SYNTHESIS, STRUCTURES AND ANTIMICROBIAL ACTIVITY OF CHLORO- AND FLUORO-SUBSTITUTED THIOCARBOXYHYDRAZONES

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ABSTRACT

A series of chloro- and fluoro-substituted thiocarboxyhydrazones, 2-(2-chlorobenzylidene)-N-methylhydrazinecarbothioamide (**1**), 2-(4-fluorobenzylidene)-N-methylhydrazinecarbothioamide (**2**), and 2-(2-chloro-4-fluorobenzylidene)-N-methylhydrazinecarbothioamide (**3**), were 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 slight modification by chloro- and fluoro-substitute groups. The crystal structures of the compounds are stabilized by hydrogen bonds and π···π interactions. The antimicrobial activity of the compounds shows that they are effective against some bacteria.

Keywords: thiocarboxyhydrazones; antimicrobial activity; crystal structure; hydrogen bonding.

INTRODUCTION

The widespread excessive use of antibacterial agents lead to development of more resistant bacteria to commonly used antibiotics. This has led to intense research 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 serious of three chloro- and fluoro-substituted thiocarboxyhydrazones, 2-(2-chlorobenzylidene)-N-methylhydrazinecarbothioamide (**1**), 2-(4-fluorobenzylidene)-N-methylhydrazinecarbothioamide (**2**), and 2-(2-chloro-4-fluorobenzylidene)-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 AR grade and used as received. 2-Chlorobenzaldehyde, 4-fluorobenzaldehyde, 2-chloro-4-fluorobenzaldehyde and 4-methyl-3-thiosemicarbazide with AR grade were purchased from Aldrich. Elemental analyses (CHN) were performed using a Perkin-Elmer 240 elemental analyzer. Infrared spectrum was recorded on a Nicolate Magna IR 750 series II FT-IR spectrophotometer as KBr pellet. UV-Vis spectra from 200 to 600 nm were recorded on a Perkin-Elmer Lambda-25 spectrophotometer. ^{1}H NMR spectra were recorded on 300 MHz Brucker Advance.

Preparation of the compounds: The compounds were prepared according to the same method. Equimolar quantities of 4-methyl-3-thiosemicarbazide were reacted with 2-chlorobenzaldhyde, 4-fluorobenzaldehyde, and 2-chloro-4-fluorobenzaldehyde, respectively, in methanol for 3 h, and the reaction progress was monitored by TLC. Then, the solvent was removed by distillation to give 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-(2-Chlorobenzylidene)-N-methylhydrazinecarbothioamide (1): Yield, 93%. Anal. Calc. for $C_9H_{10}ClN_3S$: C, 47.5; H, 4.4; N, 18.5%. Found: C, 47.3; H, 4.5; N, 18.4%. IR data (KBr, cm^{-1}): 3296m, 3123m, 2986w, 1654w, 1543s, 1523s, 1437m, 1239s, 1158w, 1090m, 1034m, 947w, 845m, 755m, 657m, 451w. UV-Vis in methanol [λ_{max}, nm (ε, L mol^{-1} cm^{-1})]: 320 (20520), 230 (10360). ^{1}H NMR (d^6-DMSO, ppm): 2.99 (s, 3H), 7.40-7.55 (m, 3H), 7.79 (d, 1H), 9.00 (s, 1H), 11.73 (s, 1H).

2-(4-Fluorobenzylidene)-N-methylhydrazinecarbothioamide (2): Yield, 87%. Anal. Calc. for $C_9H_{10}FN_3S$: C, 51.2; H, 4.8; N, 19.9%. Found: C, 51.1; H, 4.8; N, 19.7%. IR data (KBr, cm^{-1}): 3339w, 3160m, 2995w, 2931w, 1609m, 1555s, 1505s, 1400m, 1233s, 1152w, 1085m, 1034m, 929w, 830w, 582w, 526w, 477w. UV-Vis in methanol [λ_{max}, nm (ε, L mol^{-1} cm^{-1})]: 315 (18315). ^{1}H NMR (d^6-DMSO, ppm): 2.99 (s, 3H), 7.33 (d, 2H), 7.80 (d, 2H), 8.59 (s, 1H), 11.32 (s, 1H).

2-(2-Chloro-4-fluorobenzylidene)-N-methylhydrazinecarbothioamide (3): Yield, 90%. Anal. Calc. for $C_9H_9ClFN_3S$: C, 44.0; H, 3.7; N, 17.1%. Found: C, 44.1; H, 3.6; N, 17.3%. IR data (KBr, cm^{-1}): 3296m, 3141w, 2986w, 2930w, 1598m, 1555s, 1518m, 1481m, 1425w, 1394w, 1245s, 1090m, 1040m, 910w, 855m, 669w, 619w, 445w. UV-Vis in methanol [λ_{max}, nm (ε, L mol^{-1} cm^{-1})]: 318 (22525), 233 (10220). ^{1}H NMR (d^6-DMSO, ppm): 2.99 (s, 3H), 7.19 (s, 1H), 7.26 (d, 1H), 7.78 (d, 1H), 8.93 (s, 1H), 12.05 (s, 1H), 11.55 (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 2θ values, and intensity data sets were measured on a Bruker Smart 1000 CCD diffractometer with Mo Kα radiation (λ = 0.71073 Å) 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) Å. The remaining hydrogen atoms were located at the calculated positions. Crystallographic data and experimental details for structure analyses are summarized in Table 1. Selected bond lengths and angles of the complexes are listed in Table 2.

Fungal assay: C. albicans (ATCC 10231) was grown on Sabouraud dextrose agar (SDA) plates at 37 ºC and maintained at 4 ºC for short-term storage. Cultures were routinely sub-cultured every 4–6 weeks. Cultures were grown to the stationary phase (approximately 1 × 10^8 cells cm^{-3}) overnight at 37 ºC in minimal medium (2% w/v glucose, 0.5% w/v yeast nitrogen base, 0.5% w/v ammonium sulphate), again at 37 ºC. The complex (200 mg) were dissolved in DMSO (1.0 mL) and diluted by water (9.0 mL) to give a stock solution with concentration of 2.0 × 10^3 μg mL^{-1}. Doubling dilutions of the solution were made to yield a series of test solutions. Minimum inhibitory concentrations 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 ºC 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 was added sterilised Millipore water (9.5 mL) to produce a stock solution of concentration 2.0 × 10^{-3} μg mL^{-1}. Stock solution (0.5 mL) was added to sterile water (9 mL) to produce a drug solution with concentration of 100 μg mL^{-1}, and with the concentration of DMSO being 0.5%. This solution (100 μL) was added to a microtiter plate. 1:1 serial dilutions were made so as to produce a test concentration range of 50–0.1 μg mL^{-1}. Both *E. coli* and MRSA were grown in liquid broth at 37 ºC and 200 rpm to an OD_{600} of 1.0. The microtiter plate was inoculated with 100 μL of bacterial cells (OD_{600} = 1.0). The plates were incubated at 37 ºC for 24 h and OD_{600} values were read using an RMX plate reader to give MIC_{50} values (minimum concentration required to inhibit 50% of cell growth).

Table 1. Crystallographic data for the compounds.

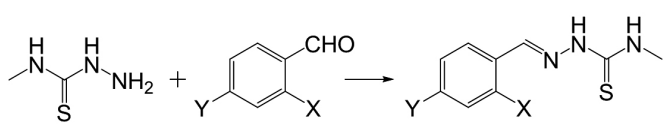
	1	**2**	**3**
Empirical formula	$C_9H_{10}ClN_3S$	$C_9H_{10}FN_3S$	$C_9H_9ClFN_3S$
Formula weight	227.7	211.3	245.7
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	*C*2/*c*	$P2_1/c$	*C*2/*c*
Unit cell dimensions			
a (Å)	14.3289(8)	7.4960(6)	13.850(2)
b (Å)	8.0603(5)	14.8095(10)	8.9415(11)
c (Å)	18.7173(11)	9.3833(6)	18.167(3)
β (º)	90.892(2)	97.590(2)	92.963(2)
Cell volume (Å^3)	2161.5(2)	1032.5(1)	2246.7(5)
Number of formula units/cell (*Z*)	8	4	8
D_{calc} (g cm^{-3})	1.399	1.359	1.453
F(000)	944	440	1008
Absorption coefficient (mm^{-1})	0.510	0.291	0.509
Temperature (K)	298(2)	298(2)	298(2)
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal size (mm)	0.23×0.21×0.20	0.23×0.23×0.22	0.17×0.15×0.15
θ Range for data collection (º)	2.18-26.42	2.59-25.50	2.71-25.50
Index ranges (*h*, *k*, *l*)	-17, 17; -10, 10; -23, 19	-9, 9; -16, 17; -11, 11	-16, 14; -10, 9; -21, 20
Completeness to theta	26.42 (98.7%)	25.50 (99.6%)	25.50 (99.7%)
Maximum and minimum transmission	0.8917, 0.9048	0.9360, 0.9387	0.9185, 0.9276
Total number of reflections measured	10522	8784	9040
Totle number of unique reflections (R_{int})	2195 (0.0269)	1915 (0.0368)	2077 (0.0305)
Number of observed reflections [$I > 2\sigma(I)$]	1885	1519	1644
Data/restraints/parameters	2195/2/134	1915/2/134	2077/2/143
Goodness-of-fit on F^2	1.053	1.050	1.037
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0319$, $wR_2 = 0.0847$	$R_1 = 0.0414$, $wR_2 = 0.1021$	$R_1 = 0.0359$, $wR_2 = 0.0882$
R indices (all data)	$R_1 = 0.0397$, $wR_2 = 0.0917$	$R_1 = 0.0549$, $wR_2 = 0.1113$	$R_1 = 0.0513$, $wR_2 = 0.0969$
Largest diff. peak and hole (e Å^{-3})	0.203, -0.176	0.217, -0.182	0.274, -0.226

Table 2. Selected bond lengths (Å) and bond angles (°) for the compounds.

1			
C7-N1	1.275(2)	N1-N2	1.373(2)
C8-N2	1.351(2)	C8-S1	1.692(2)
C8-N3	1.319(2)		
C7-N1-N2	116.1(1)	N1-N2-C8	119.3(1)
N2-C8-S1	119.1(1)	N2-C8-N3	116.8(1)
2			
C7-N1	1.272(2)	N1-N2	1.369(2)
N2-C8	1.352(3)	C8-S1	1.682(2)
C8-N3	1.325(2)		
C7-N1-N2	117.1(2)	N1-N2-C8	120.2(2)
N2-C8-S1	123.8(2)	N2-C8-N3	117.1(2)
3			
C7-N1	1.281(2)	N1-N2	1.368(2)
N2-C8	1.355(2)	C8-S1	1.687(2)
C8-N3	1.315(3)		
C7-N1-N2	115.8(2)	N1-N2-C8	120.5(2)
N2-C8-S1	119.0(2)	N2-C8-N3	116.2(2)

RESULTS AND DISCUSSION

Chemistry: 4-Methyl-3-thiosemicarbazide 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 and UV-vis spectra, and ^{1}H NMR spectra.



Scheme 1. Synthesis of the compounds. **1**: X = Cl, Y = H; **2**: X = H, Y = F; **3**: X = Cl, Y = F.

Spectra analysis: The ^{1}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 bonds appear at about 8.5-9.0 ppm, and the aromatic protons occur in the range 7.2-7.8 ppm. The signals indicative of the protons of the CH_3 groups are located at 2.99 ppm.

The IR and UV-vis spectra (Fig. 1) of the compounds are very similar. The medium and sharp bands at about 3300 cm^{-1} are assigned to the $\nu_{N\text{-}H}$. The bands observed at slight over and below 3000 cm^{-1} are assigned to the aromatic and aliphatic C-H vibrations. The intense bands at about 1550 cm^{-1} are due to the absorption of the C=N bonds.[17] The medium bands in the region 830-860

cm^{-1} are attributed to the $\nu_{C\text{-}S}$. The absorptions of the electronic spectra may be assigned to the n→π* transitions.

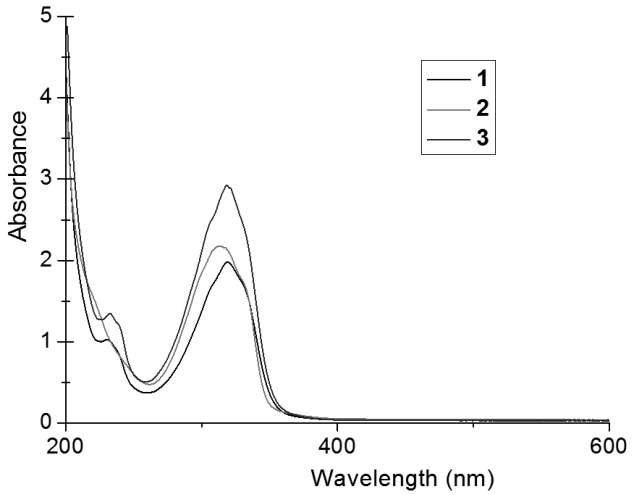


Figure 1. UV-vis spectra of the compounds.

Crystal structure description: The molecular structures of the compounds **1**, **2**, and **3** are shown in Figures 2, 3, and 4, respectively. In each of the compounds, the sulphur 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 [14.0(4)º for **1**, 16.7(3) º for **2**, and 5.2(3)º 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 present in the thionic form.[17] There is no obvious charge delocalization in the molecules, as evidenced by the typical C-N single bonds between atoms C8 and N3.

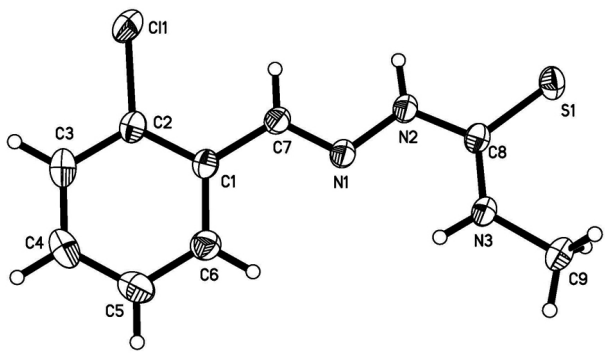


Figure 2. ORTEP view of the molecular structure of **1**, showing the atom numbering scheme for non-hydrogen atoms.

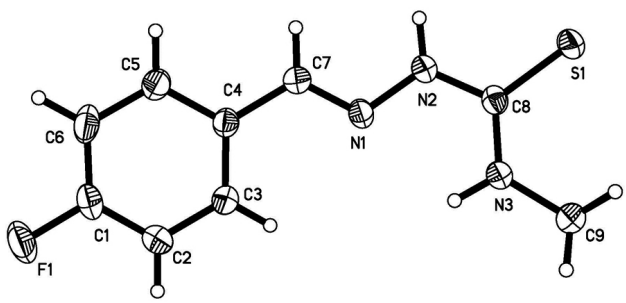


Figure 3. ORTEP view of the molecular structure of **2**, showing the atom numbering scheme for non-hydrogen atoms.

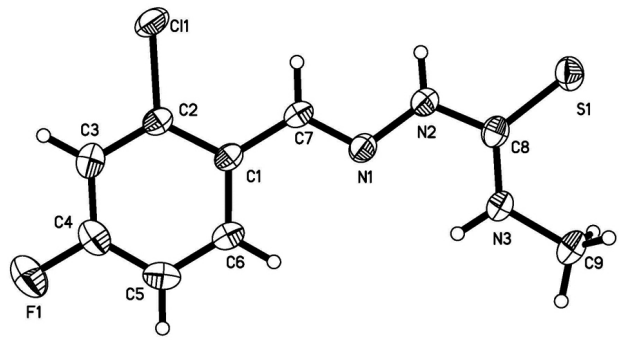


Figure 4. ORTEP view of the molecular structure of **3**, showing the atom numbering scheme for non-hydrogen atoms.

In the crystal packing diagram of the compounds (Figure 5 for **1**, Figure 6 for **2**, Figure 7 for **3**), molecules are linked through intermolecular N–H···S hydrogen bonds (Table 3) and π···π interactions (Table 4), to form 1D chains.

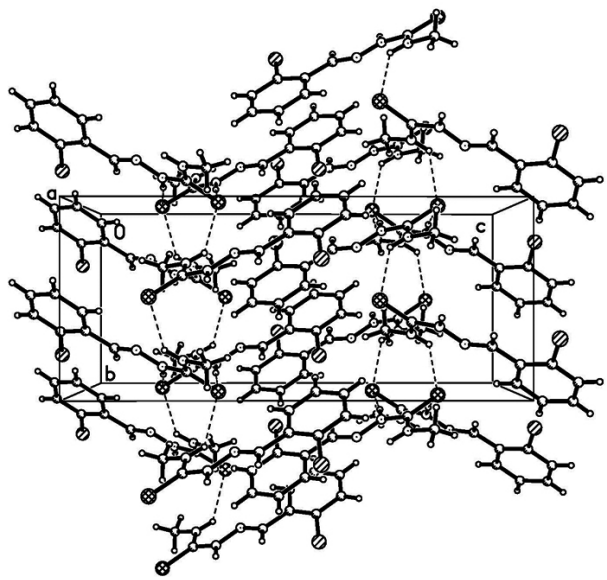


Figure 5. Molecular packing diagram of **1**. Hydrogen bonds are drawn as dashed lines.

Table 3. Hydrogen-bond geometry (Å, °) for the compounds.

D–H···*A*	*D*–H	H···*A*	*D*···*A*	*D*–H···*A*
1				
N3–H3A···S1^{i}	0.90(1)	2.83(2)	3.497(2)	133(2)
N2–H2···S1^{ii}	0.90(1)	2.526(10)	3.414(1)	171(2)
2				
N3–H3···S1^{iii}	0.90(1)	2.86(2)	3.499(2)	130(2)
3				
N2–H2···S1^{ii}	0.90(1)	2.58(1)	3.445(2)	165(2)

Symmetry codes: (i) 1/2 – *x*, 1/2 + *y*, 3/2 – *z*; (ii) 1 – *x*, *y*, 3/2 – *z*; (iii) *x*, 3/2 – *y*, –1/2 + *z*.

Table 4. Parameters between the planes for the compounds.

Cg	Distance between ring centroids (Å)	Dihedral angle (º)	Perpendicular distance of *Cg*(I) on *Cg*(J) (Å)	Beta angle (º)	Gamma angle (º)	Slippage	Perpendicular distance of *Cg*(J) on *Cg*(I) (Å)
1							
Cg(1)-*Cg*(1)^{iv}	4.2739	0	-3.5826	33.0	33.0	2.331	-3.5826
Cg(1)-*Cg*(1)^{v}	4.6898	0	3.4799	42.1	42.1	3.144	3.4799
3							
Cg(2)-*Cg*(2)^{vi}	3.7558	0	3.4979	21.4	21.4	1.368	3.4979
Cg(2)-*Cg*(2)^{vii}	4.8459	0	-3.3297	46.6	46.6	3.521	-3.3297
Symmetry codes: (iv): $-x, -y, -z$; (v): $1/2-x, 1/2-y, -z$; (vi): $1-x, 1-y, -z$; (vii): $1/2-x, 1/2-y, -z$. *Cg*(1) and *Cg*(2) are the centroids of C1-C2-C3-C4-C5-C6 (**1**) and C1-C2-C3-C4-C5-C6 (**3**), respectively.							

Biological assay results: The biological assay results are summarized in Table 5. Compound **1** has medium activity against *C. albicans* and *E. coli*, but no activity against MRSA. Compounds **2** and **3** have similar activities against *C. albicans* and *E. coli*. As for the activity on MRSA, compound **3** is better than compound **2**. From the results, it is not difficult to see that the 4-fluoro containing compound **2** has stronger activities than the 2-chloro containing compound **1**. When the chloro and fluoro groups are combined together to one new compound **3**, the activities are not improved obviously, except for MRSA.

Table 5. Antimicrobial assay results (μmol L^{-1}).

Compound	*C. albicans* MIC_{100}	MRSA MIC_{50}	*E. coli* MIC_{50}
1	12.5	>50	25
2	3.12	25	12.5
3	3.12	12.5	12.5

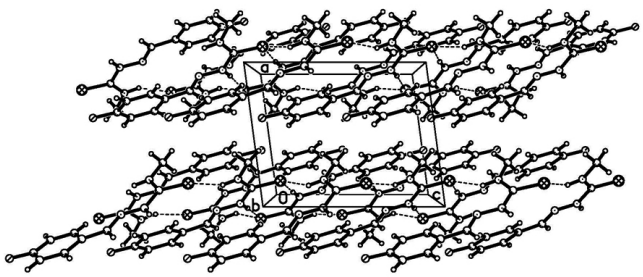


Figure 6. Molecular packing diagram of **2**. Hydrogen bonds are drawn as dashed lines.

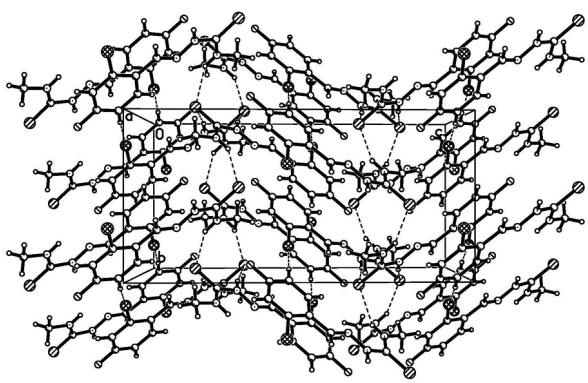


Figure 7. Molecular packing diagram of **3**. Hydrogen bonds are drawn as dashed lines.

CONCLUSION

In summary, three new similar chloro- and fluoro-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.

Supplementary data

Crystallographic data for structural analyses have been deposited with the Cambridge Crystallographic Data Centre, CCDC Nos. 981995 (**1**), 981996 (**2**), and 981997 (**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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