Journal of Structural Chemistry. Vol. 56, No. 7, pp. 1420-1425, 2015.

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

Z.-X. Liu

UDC 547.53:548.73

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 π···π interactions. The antimicrobial activity of the compounds shows that they are effective against some bacteria.

DOI: 10.1134/S0022476615070276

Keywords: synthesis, hiocarboxyhydrazones, 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 serious 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 Nicolate 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}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 3-fluorobenzaldhyde, 2,3-difluorobenzaldehyde, and

School of Chemistry and Chemical Engineering, Linyi University, Linyi Shandong, P. R. China; zengxin_liu@163.com. The text was submitted by the authors in English. *Zhurnal Strukturnoi Khimii*, Vol. 56, No. 7, pp. 1476-1480, November-December, 2015. Original article submitted April 22, 2014; revised May 14, 2014.

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 [λ_{max}, nm (ε, $L \cdot mol^{-1} \cdot cm^{-1}$)]: 317 (21325). ^{1}H 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, 17.1; 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 [λ_{max}, nm (ε, $L \cdot mol^{-1} \cdot cm^{-1}$)]: 316 (19980). ^{1}H 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 [λ_{max}, nm (ε, $L \cdot mol^{-1} \cdot cm^{-1}$)]: 332 (21723), 303 (16350). ^{1}H 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 2θ values, and intensity data sets were measured on a Bruker Smart 1000 CCD diffractometer with MoK_α 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.

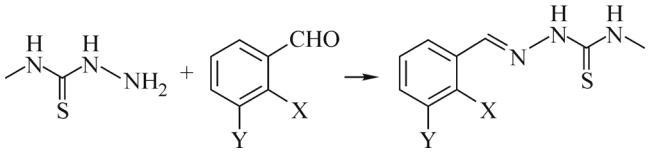
Fungal assay. *C. albicans* (ATCC 10231) was grown on Sabouraud dextrose agar (SDA) plates at 37°C and maintained at 4°C for short-term storage. Cultures were routinely sub-cultured every 4-6 weeks. Cultures were grown to the stationary phase (approximately 1×10^8 $cells \cdot cm^{-3}$) overnight at 37°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°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 2.0×10^3 $\mu g \cdot ml^{-1}$. 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°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 2.0×10^3 $\mu g \cdot ml^{-1}$. The stock solution (0.5 ml) was added to sterile water (9 ml) to produce a drug solution with a concentration of 100 $\mu g \cdot ml^{-1}$, and with a concentration of DMSO of 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 $\mu g \cdot ml^{-1}$. Both *E. coli* and MRSA were grown in liquid broth at 37°C 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°C 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

Parameter	**1**	**2**	**3**
Empirical formula	$C_9H_{10}FN_3S$	$C_9H_9F_2N_3S$	$C_9H_{11}N_3OS$
Formula weight	211.3	229.2	209.3
Crystal system	Triclinic	Monoclinic	Triclinic
Space group	*P*-1	$P2_1/c$	*P*-1
Unit cell dimensions *a*, Å	4.5437(12)	8.7200(7)	6.0340(11)
b, Å	10.6756(15)	8.0308(8)	8.5636(16)
c, Å	11.2586(19)	14.8960(13)	10.4989(19)
α, deg	107.588(2)	90	75.826(2)
β, deg	90.856(2)	92.373(2)	75.707(2)
γ, deg	101.029(2)	90	82.160(2)
Cell volume, Å^3	509.43(17)	1042.25(16)	508.04(16)
Number of formula units/cell *Z*	2	4	2
D_{calc}, g/cm^3	1.377	1.461	1.368
F(000)	220	472	220
Absorption coefficient, mm^{-1}	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
θ Range for data collection, deg	1.90-25.50	2.88-25.50	2.46-25.50
Index ranges (*h*, *k*, *l*)	–5, 5; –12, 11; –13, 13	–10, 9; –9, 9; –15, 18	–7, 7; –10, 10; –12, 12
Completeness to θ, %	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
Totle number of measured / unique reflections (R_{int})	3564 / 1832 (0.0308)	9513 / 1932 (0.0271)	4673 / 1869 (0.0255)
Number of observed reflections [$I > 2\sigma(I)$]	1605	1708	1636
Data / restraints / parameters	1832 / 2 / 134	1932 / 2 / 143	1869 / 2 / 135
Goodness-of-fit on F^2	1.175	1.087	1.217
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0686, wR_2 = 0.2637	R_1 = 0.0334, wR_2 = 0.0867	R_1 = 0.0601, wR_2 = 0.1605
R indices (all data)	R_1 = 0.0779, wR_2 = 0.2795	R_1 = 0.0391, wR_2 = 0.0923	R_1 = 0.0675, wR_2 = 0.1640
Largest diff. peak and hole, e/Å^3	0.444, –0.416	0.206, –0.189	0.350, –0.218

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 ^{1}H NMR spectra.



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

Spectral 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 8.6 ppm, and the aromatic protons occur in the range 7.2-8.1 ppm. The signals indicative of the protons of the CH_3 groups are located at about 3.0 ppm.

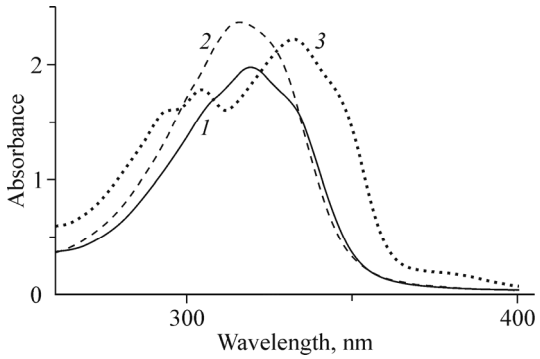


Fig. 1. UV-Vis spectra of the compounds.

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 ν_{N-H}. The bands observed at slight over and below 3000 cm^{-1} are assigned to the aromatic and aliphatic C–H vibrations. The in tense bands at about 1550 cm^{-1} are due to the absorption of the C=N bonds [17]. The medium bands at about 830-860 cm^{-1} are attributed to ν_{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.

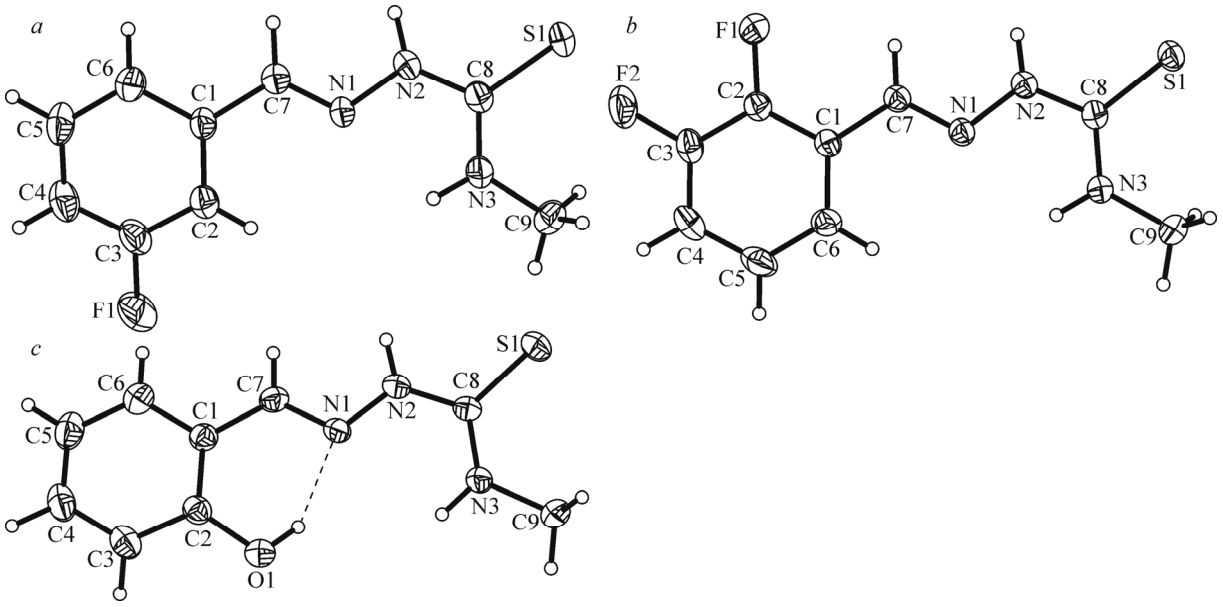


Fig. 2. ORTEP view of the molecular structure, showing the atom numbering scheme for non-hydrogen atoms. Selected bond lengths (Å) and bond angles (deg) for **1**: C7–N1 1.268(6), N1–N2 1.363(5), C8–N2 1.347(6), C8–S1 1.683(4), C8–N3 1.322(6); C7–N1–N2 116.5(4), N1–N2–C8 119.5(4), N2–C8–S1 120.0(3), 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···A	D–H	H···A	D···A	D–H···A
		1		
N2–H2···S1[i]	0.90(1)	2.553(15)	3.444(4)	172(6)
N3–H3···F1[ii]	0.90(1)	2.35(4)	3.110(5)	142(6)
		2		
N3–H3···S1[iii]	0.90(1)	2.877(19)	3.565(2)	135(2)
		3		
N3–H3···S1[iv]	0.90(1)	2.80(3)	3.474(3)	134(4)
O1–H1···N1	0.82	1.96	2.685(4)	146

Symmetry codes: [i] 3–*x*, 1–*y*, 1–*z*; [ii] 1–*x*, 1–*y*, –*z*; [iii] 2–*x*, –1/2+*y*, 1/2–*z*; [iv] –1+*x*, *y*, *z*.

TABLE 3. Antimicrobial Assay Results (μmol·l^{-1})

Compound	*C. albicans* MIC_{100}	MRSA MIC_{50}	*E. coli* MIC_{50}
1	6.25	25	25
2	3.12	25	12.5
3	25	>50	>50
Ketoconazole	4.50	>50	>50

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, π···π 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.

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 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).

The author acknowledges the Linyi University for supporting this work.

REFERENCES

1. M. Alagesan, N. S. P. Bhuvanesh, and N. Dharmaraj, *Dalton Trans.*, **42**, No. 19, 7210-7223 (2013).
2. M. D. Altintop, A. Ozdemir, G. Turan-Zitouni, S. Ilgin, O. Atli, G. Iscan, and Z. A. Kaplancikli, *Eur. J. Med. Chem.*, **58**, 299-307 (2012).

3. P. Yang, Y. Wang, Z.-C. Duan, Y. Shao, G.-H. Nie, X.-J. Song, and D.-T. Tian, *Chin. J. Struct. Chem.*, **32**, No. 7, 1023-1030 (2013).
4. Z. A. Kaplancikli, M. D. Altintop, A. Ozdemir, G. Turan-Zitouni, S. I. Khan, and N. Tabanca, *Lett. Drug Des. Discovery*, **9**, No. 3, 310-315 (2012).
5. D. Blanot, J. Lee, and S. E. Girardin, *Chem. Biol. Drug Des.*, **79**, No. 1, 2-8 (2012).
6. T. Horiuchi, Y. Takeda, N. Haginoya, M. Miyazaki, M. Nagata, M. Kitagawa, K. Akahane, and K. Uoto, *Chem. Pharm. Bull.*, **59**, No. 8, 991-1002 (2011).
7. G. D. K. Kumar, G. E. Chavarria, A. K. Charlton-Sevcik, W. M. Arispe, M. T. MacDonough, T. E. Strecker, S. E. Chen, B. G. Siim, D. J. Chaplin, and M. L. Trawick, *Bioorg. Med. Chem. Lett.*, **20**, No. 4, 1415-1419 (2010).
8. Y. Li, Z.-Y. Yang, and J.-C. Wu, *Eur. J. Med. Chem.*, **45**, No. 12, 5692-5701 (2010).
9. A. Kumar, S. Singh, H. Bordbar, T. Cartledge, and S. Ahmed, *Lett. Drug Des. Discovery.*, **8**, No. 3, 241-245 (2011).
10. D. S. Raja, N. S. P. Bhuvanesh, and K. Natarajan, *Eur. J. Med. Chem.*, **46**, No. 9, 4584-4594 (2011).
11. K. Hu, Z.-H. Yang, S.-S. Pan, H.-J. Xu, and J. Ren, *Eur. J. Med. Chem.*, **45**, No. 8, 3453-3458 (2010).
12. A. I. Matesanz, and P. Souza, *Mini-Rev. Med. Chem.*, **9**, No. 12, 1389-1396 (2009).
13. I. Dilovic, M. Rubcic, V. Vrdoljak, S. K. Pavelic, M. Kralj, I. Piantanida, and M. Cindric, *Bioorg. Med. Chem.*, **16**, No. 9, 5189-5198 (2008).
14. C. Congiu and V. Onnis, *Bioorg. Med. Chem.*, **21**, No. 21, 6592-6599 (2013).
15. M. Zhang, D.-M. Xian, H.-H. Li, J.-C. Zhang, and Z.-L. You, *Aust. J. Chem.*, **65**, No. 4, 343-350 (2012).
16. G. M. Sheldrick, *Acta Crystallogr.*, **A64**, No. 1, 112-122 (2008).
17. M. B. Ferrari, S. Capacchi, G. Reffo, G. Pelosi, P. Tarasconi, R. Albertini, S. Pinelli, and P. Lunghi, *J. Inorg. Biochem.*, **81**, No. 1, 89-97 (2000).