

## КРАТКИЕ СООБЩЕНИЯ

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## STRUCTURES AND ANTIMICROBIAL ACTIVITY OF FLUORO- AND HYDROXY-SUBSTITUTED THIOCARBOXYHYDRAZONES

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A series of fluoro- and hydroxy-substituted thiocarboxyhydrazones, 2-(3-fluorobenzylidene)-N-methylhydrazinecarbothioamide (**1**), 2-(2,3-difluorobenzylidene)-N-methylhydrazinecarbothioamide (**2**), and 2-(2-hydroxybenzylidene)-N-methylhydrazinecarbothioamide (**3**), are synthesized and characterized by elemental analysis, IR and UV-vis spectra, and single crystal X-ray diffraction. Structures of the three compounds are similar, but with a slight modification due to fluoro substituting groups. The crystal structures of the compounds are stabilized by hydrogen bonds and  $\pi \cdots \pi$  interactions. The antimicrobial activity of the compounds shows that they are effective against some bacteria.

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**Keywords:** synthesis, thiocarboxyhydrazones, antimicrobial activity, crystal structure, hydrogen bonding.

The widespread excessive use of antibacterial agents leads to the development of more resistant bacteria to commonly used antibiotics. This has resulted in the intense search for new types of antibiotics. Hydrazones are a kind of interesting biological active compounds. In recent years, a number of hydrazones have been reported to have various antimicrobial activities [ 1—6 ]. Thiocarboxyhydrazones are a special kind of hydrazone compounds, with the C=O groups replaced by C=S groups. The slight modification of the structures leads to more potent antimicrobial activities [ 7—10 ]. In addition, such compounds have potential antitumor and cytotoxic properties [ 11—13 ]. Recent research indicated that halido-substituted hydrazones have more potent activities than those without such substitute groups [ 14, 15 ]. In order to explore more effective antimicrobial materials, in the present work, a series of three thiocarboxyhydrazones, 2-(3-fluorobenzylidene)-N-methylhydrazinecarbothioamide (**1**), 2-(2,3-difluorobenzylidene)-N-methylhydrazinecarbothioamide (**2**), and 2-(2-hydroxybenzylidene)-N-methylhydrazinecarbothioamide (**3**), have been prepared, characterized, and studied on their antimicrobial activities.

**Experimental. General.** All chemicals and solvents used during the synthesis were of analytical grade and used as received. 3-Fluorobenzaldehyde, 2,3-difluorobenzaldehyde, 2-hydroxybenzaldehyde, and 4-methyl-3-thiosemicarbazide of analytical grade were purchased from Aldrich. Elemental analyses (CHN) were performed using a Perkin-Elmer 240 elemental analyzer. Infrared spectrum was recorded on a Nicolet Magna IR 750 series II FT-IR spectrophotometer as KBr pellets. UV-Vis spectra in the range from 200 nm to 600 nm were recorded on a Perkin-Elmer Lambda-25 spectrophotometer.  $^1\text{H}$  NMR spectra were recorded on 300 MHz Bruker Advance.

**Preparation of the compounds.** The compounds were prepared according to the same method. Equimolar quantities of 4-methyl-3-thiosemicarbazide were reacted with 3-fluorobenzaldehyde, 2,3-

difluorobenzaldehyde, and 2-hydroxybenzaldehyde, respectively, in methanol for 3 h, and the reaction progress was monitored by TLC. Then, the solvent was removed by distillation to give a colorless solid. The precipitate formed was filtered and washed with methanol and recrystallized from methanol. Single crystals suitable for X-ray diffraction were obtained by slow evaporation of the methanol solution of the compounds.

**2-(3-Fluorobenzylidene)-N-methylhydrazinecarbothioamide (1).** Yield, 89 %. Anal. Calc. for  $C_9H_{10}FN_3S$ : C, 51.2; H, 4.8; N, 19.9 %. Found: C, 51.0; H, 4.7; N, 20.1 %. IR data (KBr,  $cm^{-1}$ ): 3382m, 3160w, 2998w, 2949w, 1543s, 1480w, 1443m, 1263s, 1133w, 1096w, 1034m, 966w, 923w, 880w, 817w, 775w, 681w, 570m, 520w, 445w. UV-Vis in methanol [ $\lambda_{max}$ , nm ( $\epsilon$ ,  $L \cdot mol^{-1} \cdot cm^{-1}$ ): 317 (21325).  $^1H$  NMR ( $d^6$ -DMSO, ppm): 3.03 (s, 3H), 7.20 (d, 1H), 7.40—7.8.03 (m, 3H), 8.64 (s, 1H), 11.60 (s, 1H).

**2-(2,3-Difluorobenzylidene)-N-methylhydrazinecarbothioamide (2).** Yield, 92 %. Anal. Calc. for  $C_9H_9F_2N_3S$ : C, 47.2; H, 4.0; N, 18.3 %. Found: C, 47.1; H, 3.9; N, 18.5 %. IR data (KBr,  $cm^{-1}$ ): 3287m, 3126m, 2986w, 1656w, 1544s, 1523s, 1436m, 1239s, 1173w, 1090m, 1029m, 943w, 753m, 657m, 455w. UV-Vis in methanol [ $\lambda_{max}$ , nm ( $\epsilon$ ,  $L \cdot mol^{-1} \cdot cm^{-1}$ ): 316 (19980).  $^1H$  NMR ( $d^6$ -DMSO, ppm): 3.03 (s, 3H), 7.23 (d, 1H), 7.36 (m, 1H), 7.62 (d, 1H), 8.64 (s, 1H), 11.71 (s, 1H).

**2-(2-Hydroxybenzylidene)-N-methylhydrazinecarbothioamide (3).** Yield, 85 %. Anal. Calc. for  $C_9H_{11}N_3OS$ : C, 51.7; H, 5.3; N, 20.1 %. Found: C, 51.9; H, 5.3; N, 19.9 %. IR data (KBr,  $cm^{-1}$ ): 3483w, 3305w, 3118m, 2978w, 1655w, 1541s, 1522s, 1437m, 1241s, 1158w, 1088m, 1033m, 945w, 752m, 645m, 457w. UV-Vis in methanol [ $\lambda_{max}$ , nm ( $\epsilon$ ,  $L \cdot mol^{-1} \cdot cm^{-1}$ ): 332 (21723), 303 (16350).  $^1H$  NMR ( $d^6$ -DMSO, ppm): 3.02 (s, 3H), 7.47—7.60 (m, 3H), 7.88 (d, 2H), 8.62 (s, 1H), 11.37 (s, 1H).

**X-ray data collection and structure refinement.** Suitable single crystals of the compounds were selected and mounted in air onto thin glass fibers. Accurate unit cell parameters were determined by a least-squares fit of  $2\theta$  values, and intensity data sets were measured on a Bruker Smart 1000 CCD diffractometer with  $MoK_{\alpha}$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ) at room temperature. The intensities were corrected for Lorentz and polarization effects, but no corrections for extinction were made. The structures of the compounds were solved by direct methods using the SHELXL 97 program [16]. The non-hydrogen atoms were located in successive difference Fourier syntheses. The final refinement was performed by full matrix least-squares methods with anisotropic thermal parameters for non-hydrogen atoms on  $F^2$ . The amino hydrogen atoms were located from difference Fourier maps and refined isotropically, with N—H distances restrained to  $0.90(1) \text{ \AA}$ . The remaining hydrogen atoms were located at the calculated positions. Crystallographic data and experimental details for structure analyses are summarized in Table 1.

**Fungal assay.** *C. albicans* (ATCC 10231) was grown on Sabouraud dextrose agar (SDA) plates at  $37 \text{ }^{\circ}C$  and maintained at  $4 \text{ }^{\circ}C$  for short-term storage. Cultures were routinely sub-cultured every 4—6 weeks. Cultures were grown to the stationary phase (approximately  $1 \times 10^8 \text{ cells} \cdot \text{cm}^{-3}$ ) overnight at  $37 \text{ }^{\circ}C$  in the minimal medium (2 % w/v glucose, 0.5 % w/v yeast nitrogen base, 0.5 % w/v ammonium sulphate), again at  $37 \text{ }^{\circ}C$ . The complex (200 mg) was dissolved in DMSO (1.0 ml) and diluted by water (9.0 ml) to give a stock solution with a concentration of  $2.0 \times 10^3 \mu\text{g} \cdot \text{ml}^{-1}$ . Doubling dilutions of the solution were made to yield a series of test solutions. Minimum inhibitory concentration  $MIC_{100}$  values (minimum concentration required to inhibit 100 % of cell growth) were then determined using the broth microdilution method.

**Bacterial screening.** Bacteria were maintained on Nutrient Agar plates at  $4 \text{ }^{\circ}C$  and cultured in liquid broth when required. Liquid broth was used for the antibacterial testing. Liquid broth (13 g) was dissolved in water (1000 ml) in a Duran bottle, and then dispensed into 250 ml conical flasks, autoclaved, and allowed to cool. Solutions of the complex were prepared by dissolving the complex (20 mg) in DMSO (0.5 ml). To the solution sterilized Millipore water (9.5 ml) was added to produce a stock solution of a concentration  $2.0 \times 10^3 \mu\text{g} \cdot \text{ml}^{-1}$ . The stock solution (0.5 ml) was added to sterile water (9 ml) to produce a drug solution with a concentration of  $100 \mu\text{g} \cdot \text{ml}^{-1}$ , and with a concentration of DMSO of 0.5 %. This solution (100  $\mu\text{l}$ ) was added to a microtiter plate. 1:1 serial dilutions were made so as to produce a test concentration range of 50— $0.1 \mu\text{g} \cdot \text{ml}^{-1}$ . Both *E. coli* and MRSA were

Table 1

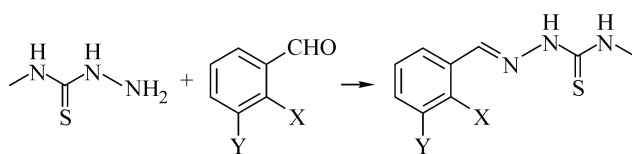
## Crystallographic data for the compounds

Parameter	1	2	3
Empirical formula	C <sub>9</sub> H <sub>10</sub> FN <sub>3</sub> S	C <sub>9</sub> H <sub>9</sub> F <sub>2</sub> N <sub>3</sub> S	C <sub>9</sub> H <sub>11</sub> N <sub>3</sub> OS
Formula weight	211.3	229.2	209.3
Crystal system	Triclinic	Monoclinic	Triclinic
Space group	<i>P</i> -1	<i>P</i> 2 <sub>1</sub> / <i>c</i>	<i>P</i> -1
Unit cell dimensions <i>a</i> , Å	4.5437(12)	8.7200(7)	6.0340(11)
<i>b</i> , Å	10.6756(15)	8.0308(8)	8.5636(16)
<i>c</i> , Å	11.2586(19)	14.8960(13)	10.4989(19)
$\alpha$ , deg.	107.588(2)	90	75.826(2)
$\beta$ , deg.	90.856(2)	92.373(2)	75.707(2)
$\gamma$ , deg.	101.029(2)	90	82.160(2)
Cell volume, Å <sup>3</sup>	509.43(17)	1042.25(16)	508.04(16)
Number of formula units/cell <i>Z</i>	2	4	2
<i>D</i> <sub>calc</sub> , g/cm <sup>-3</sup>	1.377	1.461	1.368
<i>F</i> (000)	220	472	220
Absorption coefficient, mm <sup>-1</sup>	0.295	0.308	0.289
Temperature, K	298(2)	298(2)	298(2)
Wavelength, Å	0.71073	0.71073	0.71073
Crystal size, mm	0.17×0.13×0.12	0.30×0.27×0.23	0.33×0.33×0.27
$\theta$ Range for data collection, deg.	1.90—25.50	2.88—25.50	2.46—25.50
Index ranges ( <i>h</i> , <i>k</i> , <i>l</i> )	-5, 5; -12, 11; -13, 13	-10, 9; -9, 9; -15, 18	-7, 7; -10, 10; -12, 12
Completeness to $\theta$ , %	25.50 (96.3)	25.50 (99.3)	25.50 (98.6)
Max and min transmission	0.9515 and 0.9654	0.9132 and 0.9325	0.9107 and 0.9261
Total number of measured / unique reflections ( <i>R</i> <sub>int</sub> )	3564 / 1832 (0.0308)	9513 / 1932 (0.0271)	4673 / 1869 (0.0255)
Number of observed reflections [ <i>I</i> > 2 $\sigma$ ( <i>I</i> )]	1605	1708	1636
Data / restraints / parameters	1832 / 2 / 134	1932 / 2 / 143	1869 / 2 / 135
Goodness-of-fit on <i>F</i> <sup>2</sup>	1.175	1.087	1.217
Final <i>R</i> indices [ <i>I</i> > 2 $\sigma$ ( <i>I</i> )]	<i>R</i> <sub>1</sub> = 0.0686, <i>wR</i> <sub>2</sub> = 0.2637	<i>R</i> <sub>1</sub> = 0.0334, <i>wR</i> <sub>2</sub> = 0.0867	<i>R</i> <sub>1</sub> = 0.0601, <i>wR</i> <sub>2</sub> = 0.1605
<i>R</i> indices (all data)	<i>R</i> <sub>1</sub> = 0.0779, <i>wR</i> <sub>2</sub> = 0.2795	<i>R</i> <sub>1</sub> = 0.0391, <i>wR</i> <sub>2</sub> = 0.0923	<i>R</i> <sub>1</sub> = 0.0675, <i>wR</i> <sub>2</sub> = 0.1640
Largest diff. peak and hole, e/Å <sup>-3</sup>	0.444, -0.416	0.206, -0.189	0.350, -0.218

grown in liquid broth at 37 °C and 200 rpm to an OD<sub>600</sub> of 1.0. The microtiter plate was inoculated with 100  $\mu$ l of bacterial cells (OD<sub>600</sub> = 1.0). The plates were incubated at 37 °C for 24 h and OD<sub>600</sub> values were read using an RMX plate reader to give MIC<sub>50</sub> values (minimum concentration required to inhibit 50 % of cell growth).

**Results and discussion. Chemistry.** 4-Methyl-3-thiosemicarbazide was treated with appropriate aldehydes to produce the desired products (Scheme 1). The purity of all products was determined by TLC using several solvent systems of different polarities. The synthesized compounds were characterized by elemental analysis, IR, UV-vis, and <sup>1</sup>H NMR spectra.

**Spectral analysis.** The <sup>1</sup>H NMR spectra of the compounds show signals at about 11–12 ppm, which are related to the protons of the NH groups. The signals of the protons on the CH=N double



Scheme 1. Synthesis of the compounds.

1: X = H, Y = F; 2: X = Y = F;

3: X = OH, Y = H

Fig. 1. UV-vis spectra of the compounds

bonds appear at about 8.6 ppm, and the aromatic protons occur in the range 7.2–8.1 ppm. The signals indicative of the protons of the CH<sub>3</sub> groups are located at about 3.0 ppm.

The IR and UV-vis spectra (Fig. 1) of the compounds are very similar. The medium and sharp bands at about 3300 cm<sup>-1</sup> are assigned to  $\nu_{\text{N-H}}$ . The bands observed at slight over and below 3000 cm<sup>-1</sup> are assigned to the aromatic and aliphatic C—H vibrations. The intense bands at about 1550 cm<sup>-1</sup> are due to the absorption of the C=N bonds [17]. The medium bands at about 830–860 cm<sup>-1</sup> are attributed to  $\nu_{\text{C-S}}$ . The absorptions of the electronic spectra may be assigned to  $n \rightarrow \pi^*$  transitions.

**Crystal structure description.** The molecular structures of compounds **1**, **2**, and **3** are shown in Figs. 2, *a*, *b*, and *c*, respectively. In each of the compounds, the sulfur atom and the azomethine nitrogen atom are in *trans* position with respect to the N2—C8 bond. The molecules of the compounds are not coplanar, as evidenced by the dihedral angles (2.2(5)° for **1**, 4.3(4)° for **2**, and 10.5(5)° for **3**) between the N1N2C8S1 thiourea groups and the benzene rings. The bond distances in the thiosemicarbazone side chains agree well with the values observed for similar compounds where the C=S groups are pre-sent in the thionic form [17]. There is no obvious charge delocalization in the molecules, as evidenced by the typical C—N single bonds between C8 and N3 atoms.

In the crystal structure of **1**, the molecules are linked through intermolecular N—H···S and N—H···F hydrogen bonds (Table 2) to form 1D chains running along the *ac* vector direction. In the crystal structure of **2**, the molecules are linked through intermolecular N—H···S hydrogen bonds (Table 2) to form 2D layers parallel to the *bc* plane. In addition, in this compound,  $\pi \cdots \pi$  interactions exist with a centroid to centroid distance of 3.951(2) Å. In the crystal structure of **3**, the molecules are linked through intermolecular N—H···S and O—H···N hydrogen bonds (Table 2) to form 1D chains running along the *a* axis direction.

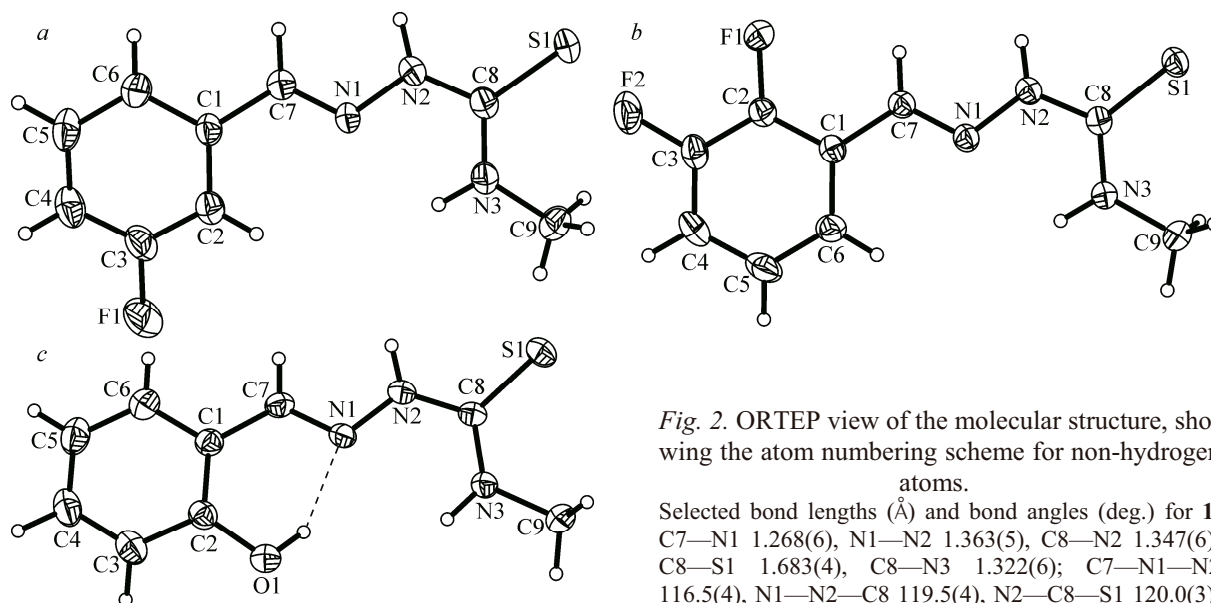


Fig. 2. ORTEP view of the molecular structure, showing the atom numbering scheme for non-hydrogen atoms.

Selected bond lengths (Å) and bond angles (deg.) for **1**: C7—N1 1.268(6), N1—N2 1.363(5), C8—N2 1.347(6), C8—S1 1.683(4), C8—N3 1.322(6); C7—N1—N2 116.5(4), N1—N2—C8 119.5(4), N2—C8—S1 120.0(3), N2—C8—N3 116.2(4), for **2**: C7—N1 1.273(2), N1—N2 1.373(2), N2—C8 1.351(2), C8—S1 1.690(2), C8—N3 1.315(2); C7—N1—N2 115.9(1), N1—N2—C8 119.8(1), N2—C8—S1 119.1(1), N2—C8—N3 117.2(1), for **3**: C7—N1 1.285(4), N1—N2 1.378(4), N2—C8 1.348(4), C8—S1 1.688(3), C8—N3 1.325(4); C7—N1—N2 115.2(3), N1—N2—C8 121.9(3), N2—C8—S1 118.6(2), N2—C8—N3 117.5(3)

Table 2

Hydrogen bond geometry (Å, deg.) for the compounds

$D-H\cdots A$	$D-H$	$H\cdots A$	$D\cdots A$	$D-H\cdots A$
<b>1</b>				
$N2-H2\cdots S1^i$	0.90(1)	2.553(15)	3.444(4)	172(6)
$N3-H3\cdots F1^{ii}$	0.90(1)	2.35(4)	3.110(5)	142(6)
<b>2</b>				
$N3-H3\cdots S1^{iii}$	0.90(1)	2.877(19)	3.565(2)	135(2)
<b>3</b>				
$N3-H3\cdots S1^{iv}$	0.90(1)	2.80(3)	3.474(3)	134(4)
$O1-H1\cdots N1$	0.82	1.96	2.685(4)	146

Symmetry codes: <sup>i</sup> 3-x, 1-y, 1-z; <sup>ii</sup> 1-x, 1-y, -z; <sup>iii</sup> 2-x, -1/2+y, 1/2-z; <sup>iv</sup> -1+x, y, z.

MRSA and *E. coli*. Thus, it is easy to see that the fluoro groups in the compounds may facilitate the antimicrobial activities. Compound **2** has the strongest activity against *C. albicans* as compared to the other two compounds, and is stronger than Ketoconazole.

In summary, three new similar fluoro- and hydroxy-substituted thiocarboxyhydrazones have been prepared and characterized. Single crystal structures of the compounds are presented. The antimicrobial activity of the compounds shows that they are effective against some bacteria.

Crystallographic data for structural analyses have been deposited with the Cambridge Crystallographic Data Center, CCDC Nos. 981998 (**1**), 981999 (**2**), and 982000 (**3**). Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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Table 3

Antimicrobial assay results ( $\mu\text{mol}\cdot\Gamma^{-1}$ )

Compound	<i>C. albicans</i> MIC <sub>100</sub>	MRSA MIC <sub>50</sub>	<i>E. coli</i> MIC <sub>50</sub>
<b>1</b>	6.25	25	25
<b>2</b>	3.12	25	12.5
<b>3</b>	25	>50	>50
Ketoconazole	4.50	>50	>50

**Biological assay results.** The biological assay results are summarized in Table 3. Compounds **1** and **2** have strong activities against *C. albicans* and *E. coli*, and medium activities against MRSA. Compound **3** has a weak activity against *C. albicans*, and no activity against