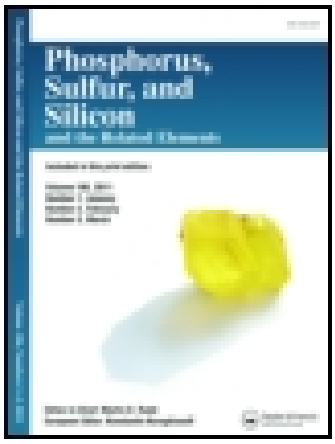

Phosphorus, Sulfur, and Silicon and the Related Elements

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Asynthesis, Crystal Structures and in vitro Anticancer Studies of New Thiosemicarbazone Derivatives

Mouayed A. Hussein[a], Muhammad Adnan Iqbal[a], Muhammad Asif[b], Rosenani A. Haque[a], Mohammed B. Khadeer Ahamed[b], Amin M. S. Abdul Majid[b] & Teoh Siang Guan[a]

[a] School of Chemical Science, Universiti Sains Malaysia, 11800 - Mindern, Pulau Pinang, Malaysia

[b] EMAN Testing and Research Laboratory, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800-Minden, Pulau Pinanag, Malaysia

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ASYNTHESIS, CRYSTAL STRUCTURES AND *IN VITRO* ANTICANCER STUDIES OF NEW THIOSEMICARBAZONE DERIVATIVES

Mouayed A. Hussein,[1] Muhammad Adnan Iqbal,[1] Muhammad Asif,[2] Rosenani A. Haque,[1] Mohammed B. Khadeer Ahamed,[2] Amin M. S. Abdul Majid,[2] Teoh Siang Guan[1 *]

[1]*School of Chemical Science, Universiti Sains Malaysia, 11800 – Mindern, Pulau Pinang, Malaysia*

[2]*EMAN Testing and Research Laboratory, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800-Minden, Pulau Pinanag, Malaysia*

*Corresponding author: Dr. Teoh Siang Guan, PhD,

Professor

School of Chemical Sciences

Universiti Sains Malaysia

Penang-11800, Malaysia

E-mail: sgteoh@usm.my

teohsiang@ymail.com

H/P : (604) ï 6577888

Fax : (604) ï 6574854

Abstract Thiosemicarbazones are an important class of compounds having significant biological properties. These compounds are used as stable ligands for the synthesis of coordination compounds of biological worth. The current study describes syntheses of four new N(3)-ethyl thiosemicarbazone derivatives and their *in vitro* anticancer testing. The purpose of the study was to observe the effect of various subtitutions at the aromatic ring against the growth of cancerous cell. The synthesized compounds were characterized by NMR (^{1}H & ^{13}C) spectroscopy and the molecular structures of each of the compounds were determined using single crystal X-ray crystallographic technique. Interestingly, compound **4** appeared to have two crystallographically different units (A and B), having slightly different bond lengths and angles compared to each other. All the compounds were tested for their *in vitro* anticancer properties against Human Colon Cancer (HCT 116) and Breast Cancer (MCF-7) cell lines. The compounds (**1-2**), having halide (Cl and Br) substitutions, showed significant activity compared to those having ethoxy and allyl substitutions (**3-4**), indicating the possible involvement of halides against cancerous cells.

Keywords Thiosemicarbazones, crystal structures, cytotoxicity, MCF-7, HCT 116, SAR

INTRODUCTION

Thiosemicarbazones are an important class of compounds having general structure R^1R^2-C=N-NH-CS-NH_2 and can be synthesized by condensation reaction of semicarbazide with a suitable aldehyde or ketone [1]. Moreover, suitable substitutions at either thioamide (**a**), sulfur (**b**) or hydrazine (**c**) nitrogen provide versatile derivatives (Figure 1). For example, according to the general structure (**a**), the substitutions R^3 and R^4 may either be alkyl, aryl or hydrogen. In the general structure (**b**), the reported R^3 substitutions are propyl and iso-propyl [2]. Such substitutions in (**b**) are adopted to inhibit the coordination of sulfur with the metal ion. In structure (**c**), the substitution R^3 is commonly a hydrogen.

Thiosemicarbazones are popular because of their metal chelating properties in order to generate metal-complexes having interesting geometries. Metal-thiosemicarbazone complexes have been found to have interesting catalytic [3-6] as well as biological applications [5,7-24]. However, it has been also reported that by suitable structural variations thiosemicarbazone ligands could also become potential anticarcinogenic agents [25-30].

Thiosemicarbazones belong to a large group of thiourea derivatives, whose biological activities are a function of parent aldehyde or ketone moiety [31-33]. Conjugated N-N-S tridentate ligand system of thiosemicarbazone seems necessary for anticancer activity, probably due to the observation that structural changes that hamper a thiosemicarbazoneôs capability to function as a chelating agent to demolish or reduce its medicinal activity [34]. Recent studies showed that the substitutions at the terminal positions (R^1R^2 and R^3R^4 in Figure 1**a**) of thiosemicarbazones have much improved their anticancer activity [35-37]. For example, the presence of aromatic group at

N(4) of isatin-ϐ thiosemicarbazones derivatives have shown increased cytotoxicity against the parental KB-3-1 cell lines and the P-glycoprotein-expressing cell line KB-V1 [38]. Herein, we report the synthesis and characterization of some new thiosemicarbazone derivatives having variation of substitutions at the benzene ring at R^1R^2-terminal position (scheme 1) to further observe this phenomenon.

RESULTS AND DISCUSSION

Synthesis

New thiosemicarbazones (**1-4**) were prepared by condensation of 4-ethyl-3-thiosemicarbazide with corresponding aldehyde derivatives. Compounds were collected either as white or yellow powderous material. Each of the synthesized compounds was recrystalized using diethyl ether/ethanol solvent system before characterization. The compounds were characterized by ^{1}H & ^{13}C NMR and elemental analysis which confirmed their assigned structures (scheme 1). Single crystals of each of the synthesized compound were obtained by slow evaporation of respective solutions either in ethanol or DMF, see the experimental part for detail.

Characterization by NMR

In general, for the all the synthesized compounds, the aromatic C-H chemical shifts appeared in the range 6.75-8.07 ŭ ppm. Importantly, for compounds **1-2** these aromatic C-H chemical shifts appeared in relatively downfield region (7.73-8.07 ŭ ppm) which might be due to the presence of electron withdrawing effect of halide groups (R^1R^2 = Br & Cl) attached to the benzene ring. In rest of the compounds (**3-4**) this range remained relatively upfield (6.75-7.49 ŭ ppm). Furthermore, the chemical shifts for ï CH=N- group appeared in the range 8.39-9.09 ŭ

ppm which is in accordance with the previous reports [25,26] on thiosemicarbazones. ^{1}H NMR signals for -N-NH- proton peak appeared at comparable region (11.44±0.04 ŭ ppm). The chemical shifts for rest of the substitutions (methoxy and allyl) appeared in the range below 6.00 ŭ ppm.

Similarly, ^{13}C NMR chemical shifts for the aromatic carbons appeared in the range 113.9-147.8 ŭ ppm. Noticeably, for the compounds **1-2**, having halide substitutions, the Ar-C range remained relatively upfield (122.5-137.5) which might be due to resonantly electron donating ability of halides [39]. Furthermore, the ï **C**H=N- and **-C**=S groups chemical shifts appeared at 150±5 and 176.5±0.2 ŭ ppm, respectively [4,25,40]. ^{13}C NMR signals for terminal N(4)-ethyl group appeared in the range 14.4-30.6 ŭ ppm and the ethoxy group in **3** at 64.1 and 50.0 for O-CH_2CH_3 in order. The most downfield chemical shifts for the aromatic substitutions appeared for the allyl group (113.1-117.4 ŭ ppm).

Crystallographic study

The single crystals of each of the synthesized compounds were obtained by the slow evaporation method. According to this method, a saturated solution of the compound in a suitable solvent (~0.5 mL) was sealed in a small glass bottle (2.0 mL) using parafilm. Small holes, using needles, were then made to let the solvent evaporate slowly at room temperature. The crystals appeared and suitable of them were then picked up under microscope and were analyzed by Bruker SMART APEXII CCD area-detector diffractometer. Crystallographic data for the compounds have been listed in Table 1 whereas the selected bond lengths and angles are listed in Table S2 (supplementary file).

The molecular structures of compounds **1-4** have been shown in Figures 2-3. The molecular structures of all the compounds contain *anti* orientation of N1 in relation to S1 and *syn* in relation to N3. These geometrical features are in accordance with the previous reports containing compounds having similar structures [27,33,41-43]. However, in some cases this orientation inverses [27], influenced by the nearby substitutions and H-bondings. Similarly, the terminal ethyl C9 atom is *anti* in relation to N2, and *syn* in relation to S1 atom. Figure 2 shows that in compound **1**, the chain C8-C10 has positional disorder. Such disorders have been frequently observed in crystallography at the terminal chains [44,45]. Furthermore, the salicylaldimine plane of **1** which includes O1, N1 and C1-C7 found to be rotated approximately 180^{o} around C6-C7 bond compared to that in the other compounds (**2-4**). This led to change the *anti* geometry between the C1 or C5 atom and N1 atom to *syn* geometry.

The asymmetric unit of **4** contains two $C_{14}H_{19}N_3O_2S$ molecules (A and B). Figure 3 shows an arrangement for the molecular structure of **4**, which reveals that A and B are crystallographically different units having slightly different bond lengths and angles compared to each other. In Figure 3A, the *syn* conformation of C8A atom with respect to C10A atom [torsion angle C8Aï N3Aï C9Aï C10A = 101.5(4)^{o}] and the *anti* conformation of the C6A with respect to O1A atom [torsion angle C6Aï C1Aï C2Aï O1A = -179.0(3)^{o}], C3A atom with respect to C12A atom [torsion angle C3Aï C4Aï C11Aï C12A = - 90.5(4)^{o}] and C4A atom with respect to C13A atom [torsion angle C4Aï C11Aï C12Aï C13A = -119.4(4)^{o}] corresponded to, the *anti* conformation [torsion angle = -77.9(4)^{o}] and the *syn* conformations [torsion angle = 179.2(3)^{o}], [torsion angle = 174.9(3)^{o}] and [torsion angle = 116.8(4)^{o}] for ligand 4B, respectively.

The S1-C8 bond distance is longer than compared to that of N2-C8 which indicates that the compounds are present in thione form. The N3-C8 [1.268(17) Å] bond distance of **1** is shorter that compared to that of N3-C9 [1.51(2) Å] that is different compared to the other compounds (**2-4**). This phenomenon leads to decrease the S1-C8-N3 [117.5(8)^{o}] bond angle and increases the C8-N3-C9 [130.0(14)^{o}] bond angle compared to other compounds (**2-4**). Simultaneously, the N2-C8-N3 [121.4(8)^{o}] bond angle increases as long as the S1-C8-N3 bond angle decreases.

In vitro anticancer study

All the synthesized compounds were tested for their cytotoxicity against two cancerous cell lines, namely Human Colon Cancer (HCT 116) and Breast Cancer (MCF-7), using MTT assay. The compounds showed dose dependent cytotoxic behavior as the population of viable cells reduced abruptly with increasing concentration of the compounds. Please see the supplementary material file for related experimental and discussion parts.

EXPERIMENTAL

Materials and Instrumentation

The melting points were determined using a Stuart Scientific SMP1 melting point apparatus. NMR spectra (^{1}H and ^{13}C) were recorded on a Bruker spectrophotometer at 400 and 500 MHz NMR machines. Tetramethylsilane was used as an internal standard and the samples were prepared in deuterated dimethyl sulfoxide (DMSO-d_6). Elemental analysis was carried out using a Perkin Elmer 2400 series-11 CHN analyzer. X-ray crystallographic data were recorded on a Bruker SMART APEXII CCD area-detector diffractometer using graphite monochromated

Mo KŬ radiation (ə = 0.71073 Å) at 100 K. The data were collected and reduced using APEX2 and SAINT programs. The molecular structures of each of the compounds were solved using the SHELXS-97 program package, and refined using the SHELXL-97 program [46]. All non-hydrogen atoms were anisotropically refined. The molecular graphics were created using SHELXTL. All chemicals, including thiosemicarbazide, aldehydes, and solvents, were purchased from Sigma-Aldrich.

Synthesis

Compounds **1-4** were prepared by the following general procedure according to which equimolar ethanolic solutions of aldehyde derivative (4.19 mmol in 20 mL ethanol) and the corresponding thiosemicarbazide (4.19 mmol in 20 mL ethanol) were refluxed with stirring for 2 h. The product was filtered, washed with ethanol and air-dried. The crystals for **1** and **2** were obtained by slow evaporation of ethanol solution at room temperature. The crystals for **3** and **4** were obtained by slow evaporation of DMF solution at room temperature.

*N-ethyl-2-(3,5-dibromo-2-hydroxybenzylidene)-hydrazine-carbothioamide (**1**)*

A solution of 3,5-dibromo-2-hydroxybenzaldehyde (1.17 g, 4.19 mmol) in ethanol (20 mL) was added to a solution of 4-ethyl-3-thiosemicarbazide (0.5 g, 4.19 mmol) in ethanol (20 mL). The resulting yellow solution was refluxed with stirring for 2 h and then filtered. The filtrate was left to stand at room temperature for two days, and needle colorless crystals were obtained. Yield: 77% (1.22 g); mp. 194 ^{o}C; Anal. Calcd. for $C_{10}H_{10}Br_2N_3OS$: C, 31.5; H, 2.6; N, 11.0%; Found: C, 31.5; H, 3.0; N, 10.9% ; ^{1}H NMR (500MHz, DMSO-d_6, δ/ppm): 1.15 (t, J =

7.5 Hz, 3H, CH_3), 3.62 (q, 2H, CH_2), 7.74, 8.07 (s, 2H, H-aromatic), 8.27 (s, 1H, CS-NH), 8.74 (s, 1H, CH=N), 11.46 (N-NH); ^{13}C NMR (125.1 MHz, DMSO-d_6, δ/ppm): 14.4 (CH_3), 30.6 (CH_2), 122.5, 124.5, 125.0, 129.5, 137.5 (C-aromatic), 150.5 (C=N), 176.6 (C=S).

*N-ethyl-2-(3,5-dichloro-2-hydroxybenzylidene)-hydrazine-carbothioamide (**2**)*

A solution of 3,5-dichloro-2-hydroxybenzaldehyde (0.80 g, 4.19 mmol) in ethanol (20 mL) was added to a solution of 4-ethyl-3-thiosemicarbazide (0.5 g, 4.19 mmol) in ethanol (20 mL). The resulting yellow solution was refluxed with stirring for 2 h and then filtered. The filtrate was left to stand at room temperature for two days, and block colorless crystals were obtained. Yield: 79% (0.96 g); m.p. 186 ^{o}C; Anal. Calcd. for $C_{10}H_{11}Cl_2N_3OS$: C, 41.0; H, 3.7; N, 14.3%. Found: C, 40.9; H, 3.4; N, 13.9% ; ^{1}H NMR (500 MHz, DMSO-d_6, δ/ppm): 1.13 (t, J = 7.5 Hz, 3H, CH_3), 3.50-3.62 (CH_2), 7.73, 8.05 (s, 2H, H-aromatic), 8.28 (s, 1H, CS-NH), 8.73 (s, 1H, CH=N), 11.47 (s, 1H, N-NH); ^{13}C NMR (125.1 MHz, DMSO-d_6, δ/ppm): 14.4 (CH_3), 30.6 (CH_2), 122.5, 123.2, 123.7, 129.5, 137.5 (C-aromatic), 150.5 (C=N), 176.6 (C=S).

*N-ethyl-2-(3-ethoxy-2-hydroxybenzylidene)-hydrazine-carbothioamide (**3**)*

A solution of 3-ethoxy-2-hydroxybenzaldehyde (0.69 g, 4.19 mmol) in ethanol (20 mL) was added to a solution of 4-ethyl-3-thiosemicarbazide (0.5 g, 4.19 mmol) in ethanol (20 mL). The resulting yellow solution was refluxed with stirring for 2 h. A white fluffy product was formed when the solution cooled down to room temperature, then filtered, washed with ethanol and air dried. Needle colorless crystals were obtained by slow evaporation of a DMF solution at room temperature. Yield: 86% (0.96 g); m.p. 222 ^{o}C; Anal. Calcd. for $C_{12}H_{17}N_3O_2S$: C, 53.8; H, 6.3;

N, 15.7%. Found: C, 54.1; H, 6.5; N, 15.2% ; ^{1}H NMR (500 MHz, DMSO-d_6, δ/ppm): 1.13 (t, J = 7.5 Hz, 3H, CH_3), 1.36 (t, J = 7.0 Hz, 3H, ethoxy-CH_3), 3.01 (q, 2H, CH_2), 4.04 (q, 2H, ethoxy-CH_2), 6.75 (d, J = 6.5 Hz, 1H, H-aromatic), 6.97 (d, J = 6.5 Hz, 1H, H-aromatic), 7.49 (m, 1H, H-aromatic), 8.39 (s, 1H, CH=N), 11.41 (s, 1H, N-NH); ^{13}C NMR (125.1 MHz, DMSO-d_6, δ/ppm): 14.5 (CH_3), 30.8 (CH_2), 50.0 (ethoxy-CH_3), 64.1 (ethoxy-CH_2), 113.9, 118.2, 119.1, 121.2, 139.1, 146.0 (C-aromatic), 146.9 (C=N), 177.4 (C=S).

*N-ethyl-2-(5-allyl-3-methoxy-2-hydroxybenzylidene-hydrazine-carbothioamide (**4**)*

A solution of 5-allyl-3-methoxy-2-hydroxybenzaldehyde (0.80 g, 4.19 mmol) in ethanol (20 mL) was added to a solution of 4-ethyl-3-thiosemicarbazide (0.5 g, 4.19 mmol) in ethanol (20 mL). The resulting colorless solution was refluxed with stirring for 2 h. A white fluffy product was formed when the solution cooled down to room temperature, which was then filtered, washed with ethanol, and air dried. Plate colorless crystals were obtained by slow evaporation of a DMF solution at room temperature. Yield: 80% (0.98 g); m.p. 194 ^{o}C. Anal. Calcd. for $C_{14}H_{19}N_3O_2S$: C, 57.2; H, 6.4; N, 14.3%. Found: C, 57.1; H, 6.5; N, 14.2% ; ^{1}H NMR (400 MHz, DMSO-d_6, δ/ppm): 1.15 (t, J = 7.5 Hz, 3H, CH_3), 3.30 (d, J = 6.0 Hz, CH_2-Ph), 3.60 (q, 2H, CH_2), 3.79 (s, 3H, O-CH_3), 5.02 (q, 2H, CH_2=), 6.02 (m, 1H, =CH), 6.78 (s, 1H, H-romatic), 7.34 (s, 1H, H-aromatic), 8.37 (s, 1H, CS-NH), 9.09 (s, 1H, CH=N) , 11.40 (s, 1H, N-NH); ^{13}C NMR (100.1 MHz, DMSO-d_6, δ/ppm): 14.6 (CH_3), 38.2 (CH_2), 55.8 (O-CH_3), 113.1 (CH_2-Ph), 115.4 (CH_2=), 117.4 (=CH), 120.8, 130.3, 138.1, 139.2, 147.8 (C-aromatic), 155.8 (C=N), 177.5 (C=S).

CONCLUSIONS

Four new thiosemicarbazone derivatives were synthesized characterized by NMR and structurally elucidated using X-ray crystallographic technique. The crystal structures of compounds revealed *syn* and *anti* geometries compared to each other. Specifically, compound **4** revealed rarely observed crystallographically different units of the same molecule which have slightly different bond lengths and angles. Such crystallographically different units have been rarely isolated [47]. Furthermore, the compounds were tested for their cytotoxic properties and found to have considerable antiproliferative abilities. However, among the tested compounds, **1-2** (having Br and Cl substitution on benzene ring) showed more pronounced anti-proliferative effects than compared to that of **3-4**. The current research concludes that as the electronegativity of substituted halide group increases the biological significance of the compounds further enhances.

Supplementary Material

CCDC 885657, 885660, 885704 and 958080 contain the supplementary data for **1**, **2**, **3** and **4**, respectively. These data can be obtained free of charge at http://www.ccdc.cam.ac.uk/data_request/cif. A supplementary file has been provided which contains the additional spectral and crystallographic features.

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Table Captions

Table 1: Crystallographic data of compounds **1-4**.

Parameter	**1**	**2**	**3**	**4**
Empirical formula	$C_{10}H_{10}Br_2N_3OS$	$C_{10}H_{11}Cl_2N_3OS$	$C_{12}H_{17}N_3O_2S$	$C_{14}H_{19}N_3O_2S$
Formula weight	380.08	292.19	267.36	293.39
Temperature (K)	100	100.0	100.0	100.0
Radiation/wavelength	MoKŭ / 0.71073 Å	MoKŭ/ 0.71073 Å	MoKŭ / 0.71073 Å	MoKŭ / 0.71073 Å
Crystal morphology and colour	Needles / colorless	Blocks / colorless	Needles / colorless	Plates / colorless
Crystal system, space group	triclinic, *P*$\bar{1}$	triclinic, *P*$\bar{1}$	Monoclinic, *P2ī/c*	triclinic, *P*$\bar{1}$
Unit cell dimensions	a = 4.288(2) Å	a = 5.3023(2) Å	a = 12.9464(3) Å	a = 5.9197(10) Å
	b = 11.893(5) Å	b = 9.3655(3) Å	b = 5.9878(1) Å	b = 13.667(2) Å
	c = 13.181(6) Å	c = 12.9787(5)Å	c = 17.3365(3)Å	c = 18.725(3) Å
	α = 97.082(9)°	α = 104.444(2)°	α = 90°	α = 92.552(4)°
	β = 97.600(9)°	β = 95.345(2)°	β = 99.371(1)°	β = 90.300(6)°
	γ = 99.750(9)°	γ = 91.663(2)°	γ = 90°	γ = 102.110(3)°

Volume ($Å^3$)	649.3(5)	620.51(4)	1326.00(4)	1479.6(4)
Z, Calculated density (Mg/m^3)	2, 1.944	2, 1.564	4, 1.339	4, 1.317
F(000)	370	300	568	624
Crystal size (mm)	0.09 x 0.12 x 0.50	0.12 x 0.23 x 0.44	0.11 x 0.14 x 0.52	0.09 x 0.15 x 0.43
Reflections collected / unique	12601 / 3789	13860 / 3581	24246 / 3582	27095 / 8497
R(int)	0.067	0.029	0.030	0.061
Goodness of fit (S)	1.06	1.10	1.05	1.07
$R[F^2 > 2ů(F^2)]$, $wR(F^2)$	0.0415, 0.1306	0.0335, 0.0869	0.0385, 0.1006	0.0745, 0.2575
Observed data [I>2ů(I)]	2914	3049	3044	6499

Figure Captions

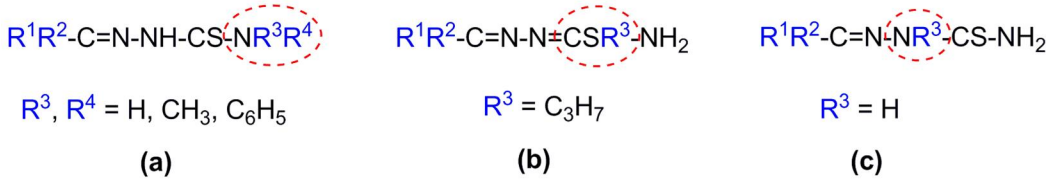


Figure 1: The derivatives of thiosemicarbzides which can be obtained by substituting at, (**a**) thioamide nitrogen, (**b**) sulfur, and (**c**) hydrazine nitrogen. The substitutions, R^1 & R^2 are either alkyl or aryl groups.

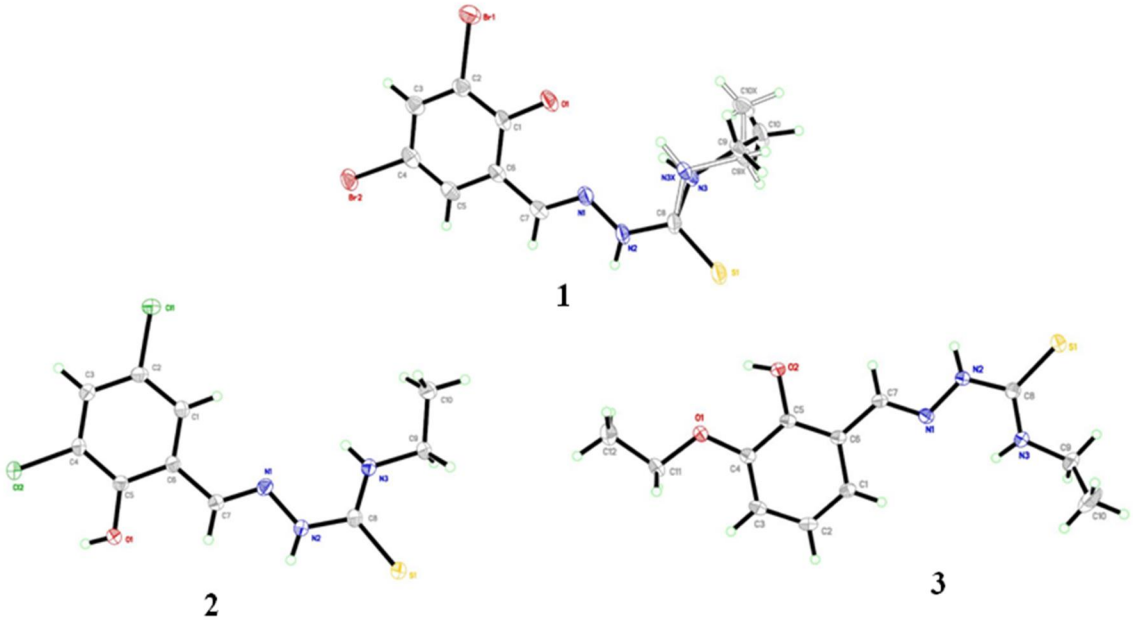


Figure 2: Molecular structures of **1-3** drawn at 50% probability. The N^4-ethyl unit in **1** has an occupancy disorder.

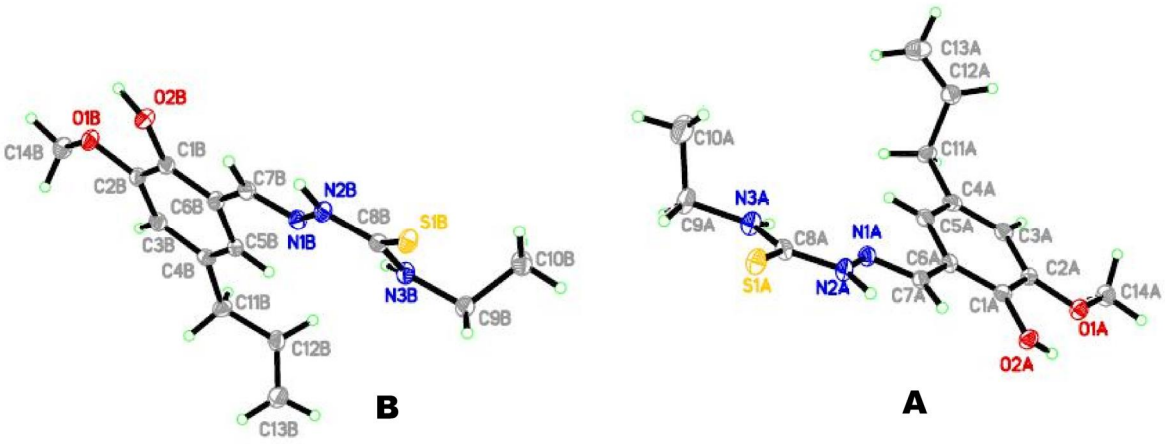
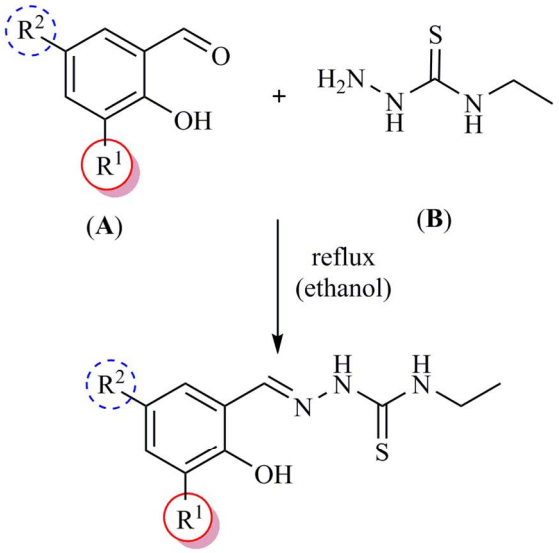
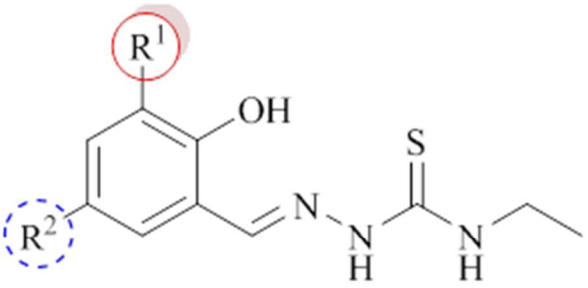


Figure 3: Molecular structure of **4** drawn at 50% probability. The structure was found to have two crystallographically different units (A and B) which have slightly different bond lengths and angles compare to each other.



	R^1	R^2
1	Br	Br
2	Cl	Cl
3	OCH_2CH_3	H
4	OCH_3	CH_2=CH-CH_2

Scheme 1: Syntheses of thiosemicarbazone derivatives (**1-4**) by refluxing different hydroxybenzaldehydes (**A**) and 4-ethyl-3- thiosemicarbazide (**B**) in ethanol.



	R^1	R^2
1	Br	Br
2	Cl	Cl
3	$OCH2CH_3$	H
4	OCH_3	CH_2=CH-CH_2