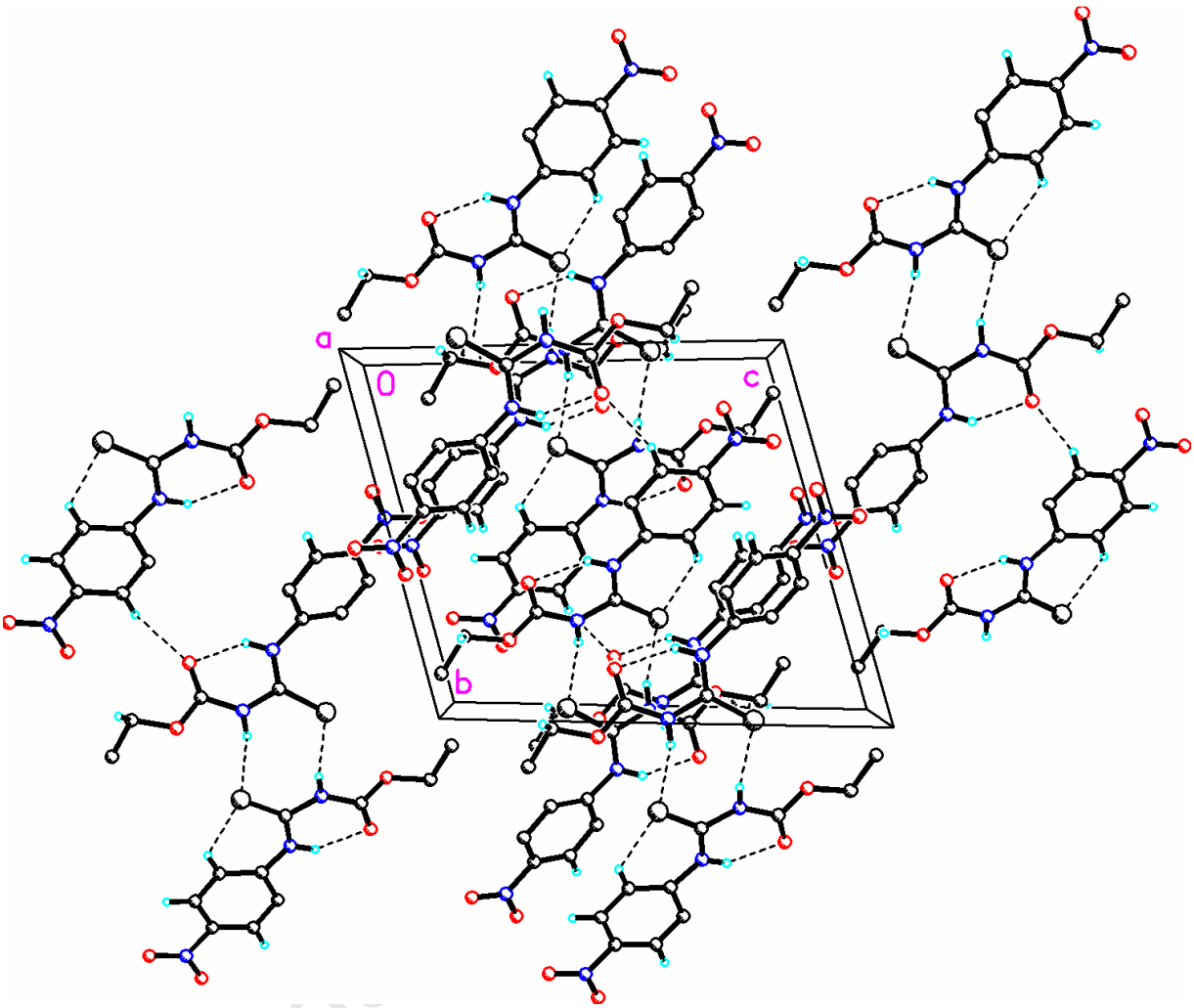


(b)

Fig. 1 ORTEP views of **(a)** Compound **1** and **(b)** Compound **3** with probability thermal ellipsoids at the 50% level.



(a)



(b)

Fig. 2 Crystal packing of **(a)** Compound **1** and **(b)** Compound **3** viewed along the b-axis.

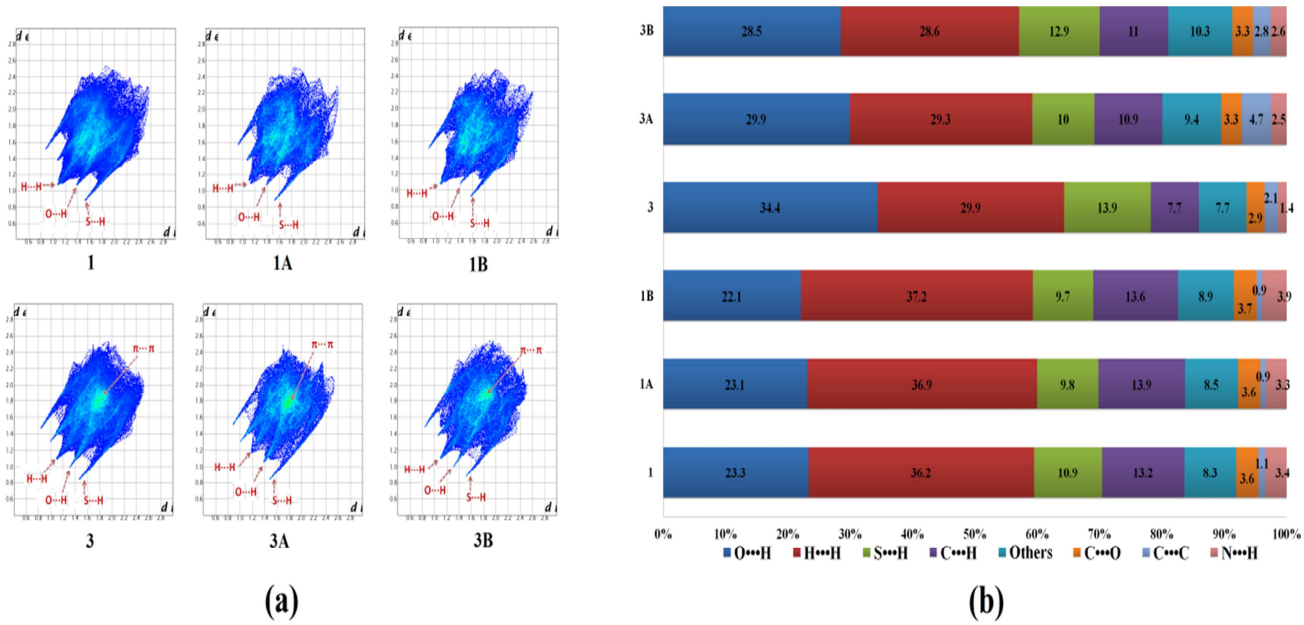


Fig. 3 (a) 2D fingerprint plot of compounds **1** and **3**, (**1A**, **1B** and **3A**, **3B** represent each individual molecules in asymmetric unit of **1** and **3**, respectively) **(b)** Percentage contribution of various intermolecular interactions obtained from decomposed fingerprint plots.

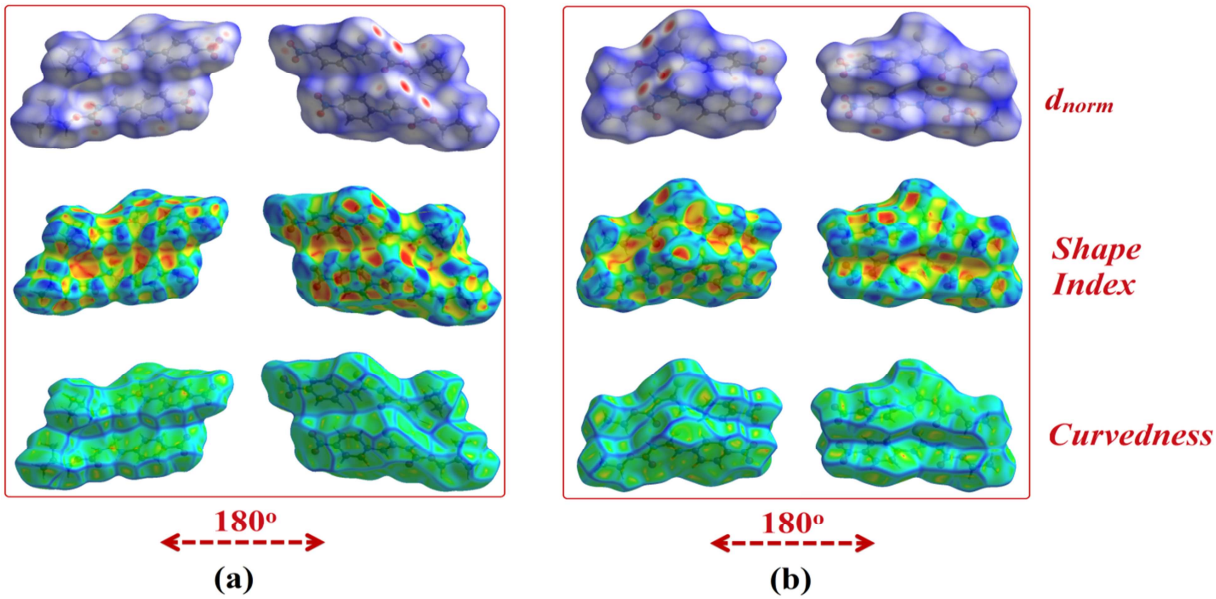


Fig. 4 (a) and **(b)** represent the Hirshfeld surfaces mapped over d_{norm}, *Shape Index* and *Curvedness* for compounds **1** and **3**, respectively.

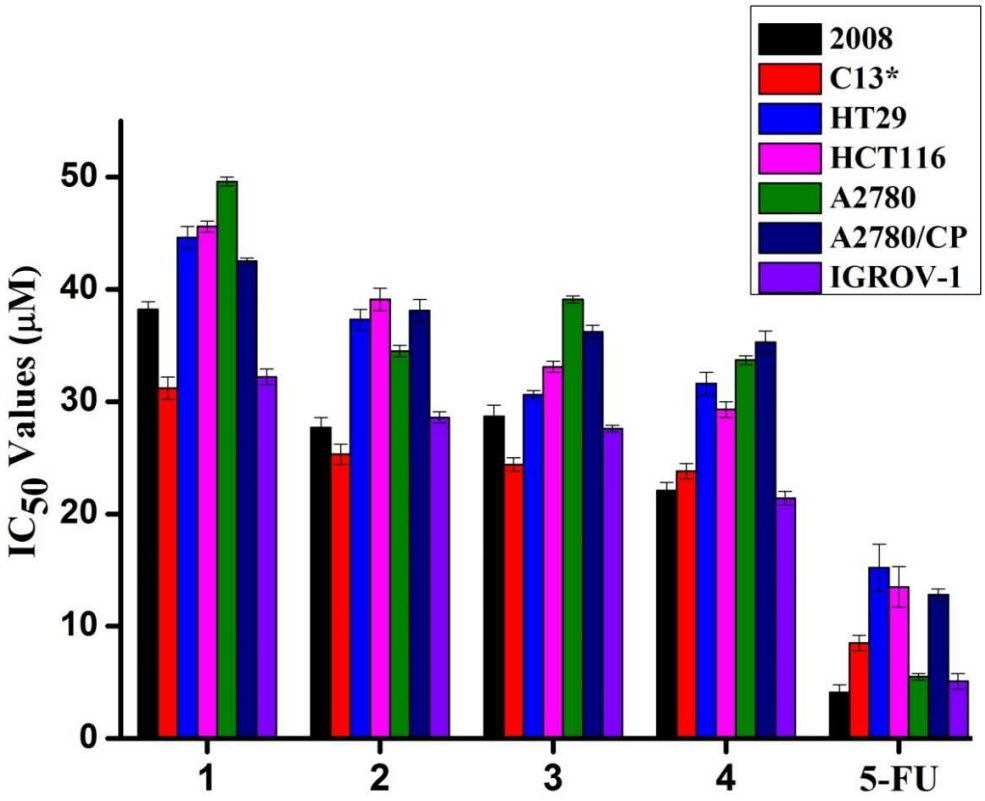


Fig. 5 Comparative *in vitro* cytotoxicity of compounds **1**−**4** for a panel of seven human cancer cell lines; Cervical (**2008** and **C13*),** Colorectal (**HT29** and **HCT116**) and Ovarian carcinoma (**A2780**, **A2780/CP** and **IGROV-1**).

Highlights:

- ❖ Single crystal X-ray analysis of compounds **1, 3** and various spectroscopic techniques were used to establish the structure of the compounds.
- ❖ The presence of intramolecular hydrogen bond (N─H···O═C) compels planarity of the molecule.
- ❖ Hirshfeld surfaces analysis of compounds **1, 3** were performed to understand hydrogen bonding and other weak interactions.
- ❖ 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 cancer cell lines exhibited promising anticancer activity

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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