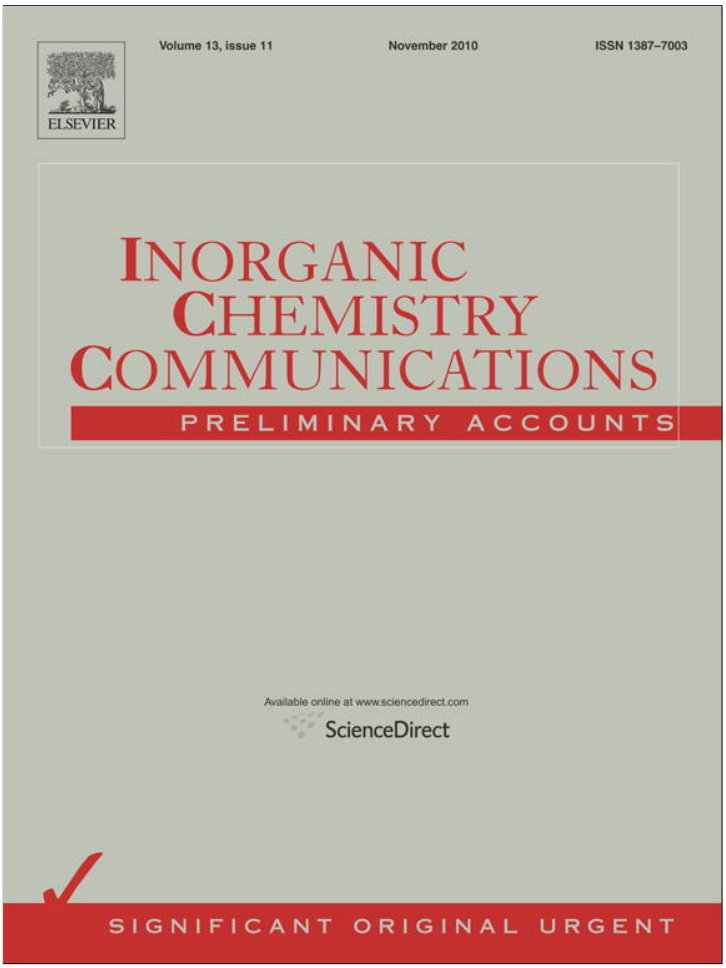


Inorganic Chemistry Communications 13 (2010) 1268–1271

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

Synthesis, crystal structures, and biological activities of 2-thiophene N(4)-methylthiosemicarbazone and its unusual hexanuclear silver(I) cluster

Ming-Xue Li [a,b], Dong Zhang [a], Li-Zhi Zhang [a], Jing-Yang Niu [a,*]

[a] Institute of Molecular and Crystal Engineering, College of Chemistry and Chemical Engineering, Henan University, Kaifeng, 475004, PR China
[b] Key Laboratory of Natural Medicines and Immuno-Engineering, Henan University, Kaifeng, 475004, PR China

ARTICLE INFO

Article history:
Received 6 May 2010
Accepted 8 July 2010
Available online 15 July 2010

Keywords:
Thiosemicarbazone
Silver(I)
Crystal structure
Cytotoxic activity

ABSTRACT

2-Thiophene N(4)-methylthiosemicarbazone (HL) and its Ag(I) complex of formula $[Ag_6(\mathbf{L})_6 \cdot 4DMF]$ **1** have been synthesized and characterized by elemental analysis, IR spectra and single-crystal X-ray diffraction studies. The silver(I) complex **1** with a 1:1 metal–ligand molar ratio is an unusual hexanuclear cluster with 6 silver atoms in different environments: four silver atoms of them are considered to be 3-coordinate form, which are coordinated by two thiolate sulfur atoms and one imine nitrogen and the other two silver atoms are considered to be 4-coordinate form, which are coordinated by three thiolate sulfur atoms and one imine nitrogen, with four thiolate sulfur bridging two silver atoms and two thiolate sulfur bridging three silver atoms. Biological studies, carried out *in vitro* against bacteria, fungi and SMMC-7721 liver cancer cell line, respectively, have shown that the free ligand and the title complex show distinct difference in the biological property.

Thiosemicarbazones and their transition metal complexes have received considerable attention in chemistry and biology, primarily because of their marked and various biological properties [1–6]. The biological activities of thiosemicarbazones often depend on the parent aldehyde or ketone. On the other hand, the biological properties of metal thiosemicarbazones often differ from those of either ligands or the metal ions which are considered to be related to metal ion coordination [7–9]. In some cases the highest *in vivo* activity is associated with a metal complex rather than the parent ligand and some side effects may decrease upon complexation [10–12]. In addition, thiosemicarbazones are versatile ligands that can coordinate as neutral ligands or in the deprotonated form and are flexible spacers with potential multiple binding sites that can be used to construct coordination polymers with multiple dimensions and various topologies[13,14]. Their coordinative behaviour markedly depends on the transition metal ions employed. More importantly, the design of silver (I) complexes has attracted particular attention due to their fascinating structures, photoluminescent properties, biological and pharmacological activities, such as antibacterial and antifungal properties [15–20].

In the recent years we have been working on the structural and biological properties of heterocyclic thiosemicarbazones and their metal complexes [21]. After a careful literature search, we can affirm that thiosemicarbazone silver(I) complexes are scarce [22–24]. Therefore, it seemed important for us to fulfil this work.

As a part of our ongoing studies, in the present paper, we describe synthesis, IR spectra and single-crystal X-ray crystal structures of 2-thiophene N(4)-methylthiosemicarbazone (Scheme 1) and its Ag(I) complex of formula $[Ag_6(\mathbf{L})_6 \cdot 4DMF]$ **1**. Biological studies of the free ligand and the title complex were carried out *in vitro* against bacteria, fungi and SMMC-7721 liver cancer cell line, respectively.

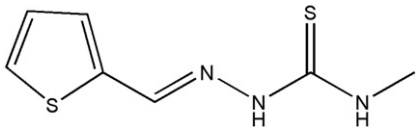
The ligand HL was synthesized by reacting thiophene-2-carboxaldehyde and 4-methyl-3-thiosemicarbazide (1:1 molar ratio) in ethanol [25]. Complex **1** was synthesized by reacting 2-thiophene N (4)-methylthiosemicarbazone and $AgNO_3$ (1:1 molar ratio) in ethanol [26].

Single-crystal X-ray analysis [27–29] reveals that the HL crystallizes in monoclinic system, with space group $P2_1/c$. As shown in Fig. 1, the molecule exists in the E conformation around the N(2)–C(2) bond. From the imine and the thioamide bond distances, along with the rest of the structural parameters of 2-thiophene N(4)-methylthiosemicarbazone, we may conclude that the ligand exists in thione form in its solid state as observed in other free unsubstituted thiosemicarbazides [30,31]. On the other hand, the molecules of 2-thiophene N(4)-methylthiosemicarbazone are held together in the crystal packing through an extended network of intermolecular hydrogen bonds involving the amino nitrogen atom N(1), the hydrazine nitrogen atom N(2) and the sulfur atom S(1) [N(1)–H(1A)···S(1) 3.536 Å ; $x, -y+3/2, z-1/2$; N(2) –H(2A) ··· S(1) 3.381 Å ; $-x,-y+1, -z$](Fig. 2).

Single-crystal X-ray analysis [32] reveals that complex **1** crystallizes in triclinic system, with space group P_{-1}. As shown in Fig. 3, the structure of complex **1** consists of six silver atoms, six molecules of ligand which are each singly deprotonated and four DMF solvate molecules. Four silver atoms of them are considered to be 3-

* Corresponding author.
E-mail addresses: limingxue@henu.edu.cn (M.-X. Li), jyniu@henu.edu.cn (J.-Y. Niu).

1387-7003/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.inoche.2010.07.012



Scheme 1. 2-Thiophone N(4)-methylthiosemicarbazone, HL.

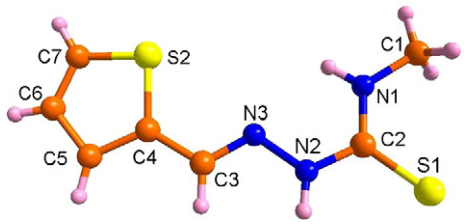


Fig. 1. Crystal structure of HL with atomic numbering scheme. Selected bond lengths (Å) and angles (°): S(1)–C(2) 1.686(3), N(2)–N(3) 1.373(3), N(1)–C(1) 1.440(4), N(1)–C(2) 1.321(4), N(2)–C(2) 1.350(4), N(3)–C(3) 1.282(4) and C(3)–N(3)–N(2) 116.4(2), C(2)–N(2)–N(3) 119.9(2), N(1)–C(2)– N(2) 117.3(2), C(2)–N(1)–C(1) 123.8(3), N(1)–C(2)–S(1) 123.8(2).

coordinate form, which are coordinated by two thiolate sulfur atoms and one imine nitrogen and the other two silver atoms are considered to be 4-coordinate form, which are coordinated by three thiolate sulfur atoms and one imine nitrogen, with four thiolate sulfur bridging two silver atoms and two thiolate sulfur bridging three silver atoms. The two six-membered Ag_3S_3 rings adopt a chair configuration stacked one above the other and linked together by two bridging AgS units, while the two six-membered Ag_3S_3 rings of $[Ag_6(\mathbf{L^1}H)_6]$ with a wheel-type conformation were linked together by 6 bridging NCS units of the $\mathbf{L^1}H$ ligands [24[a]]. Therefore, the complex **1** under the analogous conditions contains an unusual hexanuclear silver cluster [23]. The central core Ag_2S_2 is almost square planar(S–Ag–S, Ag–S–Ag, 87.43°, 92.57°). Within the ring systems the Ag···Ag distances are 2.966(1), 2.993(1) and 3.197(1) Å, these distances are similar to that in metallic silver (2.889 Å), shorter than the sum of the van der Waals radii of two silver atoms (3.44 Å) [22], suggesting the existence of some metal–metal interaction between the silver atoms. The coordination of a potentially tridentate thiosemicarbazone in silver(I) complex without using its thiophene sulfur is unusual. It is interesting to compare the structure of the ligand in the free state and in the complex **1**. The largest difference may be due to the distance around atom C(2). In the free ligand, the bond distances of C(2)–N(2) of 1.35 Å and C(2)–S(1) of 1.69 Å suggest that C(2)–N(2) is the single bond and C(2)–S(1) is the double bond, while in the complex **1**, the bond distances of C(2)–N(2) of 1.31 Å and C(2)–S(1) of 1.78 Å show that C(2)–N(2) is the double bond and C(2)–S(1) is the single bond [21[b]]. These results indicate the presence of the Ag–S–Ag, formed by the elongation of the –NH–C=S group in the free ligand to –N=C–S–, and the ligand coordinates to the metal through the sulfur after deprotonation. The Ag–N bond distance is 2.420(1) Å, similar to those found in the complexes of Ag(I) and nitrogen-containing heterocyclic ligands [33]. Each thiolate sulfur atom bridges asymmetrically silver atoms with Ag–S bond lengths of 2.467(1) and 2.568(1) Å, in the same range as other polynuclear silver complexes [23,34–36].

Since the thiosemicarbazone moieties have both the hydrogen bond donors (and/or acceptors), the species provide the possibility to form hydrogen bonds in the crystal. As shown in Fig. 4, the oxygen atoms in the DMF molecules each interact with hydrogen atoms from the thioamide groups of different molecules to form the hydrogen bonding. The separations for N(1)···O(1) , N(4)···O(1) (symmetry code A: $-x+1, -y+1, -z$) and N(7)···O(1) (symmetry code A: $x+1, y, z$) are 3.003, 3.126 and 3.087 Å with the N–H···O angles being 147.4, 156.3 and 163.4°, respectively.

The infrared spectral bands most useful for determining the mode of coordination of 2-thiophene N(4)-methylthiosemicarbazone are the ν(C=N) and ν(C=S) vibrations. The ν(C=N) band of the ligand and the complex is found at 1601 and 1577 cm^{-1}, respectively. The decrease in the frequency of this band in the spectra of the complex is an evidence for the coordination *via* the azomethine nitrogen atom [37]. In the free ligand, a band at 858 cm^{-1} is assigned to ν(C=S), whereas in the complex **1** this band is found to be shifted to lower frequency (822 cm^{-1}), indicating the coordination of sulfur to the silver center. These observations have also been confirmed by X-ray single-crystal structure analysis.

In view of the antimicrobial activity of thiosemicarbazone [38–40], we have tested the ability of the parent ligand and its silver complex against representative bacteria and fungi by the disc diffusion method [41,42]. Based on the minimum inhibitory concentration (Table 1), generally, the title two compounds display moderate antibacterial effect and more inhibitory properties against Gram positive bacteria than against Gram negative bacteria. In addition, the free ligand showed enhanced activity compared to its silver complex. In contrast, both the ligand and the title complex exert a poor growth inhibition against fungi. Detailed studies of cytotoxicity mechanisms are in progress.

IC_{50} values (compound concentration that produces 50% of cell death) were calculated for the free ligand and the title complex against liver cancer SMMC-7721 cell lines [43]. Complex **1** exhibited

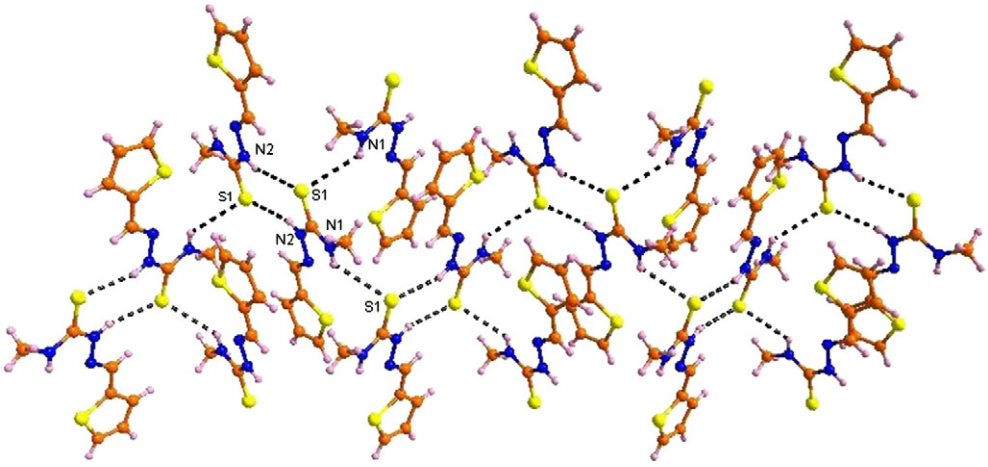


Fig. 2. Molecular packing of HL. Dashed lines indicate hydrogen bonds.

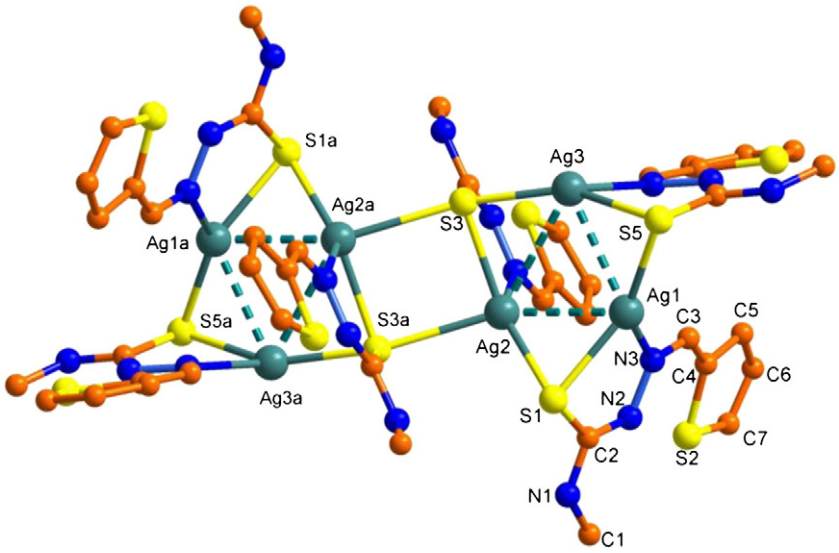


Fig. 3. Crystal structure of complex **1** with atomic numbering scheme, solvent molecules and all hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (°): Ag(1)–N(3) 2.326(4), Ag(1)–N(5) 2.420(1), Ag(2)–N(6) 2.324(4), Ag(1)–S(1) 2.496(2), Ag(2)–S(1) 2.467(1), Ag(2)–S(3) 2.568(1), S(1)–C(2) 1.778(5), S(3)–C(9) 1.745(5), S(5)–C(16) 1.780(5) and N(3)–Ag(1)–S(5) 135.0(1), N(3)–Ag(1)–S(1) 77.30(11), N(5)–Ag(1)–S(1) 147.4(1), S(1)–Ag(2)–S((3) 162.3(1), S(3)–Ag(3)–S(5) 125.7(1), Ag(2)–S(1)–Ag(1) 73.40(4), Ag (3)–S(3)–Ag(2) 79.59(4), Ag(1)–S (5)–Ag(3) 74.46(4).

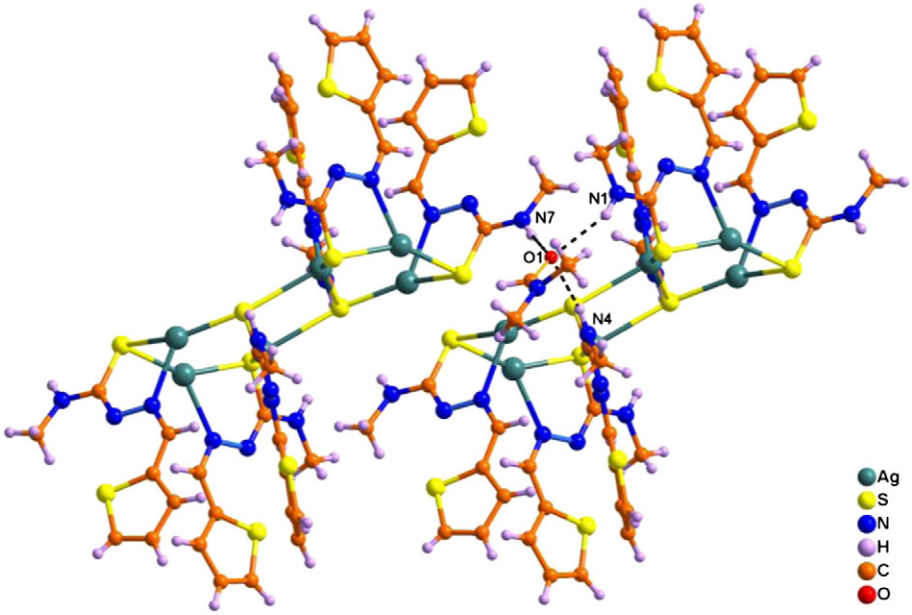


Fig. 4. Hydrogen bond in dashed lines in complex **1.**

significant antitumor activity with $IC_{50} = 13.2\,\mu M$ while the free ligand had no antitumor activity at all. The distinct difference in the antitumor potency of the free ligand and the title complex further justifies the purpose of this study. A thorough investigation regarding the toxicity and biological effects of the title silver complex is essential for medical practice as a metal-based drug.

In summary, 2-thiophene N(4)-methylthiosemicarbazone and its hexanuclear Ag(I) complex were synthesized and fully characterized. The title two compounds show distinct difference in the biological property.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China, the Natural Science Foundation of Henan Province(102300410093), the China Postdoctoral Science Foundation

Table 1
Antibacterial and antifungal activities of the tested compounds.

Microorganism	MIC mg/mL	
	HL	1
B. subtilis	0.25	0.5
S. aureus	0.25	0.5
A.tumefaciens	0.25	1.0
E.coli	1.0	–[a]
S. typhimurium	1.0	1.0
P.aeruginosa	0.25	1.0
A.niger	0.5	–
M.mucedo	–	–
P.oxalicum	–	–
C.lusitaniae	–	–
C.albicans	1.0	–

[a] No inhibition or MIC>2 mg/mL.

(20090460847), the Foundation for University Young Key Teacher by Henan Province (2009GGJS-025) and the Natural Science Foundation of the Educational Department of Henan Province (2010B150003).

Appendix A. Supplementary data

CCDC 770639 and 770640 contain the supplementary data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Center via www.ccdc.cam.ac.uk/data_request/cif.

References

[1] S.B. Padhye, G.B. Kauffman, Coord. Chem. Rev. 63 (1985) 127.
[2] D.X. West, A.E. Liberta, S.B. Padhye, R.C. Chikate, P.B. Sonawane, A.S. Kumbhar, R.G. Xerande, Coord. Chem. Rev. 123 (1993) 49.
[3] A. Murugkar, S. Padhye, S. Guha-Roy, U. Wagh, Inorg. Chem. Commun. 2 (1999) 545.
[4] M. Baldini, M. Belicchi-Ferrari, F. Bisceglie, G. Pelosi, S. Pinelli, P. Tarasconi, Inorg. Chem. 42 (2003) 2049.
[5] S. Padhye, Z. Afrasiabi, E. Sinn, J. Fok, K. Mehta, N. Rath, Inorg. Chem. 44 (2005) 1154.
[6] C.R. Kowol, R. Trondl, P. Heffeter, V.B. Arion, M.A. Jakupec, A. Roller, M. Galanski, W. Berger, B.K. Keppler, J. Med. Chem. 52 (2009) 5032.
[7] A.P. Rebolledo, M. Vieites, D. Gambino, O.E. Piro, E.E. Castellano, C.L. Zani, E.M. Souza-Fagundes, L.R. Teixeira, A.A. Batista, H. Beraldo, J. Inorg. Biochem. 99 (2005) 698.
[8] D.X. West, G.A. Bain, R.J. Butcher, J.P. Jasinski, Y. Li, R.Y. Pozdniakiv, J. Valdes-Martinez, R.A. Toscano, S. Hernandes-Ortega, Polyhedron 15 (1996) 665.
[9] D. Kovala-Demertzi, A. Domopoulou, M. Demertzis, C.P. Paptopoulou, A. Terzis, Polyhedron 13 (1994) 1917.
[10] N. Farrell, Coord. Chem. Rev. 232 (2002) 1.
[11] D. Kovala-Demertzi, A. Papageorgiou, L. Papathanasis, A. Alexandratos, P. Dalezis, J.R. Miller, M.A. Demertzis, Bioorg. Med. Chem. Lett. 44 (2009) 1296.
[12] Z. Iakovidou, E. Mioglou, D. Mourelatos, A. Kotsis, M.A. Demertzis, A. Papagoergiou, J.R. Miller, D. Kovala-Demertzi, Anticancer Drugs 12 (2001) 65.
[13] E. López-Torres, M.A. Mendiola, C.J. Pastor, B.S. Pérez, Inorg. Chem. 43 (2004) 5222.
[14] I. Pal, F. Basuli, T.C.W. Mak, S. Bhattacharya, Angew. Chem. Int. Ed. 40 (2001) 2923.
[15] R. Curran, J. Lenehan, M. McCann, K. Kavanagh, M. Devereux, D.A. Egan, G. Clifford, K. Keane, B.S. Creaven, V. McKee, Inorg. Chem. Commun. 10 (2007) 1149.
[16] K. Nomiya, S. Takahashi, R. Noguchi, J. Chem. Soc, Dalton Trans, 2000 2091.
[17] K. Nomiya, S. Takahashi, S.R. Noguchi, S. Nemoto, T. Takayama, M. Oda, Inorg. Chem. 39 (2000) 3301.
[18] W. Su, M. Hong, J. Weng, Y. Liang, Y. Zhao, R. Cao, Z. Zhou, A.S.C. Chan, Inorg. Chim. Acta 331 (2002) 8.
[19] T.S. Lobana, R. Sharma, R.J. Butcher, Polyhedron 27 (2008) 1375.
[20] B. Liu, G.-W. Zhou, M.-L. Fu, L. Xu, G.-C. Guo, J.-S. Huang, Bull. Korean Chem. Soc. 25 (2004) 1937.
[21] (a) M.-X. Li, C.-L. Chen, C.-S. Ling, J. Zhou, B.-S. Ji, Y.-J. Wu, J.-Y. Niu, Bioorg. Med. Chem. Lett. 19 (2009) 2704;
(b) M.-X. Li, Y. Bai, B.-G. Zhang, C.-Y. Duan, J. Xu, Q.-J. Meng, Inorg. Chem. 44 (2005) 5459;
(c) M.-X. Li, Q.-Z. Sun, Y. Bai, C.-Y. Duan, B.-G. Zhang, Q.-J. Meng, Dalton Trans. (2006) 2572;
(d) M.-X. Li, J. Zhou, C.-L. Chen, J.-P. Wang, Z. Naturforsch. 63b (2008) 280;
(e) M.-X. Li, J. Zhou, Z.-L. Wang, J.-P. Wang, Chinese J. Struct. Chem. 27 (2008) 281;
(f) M.-X. Li, J. Zhou, H. Zhao, C.-L. Chen, J.-P. Wang, J. Coord. Chem. 62 (2009) 1423;
(g) D. Zhang, Q. Li, M.-X. Li, D.-Y. Chen, J.-Y. Niu, J. Coord. Chem. 63 (2010) 1063;
(h) M.-X. Li, C.-L. Chen, D. Zhang, J.-Y. Niu, B.-S. Ji, Eur. J. Med. Chem. 45 (2010) 3169.
[22] A. Castiñeiras, I. García-Santos, S. Dehnen, P. Sevillano, Polyhedron 25 (2006) 3653.
[23] L.J. Ashfield, A.R. Cowley, J.R. Dilworth, P.S. Donnelly, Inorg. Chem. 43 (2004) 4121.
[24] (a) R. Pedrido, M.J. Romero, M.R. Bermejo, M. Martínez-Calvo, A.M. González-Noya, G. Zaragoza, Dalton Trans. (2009) 8329;
(b) T.S. Lobana, R. Sharma, R.J. Butcher, Polyhedron 28 (2009) 1103.
[25] Synthesis of HL: Thiophene-2-carboxaldehyde (0.45 g, 4.0 mmol) was added dropwise to an ethanol solution (30 mL) of 4-methyl-3-thiosemicarbazide (0.44 g, 4.0 mmol) with five drops of acetic acid as catalyst. After refluxed for 2 h, the resultant solution was filtered. Brown crystals suitable for X-ray studies were obtained by slow evaporation of its ethanol solution. Anal. Calcd. for C7H9N3S2: C 42.15, H 4.52, N 21.07; found C 42.02, H 4.34, N 20.85.
[26] Synthesis of 1: AgNO3(0.07 g, 0.4 mmol) and 2-thiophene N(4)-methylthiosemicarbazone (0.08 g, 0.4 mmol) were suspended in 30 mL of ethanol and refluxed for 0.5 h with stirring. The resulting precipitate was filtered off, washed with cold ethanol and dried in a vacuum. Brown crystals suitable for X-ray diffraction were obtained by slow evaporation of its DMF solution. Anal. Calcd. for C54H76Ag6-N22O4S12: C 30.43, H 3.57, N 14.46; found C 30.21, H 3.71, N 14.19.
[27] Crystal data for HL: C7H9N3S2 (199.29), Crystal dimensions 0.36×0.31×0.27 mm3, monoclinic, P21/c, a=8.0897(7) Å, b=13.286(1) Å, c=9.454 (1) Å, β=98.770(2)°, V=1004.3(1) Å 3, Z=4, ρcalcd=1.318 Mg m–3, 5020 reflections collected, 1447 unique (Rint=0.0212), R1=0.0498 [I>2σ (I)], wR2=0.1608 (all data). The data were collected on a Bruker APEX-II CCD diffractometer (MoKa, λ=0.71073 Å) at 293(2) K. All structures were solved by the direct method and refined by the full-matrix least-squares on F2 using the SHELXL-97 [28, 29]. All of the non-hydrogen atoms were refined anisotropically.
[28] G.M. Sheldrick, SHELXS 97, Program for Crystal Structure Solution, University of Göttingen, Göttingen, 1997.
[29] G.M. Sheldrick, SHELXL 97, Program for Crystal Structure Refinement, University of Göttingen, Göttingen, 1997.
[30] A. Sreekanth, M.R.P. Kurup, Polyhedron 23 (2004) 969.
[31] D.X. West, G.A. Bain, R.J. Butcher, J.P. Jasinski, Y. Li, R.Y. Pordniakiv, Polyhedron 15 (1996) 665.
[32] Crystal data for 1: C54H76Ag6N22O4S12 (2129.31), Crystal dimensions 0.29×0.21×0.13 mm3, triclinic, space group P-1, a=10.718(2) Å, b=14.654(1) Å, c=15.048 (1) Å, α=118.851(1)°, β=95.800(2)°, γ=99.383(2)°, V=1997.7(4) Å 3, Z=1, ρcalcd=1.770 Mg·m–3, 10408 reflections collected, 5000 unique (Rint=0.0779), R1=0.0507 [I>2σ (I)], wR2=0.1190 (all data). The data were collected on a Bruker APEX-II CCD diffractometer (MoKa, λ=0.71073 Å) at 293(2) K. All structures were solved by the direct method and refined by the full-matrix least-squares on F2 using the SHELXL-97. All of the non-hydrogen atoms were refined anisotropically.
[33] Y.-B. Dong, H.-Y. Wang, J.-P. Ma, D.-Z. Shen, R.-Q. Huang, Inorg. Chem. 44 (2005) 4679.
[34] M.C. Biagini, M. Laufranchi, L. Marchiò, M.A. Pellinghelli, Inorg. Chim. Acta 305 (2000) 95.
[35] M. Yazdanbakhsh, M. Hakimi, M.M. Heravi, M. Ghassemzadeh, B. Neumüller, Z. Anorg. Allg. Chem. 631 (2005) 924.
[36] F. Adhami, M. Ghassemzadeh, M.M. Heravi, A. Taeb, Z. Anorg. Allg. Chem. 625 (1999) 1411.
[37] H.-B. Bürg, J.D. Dunitz (Eds.), Structure Correlations, VCH, Weinheim, Germany, 1994, p. 780.
[38] A. Mishra, N.K. Kaushik, A.K. Verma, R. Gupta, Eur. J. Med. Chem. 43 (2008) 2189.
[39] M. Joseph, M. Kuriakose, M.R.P. Kurup, E. Suresh, A. Kishore, S.G. Bhat, Polyhedron 25 (2006) 61.
[40] M. Joseph, V. Suni, M.R.P. Kurup, M. Nethaji, A. Kishore, S.G. Bhat, Polyhedron 23 (2004) 3069.
[41] The in vitro antibacterial activity of the free ligand and its silver complex was investigated against several representative Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, and *Agrobacterium tumefaciens*) and Gram negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*). The antifungal activity was assayed against mould (*Aspergillus niger*, *Mucor mucedo*, and *Penicillium oxalicum*) and yeast (*Candida lusitaniae*, *Candida albicans*). The minimal inhibitory concentrations (MIC, mg/mL) were estimated by the disk diffusion method [42]. The final concentration of all cultures in Mueller-Hinon agar (MHA) for bacteria and potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) for mould spores and yeast cells was adjusted to 106 cfu/mL (bacteria) or 2×105 cfu/mL (mould spores and yeast cells) and used for inoculation in the MIC test. Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO) were prepared at concentrations of 0–2000 μg/mL. To each plate was inoculated with 0.1 mL of the prepared bacterial and fungi cultures. Similarly, each plate carried a blank disc, with solvent DMSO only in the center to serve as negative control. The inoculated plates were then incubated at either 37 °C for 18–20 h (bacteria) or 28 °C for 48–96 h (fungi), respectively. The minimal inhibitory concentration (MIC) was detected as the lowest concentration of drug in plate for which no visible growth took place by macroscopic evaluation. All determinations were performed in triplicate and confirmed by three separate experiments.
[42] S.A. Khan, K. Saleem, Z. Khan. Eur. J. Med. Chem. 42 (2007) 103.
[43] SMMC-7721, a human liver cancer cell line (purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS), was cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U mL−1 of penicillin, 100 μg (200 μL per well) of streptomycin at 37 °C in humid air atmosphere of 5% CO2. Cell cytotoxicity was assessed by the MTT assay. Briefly, cells were placed into a 96-well-plate (5×103 cells per well). The next day the compound diluted in culture medium at various concentrations was added (200 μL per well) to the wells. 48 h later 20 μL of MTT (0.5 mg mL−1 MTT in PBS) was added and cells were incubated for a further 4 h. 200 μL of DMSO was added to each culture to dissolve the MTT crystals. The MTT-formazan product dissolved in DMSO was estimated by measuring absorbance at 570 nm with a micro plate reader. Then the inhibitory percentage of each compound at various concentrations was calculated, and the IC50 value was determined.