View Online / Journal Homepage / Table of Contents for this issue
Dynamic Article Links

Dalton Transactions

Cite this: *Dalton Trans.*, 2012, **41**, 12882

www.rsc.org/dalton

PAPER

# One dodecahedral bismuth(III) complex derived from 2-acetylpyridine *N*(4)-pyridylthiosemicarbazone: synthesis, crystal structure and biological evaluation†

**Mingxue Li,*[a] Yanli Lu,[a] Min Yang,[a] Yanke Li,[a] Lizhi Zhang[a] and Songqiang Xie*[b]**

***Received 12th June 2012, Accepted 27th August 2012***
**DOI: 10.1039/c2dt31256e**

Downloaded by Wuhan University on 15 October 2012
Published on 29 August 2012 on http://pubs.rsc.org | doi:10.1039/C2DT31256E

One dodecahedral bismuth(III) complex [Bi(HL)$(NO_3)_3$] (**1**) (HL = 2-acetylpyridine *N*(4)-pyridylthiosemicarbazone) has been synthesized and structurally characterized. The analytical data reveals the formation of 1 : 1 (metal : ligand) stoichiometry. The bismuth(III) ion is nine-coordinated by one electron pair ($6s^2$) of the bismuth(III) atom, two nitrogen and one sulfur atoms from the $N_2S$ tridentate ligand and five oxygen atoms from three nitrate ions. Biological studies, carried out *in vitro* against eight selected bacteria, and four human cancer cells, respectively, have indicated that **1** shows better growth-inhibiting properties. Upon further investigation, **1** might produce cytotoxicity through apoptosis.

## Introduction

Bismuth is regarded nowadays as a relatively non-toxic heavy metal. The bismuth(III) atom, with a larger ionic radius (1.16 Å),[1] has one inert electron pair ($6s^2$) and can form complexes with higher coordination numbers. Corresponding to its various coordination numbers, from 3 to 10,[2,3] the structures of Bi(III) compounds are irregular. Bismuth compounds have been widely used clinically for centuries because of their high effectiveness and low toxicity in the treatment of a variety of microbial infections, including syphilis, diarrhoea, gastritis and colitis.[4,5] The efficacy of thr recently developed bismuth-based triple therapy in the eradication of *Helicobacter pylori* (the bacterium which is mainly responsible for gastritis, peptic and duodenal ulcers, and gastric cancer) from patients exceeded the normal PPI-based (proton pump inhibitor-based) therapies.[6] Apart from antimicrobial activity, bismuth compounds also exhibit anticancer activities. $^{212}$Bi and $^{213}$Bi compounds have been used as targeted radio-therapeutic agents for cancer treatment, and furthermore they have the ability to reduce the side-effects of cisplatin in cancer therapy. Their good activities as antimicrobial and antitumour agents[7] mean that there is a scope for the development of new and effective bismuth based drugs.

Thiosemicarbazones and their metal complexes have received considerable attention due to their coordination chemistry and a broad range of pharmacological properties, notable for antiparasital, antibacterial and antitumor activities.[8–13] A typical representative of these compounds is 3-aminopyridine carbaldehyde thiosemicarbazone (Triapine), which is currently undergoing clinical trials.[14] In general, thiosemicarbazones are obtained by condensation of the corresponding thiosemicarbazide with aldehydes or ketones. The studies indicate that the biological activities of thiosemicarbazones often show a high dependence on their substituents. Minor modifications in thiosemicarbazones can lead to significant changes in biological activity. Earlier reports on N(4)-substituted thiosemicarbazones have concluded that the presence of a bulky group at the terminal nitrogen considerably increases biological activity.[15–17] Moreover, the biological properties of thiosemicarbazones are often related to metal ion coordination in different ways since some of them increase the biological activity by forming chelates with specific metal ions.[18,19] 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.[20–22] Their mechanism of action is still controversial in many respects and has been identified, including ribonucleotide reductase inhibition, metal dependent radical damage, DNA binding, and inhibition of protein synthesis.[23–26]

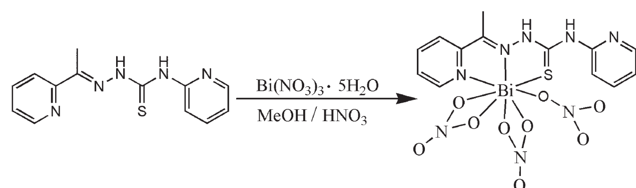
2-Acetylpyridine thiosemicarbazone is the first antimalarial thiosemicarbazone, which displays a higher activity when the N(4) position is either disubstituted or part of a ring system.[27] *N*,*N*,*S*-Tridentate thiosemicarbazones derived from 2-acetylpyridine form an important class of compounds possessing biological activity.[28,29] Although thiosemicarbazones and their metal complexes derived from 2-acetylpyridine, *N*(4)-pyridylthiosemicarbazone have been investigated,[30] to our knowledge, there are no reports in the literature on structural and biological studies of its bismuth(III) complexes. Furthermore, comparatively few structural studies of bismuth(III) complexes containing thiosemicarbazones have been published.[31] More importantly, over the years, the limited success in metal-based anticancer research appears to be caused by the relative lack in structural diversity encountered in the reported compounds.[32]

[a]*Institute of Molecular and Crystal Engineering, College of Chemistry and Chemical Engineering, Henan University, Kaifeng 475004, PR China. E-mail: limingxue@henu.edu.cn; Fax: (+86) 3783886876*
[b]*Institute of Chemical biology, Henan University, Kaifeng 475004, PR China. E-mail: kfxsq@yahoo.com.cn*

† CCDC reference number 885098. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c2dt31256e


This journal is © The Royal Society of Chemistry 2012

Published on 29 August 2012 on http://pubs.rsc.org | doi:10.1039/C2DT31256E
Downloaded by Wuhan University on 15 October 2012




**Scheme 1** The reaction scheme for the synthesis of **1**.

Stimulated by the above-mentioned encouraging and promising results, it seems useful and desirable for us to synthesize and characterize the bismuth(III) complex formulated as [Bi(HL)-$(NO_3)_3$] (**1**) of 2-acetylpyridine *N*(4)-pyridylthiosemicarbazone (Scheme 1). Also, we have evaluated its *in vitro* biological activity. It is worth noting that cytotoxic agents can induce cell death through various pathways including necrosis and apoptosis.[33] Apoptosis is a common process of programmed cell death and is the focus of oncology research at present. To provide an insight into the pharmacological properties of these compounds initially, double staining of HepG2 cells with FITC-labeled Annexin V and propidium iodide (PI) is used to illustrate the drug-induced apoptosis of **1**.

## Results and discussion

### X-Ray crystallography

Here, it should be noted that there has been a confusion in the coordination number of the bismuth(III) complexes. Some groups have counted an electron pair ($6s^2$) of the bismuth(III) atom as one of the coordination numbers,[34,35] while other groups did not.[36–38] Since an electron pair of the bismuth(III) atom is reaction inert, but forces steric pressure on the coordination interaction between the ligand and the bismuth(III) atom, *i.e.*, stereochemically active, it can be counted in the coordination number of the bismuth(III) complexes. From the viewpoint of the valence shell electron pair repulsion rule (VSEPR) for main group elements,[1] it will be also reasonable to count an inert electron pair of the bismuth(III) atom as one coordination number.

As shown in Fig. 1A, the molecular structure of **1** is depicted as a monomeric, 9-coordinate complex formed by one electron pair of the bismuth(III) atom, one tridentate neutral thiosemicarbazone ligand and five oxygen atoms from one monodentate and two bidentate nitrate ions. One dodecahedral geometry is formed (Fig. 1B). The distortion in geometry from the more regular dodecahedron may, at least in part, be due to the lone electron pair of bismuth(III). The tridentate neutral thiosemicarbazone coordinates to the bismuth(III) with its imine nitrogen, thione sulfur and pyridine nitrogen atoms, resulting in two five-membered chelate rings whose dihedral angle is 17.3°. Two bidentate nitrate ions coordinate to the bismuth(III) *via* [O,O] forming two four-membered chelate rings. While the O(5) atom on the monodentate nitrate ion coordinates to the bismuth(III) atom from one side of the plane formed by S(1), N(4), N(5) and an electron pair [the sum of angles N(5)–Bi(1)–N(4), N(4)–Bi(1)–S(1), S(1)–Bi(1)–O(2), O(2)–Bi(1)–O(7) and O(7)–Bi(1)–N(5) is 344.9°]. The bond distances around the bismuth(III) atom [Bi(1)–N(5) 2.610(9), Bi(1)–N(4) 2.435(9), Bi(1)–S(1) 2.632(3), Bi(1)–O(2) 2.566(9), Bi(1)–O(7) 2.746(7), Bi(1)–O(5) 2.508(6), Bi(1)–O(1) 2.744(9), Bi(1)–O(9) 2.671(8)] (Table 1) are compared with other data in related bismuth(III) complex $[Bi(O_2CC_9H_6N)_2(O_3N)(O_2CC_9H_6NH)]\cdot 2H_2O$ which is also a distorted dodecahedral geometry.[39]

The molecules of complex **1** are held together in the crystal packing through intramolecular hydrogen bonds involving the hydrazine nitrogen N(3) atom, the uncoordinated nitrogen N(1) atom of the pyridyl ring and intermolecular hydrogen bonds involving the terminal nitrogen N(2) atom, and the oxygen atom O(6) of the nitrate ion, respectively (Fig. 2). The distances for N(1)···N(3) and N(2)···O(6) (symmetry code: $-x + 2, -y, -z$) are 2.717(13) and 2.879(12) Å with the N(1)–H···N(3) angle being 130.6° and N(2)–H···O(6) 159.8°, respectively.

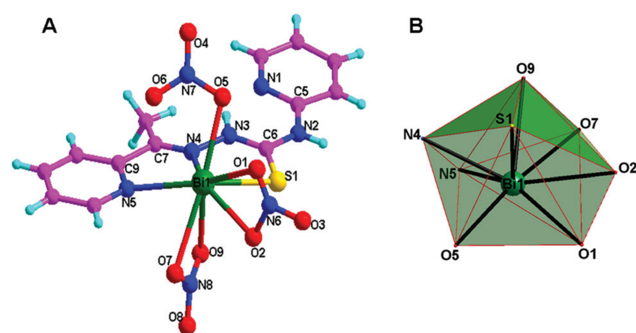



**Fig. 1** (A) Structure of complex **1** with atomic numbering scheme. (B) Polyhedron showing dodecahedral geometry around the bismuth atom of the asymmetric unit.

### Antimicrobial activity

In view of the antimicrobial activity of thiosemicarbazones,[40] we have tested the inhibition ability of the obtained compounds as well as the starting compound $Bi(NO_3)_3\cdot 5H_2O$ against the

**Table 1** Selected bond lengths [Å] and angles [°] of the complex **1**

| | | | |
|---|---|---|---|
| Bi(1)–N(4) | 2.435(9) | N(3)–C(6) | 1.318(11) |
| Bi(1)–O(5) | 2.508(6) | N(3)–N(4) | 1.294(7) |
| Bi(1)–N(5) | 2.610(9) | N(4)–C(7) | 1.326(11) |
| Bi(1)–O(2) | 2.566(9) | N(5)–C(9) | 1.324(13) |
| Bi(1)–S(1) | 2.632(3) | C(7)–C(9) | 1.506(14) |
| Bi(1)–O(9) | 2.671(8) | N(6)–O(1) | 1.222(8) |
| Bi(1)–O(7) | 2.746(7) | N(6)–O(2) | 1.233(8) |
| Bi(1)–O(1) | 2.744(9) | N(8)–O(9) | 1.197(7) |
| S(1)–C(6) | 1.748(11) | N(8)–O(7) | 1.223(8) |
| N(4)–Bi(1)–O(5) | 74.9(3) | S(1)–Bi(1)–O(9) | 74.8 (2) |
| N(4)–Bi(1)–N(5) | 64.4(3) | N(4)–Bi(1)–O(7) | 109.9(3) |
| O(5)–Bi(1)–N(5) | 108.1(3) | O(5)–Bi(1)–O(7) | 171.2(2) |
| N(4)–Bi(1)–O(2) | 141.4(3) | N(5)–Bi(1)–O(7) | 68.9(3) |
| O(5)–Bi(1)–O(2) | 115.2(3) | O(2)–Bi(1)–O(7) | 65.9(3) |
| N(5)–Bi(1)–O(2) | 134.0(3) | S(1)–Bi(1)–O(7) | 108.9(2) |
| N(4)–Bi(1)–S(1) | 72.4(2) | O(9)–Bi(1)–O(7) | 45.4(2) |
| O(5)–Bi(1)–S(1) | 79.40(18) | N(4)–Bi(1)–O(1) | 138.1(3) |
| N(5)–Bi(1)–S(1) | 131.6(2) | O(5)–Bi(1)–O(1) | 70.2(3) |
| O(2)–Bi(1)–S(1) | 73.3(2) | N(5)–Bi(1)–O(1) | 149.3(3) |
| N(4)–Bi(1)–O(9) | 71.6(3) | O(2)–Bi(1)–O(1) | 47.6(3) |
| O(5)–Bi(1)–O(9) | 142.5(2) | S(1)–Bi(1)–O(1) | 78.9(2) |
| N(5)–Bi(1)–O(9) | 71.9(3) | O(9)–Bi(1)–O(1) | 128.9(3) |
| O(2)–Bi(1)–O(9) | 82.8(3) | O(7)–Bi(1)–O(1) | 107.9(3) |

View Online

Downloaded by Wuhan University on 15 October 2012
Published on 29 August 2012 on http://pubs.rsc.org | doi:10.1039/C2DT31256E

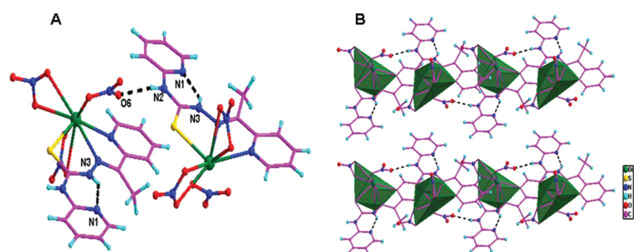



**Fig. 2** (A) Hydrogen bond in dashed lines in complex **1**. (B) The molecular packing projected along the *a*-axis of complex **1**.

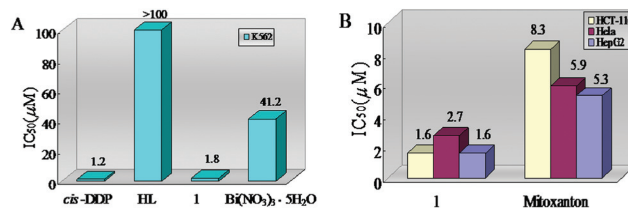



**Fig. 3** (A) The cytotoxicity of the tested compounds against K562 leukaemia cells. (B) The cytotoxicity of **1** on the other three different kinds of tumor cells, compared with Mitoxantone.

**Table 2** Minimal inhibitory concentration (μg $mL^{-1}$) of the tested compounds

| | MIC (μg $mL^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| Microorganism | HL | 1 | $Bi(NO_3)_3{\cdot}5H_2O$ | Amp | Cm | Kan |
| *B. subtilis* | 15.6 | 62.5 | 1000 | 0.24 | 15.6 | 15.6 |
| *S. aureus* | 125 | 125 | 1000 | 0.48 | 62.5 | 7.81 |
| *B. cereus* | 125 | 15.6 | 500 | —[a] | 31.2 | 62.5 |
| *S. lutea* | 125 | 125 | 500 | 0.48 | 15.6 | 125 |
| *P. aeruginosa* | 31.2 | 31.2 | 250 | 1.95 | 31.2 | 3.9 |
| *S. typhimurium* | 31.2 | 62.5 | 500 | 125 | 31.2 | 31.2 |
| *E. coli* | 125 | —[a] | 500 | 250 | 31.2 | 31.2 |
| *A. tumefaciens* | 125 | 125 | 500 | 0.48 | 15.6 | 3.9 |

[a] No inhibition or MIC > 1000 μg $mL^{-1}$.

selected four Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus* and *Sarcina lutea*) and four Gram negative bacteria (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli* and *Agrobacterium tumefaciens*) by the disc diffusion method.[41] Based on the minimum inhibitory concentrations (Table 2), generally, both the free ligand and **1** exhibit broad and effective activities against the tested microorganisms. However, $Bi(NO_3)_3{\cdot}5H_2O$ alone is inactive under the same experimental conditions. It should be emphasized that **1** showed higher activity against Gram positive bacteria *Bacillus cereus* (MIC = 15.6 μg $mL^{-1}$) than its parent ligand, positive control antibiotics ampicillin (Amp), chloramphenicol (Cm), kanamycin sulfate (Kan), respectively. Hence a synergistic effect involving the metal and the thiosemicarbazone could probably explain the improved antimicrobial activities.

## Cytotoxicity assay

In terms of the cytotoxic activity of thiosemicarbazones,[42,43] firstly, we have evaluated the ability of the obtained compounds to inhibit tumor cell growth against human leukaemia K562 cells. $IC_{50}$ values (compound concentration that produces 50% of cell death) in micromolar units are calculated (Fig. 3A). The human clinical drug, cisplatin (*cis*-DDP), is used as the reference compound for comparison. The comparison of the cytotoxic activities indicates that **1** shows a much lower $IC_{50}$ value (1.8 μM) than both HL (>100 μM) and $Bi(NO_3)_3{\cdot}5H_2O$ (41.2 μM), indicating that coupling of HL to $Bi(NO_3)_3{\cdot}5H_2O$ leads to an enhancement of the cytotoxicity of the free ligand. This confirms that complexation with a metal has a synergetic effect on the cytotoxicity.[18,19] These results are consistent with the cases of many other analogues of thiosemicarbazones.[44] Importantly, it should be emphasized that **1** shows excellent activity, similar to that of cisplatin (1.2 μM).[31d] Therefore, complex **1** merits further biological screening as well as studies of the mechanism of action.

Later on, upon further analysis, **1** exhibits prominent inhibition against the growth of HCT-116 cells (human colorectal cancer), Hela cells (human cervical carcinoma), and HepG2 cells (human hepatocellular carcinoma). Mitoxantone is a kind of antibiotic antitumor drug, and was used as the reference compound for comparison. Remarkably, the $IC_{50}$ values of complex **1** are 1.6, 2.7 and 1.6 μM, and are lower than those of Mitoxantone against HCT-116 cells, Hela cells and HepG2 cells, respectively (Fig. 3B). Therefore, a detailed mechanism study is necessary.

## Cellular apoptotic evaluation

Apoptosis or programmed cell death, a favorable ultimate fate of tumor cells, is a complicated cellular process and a major field of interest in antitumor drug development.[45] Herein, double staining of HepG2 cells with FITC-labeled Annexin V and propidium iodide (PI) is used to illustrate the drug-induced apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V, a $Ca^{2+}$ dependent phospholipid-binding protein that has a high affinity for PS, can bind to cells with exposed PS. Annexin V may be conjugated to fluorochromes such as FITC. This format retains its high affinity for PS and thus serves as a sensitive probe for the analysis of cells that are undergoing apoptosis. Since externalization of PS appears in the earlier stages of apoptosis, Annexin V-FITC staining can identify apoptosis at an earlier stage. Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI ,and the PI bind to cellular DNA. This assessment can distinguish cell apoptosis from necrosis.

Caspase-3 is one of the key proteases in early apoptosis. So, the activation of caspase-3 is measured by indirect immunofluorescence, using primary antibody against the cleaved portions of caspase-3.

In order to confirm that the cell death in HepG2 cells is due to apoptosis, the apoptotic effect of **1** is evaluated by Annexin-V-FITC apoptosis detection kit, and caspase-3 activation kit combined with a high-content screening (HCS) system. As

 This journal is © The Royal Society of Chemistry 2012

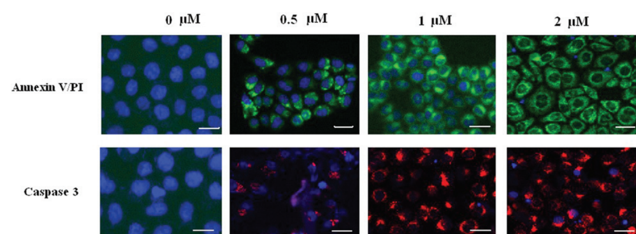



**Fig. 4** Annexin-V FITC/PI double staining and caspase-3 activation was measured in HepG2 cells by indirect immunofluorescence. Cells were plated in triplicate in 24-well plates overnight and treated with **1** (0.5, 1 and 2 μM) for 48 h, then stained for either active annexin V/PI or caspase 3 by indirect immunofluorescence using antibody against cleaved caspase-3. The secondary antibody used was conjugated with Cy3 (red) caspase-3. Images were acquired on the ArrayScan® HCS Reader using Cellomics' Target Activation BioApplication. Scale bar = 10 μm.

Published on 29 August 2012 on http://pubs.rsc.org | doi:10.1039/C2DT31256E
Downloaded by Wuhan University on 15 October 2012

shown in Fig. 4, after incubation for 48 h, **1** induces HepG2 cell apoptosis. We can see nuclear condensation, loss of cells and an increase in caspase-3 activation as evidenced by the staining of active (cleaved) caspase-3.

## Conclusions

In summary, one dodecahedral bismuth(III) complex **1** derived from 2-acetylpyridine *N*(4)-pyridylthiosemicarbazone has been synthesized and structurally characterized. Biological studies have shown that **1** shows effective growth-inhibiting activity. In a preliminary mechanistic study, **1** might produce cytotoxicity through apoptosis, which is supported by the significant variation of Annexin V and an increase in caspase-3 activation, as evidenced by the staining of active (cleaved) caspase-3. These promising results will be essential for antibacterial and anticancer drug discovery.

## Experimental

### Synthesis

All solvents and reagents are commercially available and were used without further purification. Elemental analysis of C, H and N was performed with a Perkin-Elmer 240 analyzer. The infrared spectra were recorded from KBr discs with a Nicolet 170 FT infrared spectrophotometer. The mass spectra were carried out on an Esquire 3000 LC-MS mass spectrophotometer. $^{1}$H NMR spectra were recorded in DMSO-d$_6$ using a BrukerAV-400 spectrometer.

**HL.** The ligand HL was synthesized by the literature method.[30]

**[Bi(HL)(NO$_3$)$_3$].** A Bi(NO$_3$)$_3$·5H$_2$O (0.097 g, 0.2 mmol) solution dissolved in methanol, with the help of a few drops of nitrate acid, was added dropwise to a methanol solution (20 mL) of 2-acetylpyridine *N*(4)-pyridylthiosemicarbazone (0.054 g, 0.2 mmol). After refluxing with stirring for 1 h, the resultant mixture was filtered. The obtained crude product was further recrystallized from methanol and dried over P$_4$O$_{10}$ *in vacuo*. Yield: 88%. Elemental analysis for $C_{13}H_{13}BiN_8SO_9$ (%): calcd C, 23.43; H, 1.97; N, 16.82; found C, 23.41; H, 1.85; N, 16.95. MS (ESI – negative, CH$_3$OH) (*m*/*z*): 760.0 = [Bi(HL)(NO$_3$)$_4$(CH$_3$OH)]$^-$, calc. mass = 760.1. IR [KBr, $\nu$ (cm$^{-1}$)]: 3238 and 3092 (NH), 1579 (C═N), 1164 (N–N), 776 (C═S). UV-vis [$\lambda$ (nm), CH$_3$OH]: 327, 401. $^{1}$H NMR (400 MHz, DMSO-d$_6$, ppm): 10.81 (s, 1H, NH), 9.51 (s, 1H, NH), 8.95 (s, 2H, Py), 8.38 (d, $J$ = 4 Hz, 2H, Py), 7.94 (s, 2H, Py), 7.17 (s, 2H, Py), 2.72 (s, 3H, CH$_3$). Orange crystals suitable for X-ray studies were obtained by slow evaporation of its methanol solution.

### Single-crystal X-ray diffraction measurements

Intensity data of **1** were collected on a Siemens SMART-CCD diffractometer equipped with graphite-monochromatic MoKα radiation ($\lambda$ = 0.71073 Å) using the SMART and SAINT programs.[46] The structure was solved by direct methods and refined on $F^2$ by full-matrix least-squares techniques with SHELXTL version 5.1.[47,48] All of the non-hydrogen atoms were refined with anisotropic thermal displacement parameters. The positions of hydrogen atoms were added in idealized geometrical positions. Table 3 summarizes the important crystallographic and refinement parameters for **1**. CCDC 885098 contains the supplementary crystallographic data of **1**.†

### Antimicrobial activity

We have tested the inhibition ability of the obtained compounds, as well as the starting compound Bi(NO$_3$)$_3$·5H$_2$O, against the selected four Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus* and *Sarcina lutea*) and four Gram negative bacteria (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli* and *Agrobacterium tumefaciens*) by

**Table 3** Crystallographic data for complex **1**

| Crystal data | **1** |
|---|---|
| Empirical formula | $C_{13}H_{13}BiN_8O_9S$ |
| Formula weight | 666.35 |
| Crystal system | Orthorhombic |
| Space group | $P2_12_12_1$ |
| $T$/K | 296(2) |
| $a$/Å | 9.6213(13) |
| $b$/Å | 13.3205(17) |
| $c$/Å | 15.933(2) |
| $V$/Å$^3$ | 2042.0(5) |
| $D_c$/g cm$^{-3}$ | 2.168 |
| $Z$ | 4 |
| $\mu$/mm$^{-1}$ | 8.803 |
| $\theta$/° | $1.99 \le \theta \le 26.00$ |
| $F_{000}$ | 1272 |
| Index ranges | $-11 \le h \le 11, -16 \le k \le 11, -18 \le l \le 19$ |
| Refl. collected | 4013 |
| Refl. unique | 2507 |
| $R_{int}$ | 0.0425 |
| Parameters | 289 |
| $R_1$, $wR_2$ [$I \ge 2\sigma(I)$] | 0.0406, 0.0706 |
| $R_1$, $wR_2$ (all date) | 0.0561, 0.0722 |
| Goodness-of-fit on $F^2$ | 0.937 |
| $\Delta\rho_{max}$, $\Delta\rho_{min}$/e Å$^{-3}$ | 1.023, −1.030 |

View Online

Downloaded by Wuhan University on 15 October 2012
Published on 29 August 2012 on http://pubs.rsc.org | doi:10.1039/C2DT31256E

the disc diffusion method. All microorganisms were provided by the China General Microbiological Culture Collection Center (CGMCC). The bacterial strains were grown in Mueller-Hinton Agar (MHA) plates at 37 °C. The minimal inhibitory concentrations (MIC, μg $mL^{-1}$) were estimated by the disk diffusion method.[41] The final concentration of all cultures in MHA for bacteria was adjusted to $10^6$ CFU $mL^{-1}$, and was used for inoculation in the MIC test. Serial dilutions of the test compounds (dissolved in 10% DMSO in PBS) were prepared at concentrations of 0–2000 μg $mL^{-1}$. Each plate was inoculated with 0.1 mL of the prepared bacterial cultures. Similarly, each plate carried a blank disc, with medium solvent containing 10% DMSO only in the center to serve as a control, as well as control antibiotics ampicillin (Amp), streptomycin (Str), kanamycin sulfate (Kan) for bacteria. The inoculated plates were then incubated at 37 °C for 18–20 h. The MIC was detected as the lowest concentration of drug in the plate for which no visible growth took place by macroscopic evaluation. All determinations were performed in triplicate and confirmed by three separate experiments.

### Cytotoxicity assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to evaluate cytotoxicity. Cells were plated into 96-well plates at a cell density of $1 \times 10^4$ cells per well, and were allowed to grow in a $CO_2$ incubator. After 24 h, the medium was removed and replaced by fresh medium containing the tested compounds which were dissolved in DMSO at 0.01 M and was diluted to various concentrations with phosphate-buffered saline (PBS) before the experiment, and the final concentration of DMSO is lower than 1%. After 24 h incubation, cultures were incubated in 100 μL of medium with 10 μL of 5 mg $mL^{-1}$ MTT solution for 4 h at 37 °C. The medium with MTT was removed, and 100 μL of DMSO was added to each well to dissolve the formazan. The absorbance at 570 nm was measured with a microplate reader (Bio-Tek ELX800, USA). The inhibitory percentage of each compound at various concentrations was calculated, and the $IC_{50}$ value was determined.

### Cellular apoptotic evaluation

Cell apoptosis was evaluated by an AnnexinV-FITC apoptosis detection kit, and a caspase-3 activation kit using a high content screening (HCS) technique. Briefly, the HepG2 cells were seeded in 24-well plates, and were exposed to **1** for 48 h, then harvested and stained according to manufacturer's protocol. Images were acquired on the ArrayScan® HCS Reader using Target Activation BioApplication software.

## Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (21071043).

## References


1 F. A. Cotton, G. Wilkinson and P. L. Gaus, *Basic Inorganic Chemistry*, Wiley, New York, 3rd edn, 1998.

2 F. A. Cotton, G. Wilkinson, C. A. Murillo and M. Bochmann, *Advanced Inorganic Chemistry*, John Wiley & Sons Inc., USA, 6th edn, 1999.

3 W. Levason and G. Reid, in *Comprehensive Coordination Chemistry II: Coordination Chemistry of the S, P, and F Metals*, ed. A. B. P. Lever, Elsevier Pergamon, Amsterdam, 2004, vol. 3, p. 465.

4 P. C. Andrews, R. L. Ferrero, P. C. Junk, I. Kumar, Q. Luu, K. Nguyena and J. W. Taylora, *Dalton Trans.*, 2010, **39**, 2861–2868.

5 G. G. Briand and N. Burford, *Chem. Rev.*, 1999, **99**, 2601–2657.

6 I. L. P. Beales, *BMC Gastroenterol.*, 2001, **1**, 7–15.

7 N. Yang and H. Z. Sun, *Coord. Chem. Rev.*, 2007, **251**, 2354–2366.

8 Y. Yu, D. S. Kalinowski, Z. Kovacevic, A. R. Siafakas, P. J. Jansson, C. Stefani, D. B. Lovejoy, P. C. Sharpe, P. V. Bernhardt and D. R. Richardson, *J. Med. Chem.*, 2009, **52**, 5271–5294.

9 K. Singh, M. S. Barwa and P. Tyagi, *Eur. J. Med. Chem.*, 2006, **41**, 147–153.

10 M. S. Karthikeyan, D. J. Prasad, B. Poojary, B. K. Subramanya, B. S. Holl and N. S. Kumari, *Bioorg. Med. Chem.*, 2006, **14**, 7482–7489.

11 C. R. Kowol, R. Trondl, P. Heffeter, V. B. Arion, M. A. Jakupec, A. Roller, M. Galanski, W. Berger and B. K. Keppler, *J. Med. Chem.*, 2009, **52**, 5032–5043.

12 H. Beraldo and D. Gambino, *Mini-Rev. Med. Chem.*, 2004, **4**, 31–39.

13 P. Panneerselvam, R. R. Nair, G. Vijayalakshmi, E. H. Subramanian and S. K. Sridhar, *Eur. J. Med. Chem.*, 2005, **40**, 225–229.

14 C. R. Kowol, R. Trondl, V. B. Arion, A. Michael, M. A. Jakupec, I. K. Lichtscheidl and B. K. Keppler, *Dalton Trans.*, 2010, **39**, 704–706.

15 S. Singh, N. Bharti, F. Naqvi and A. Azam, *Eur. J. Med. Chem.*, 2004, **39**, 459–465.

16 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 and H. Beraldo, *J. Inorg. Biochem.*, 2005, **99**, 698–706.

17 H. Beraldo and D. Gambino, *Mini-Rev. Med. Chem.*, 2004, **4**, 159–165.

18 U. Abram, K. Ortner, R. Gust and K. Sommer, *J. Chem. Soc., Dalton Trans.*, 2000, 735–744.

19 T. Murafuji, Y. Miyoshi, M. Ishibashi, A. F. M. Mustafizur Rahman, Y. Sugihara, I. Miyakawa and H. Uno, *J. Inorg. Biochem.*, 2004, **98**, 547–552.

20 C. R. Kowol, R. Trondl, P. Heffeter, V. B. Arion, M. A. Jakupec, A. Roller, M. Galanski, W. Berger and B. K. Keppler, *J. Med. Chem.*, 2009, **52**, 5032–5043.

21 I. C. Mendes, J. P. Moreira, J. D. Ardisson, R. G. dos Santos, P. R. O. da Silva, I. Garcia, A. Castineiras and H. Beraldo, *Eur. J. Med. Chem.*, 2008, **43**, 1454–1461.

22 D. Kovala-Demertzi, A. Papageorgiou, L. Papathanasis, A. Alexandratos, P. Dalezis, J. R. Miller and M. A. Demertzis, *Eur. J. Med. Chem.*, 2009, **44**, 1296–1302.

23 M. C. Miller III, C. N. Stineman, J. R. Vance, D. X. West and I. H. Hall, *Anticancer Res.*, 1998, **18**, 4131–4140.

24 M. Baldini, M. Belicchi-Ferrari, F. Bisceglie, P. P. Dall'aglio, G. Pelosi, S. Pinelli and P. Tarasconi, *Inorg. Chem.*, 2004, **43**, 7170–7179.

25 B. Garcia, J. Garcia-Tojal, R. Ruiz, R. Gil-Garcia, S. Ibeas, B. Donnadieu and J. M. Leal, *J. Inorg. Biochem.*, 2008, **102**, 1892–1900.

26 A. Bacchi, M. Carcelli, P. Pelagatti, C. Pelizzi, G. Pelizzi and F. Zani, *J. Inorg. Biochem.*, 1999, **75**, 123–133.

27 D. L. Klayman, J. F. Bartoserich, T. S. Griffin, C. J. Mason and J. P. Scovill, *J. Med. Chem.*, 1979, **22**, 885–893.

28 T. P. Stanojkovic, D. Kovala-Demertzi, A. Primikyri, I. Garcia-Santos, A. Castineiras, Z. Juranic and M. A. Demertzis, *J. Inorg. Biochem.*, 2010, **104**, 467–476.

29 L. Labib, T. E. Khall, M. F. Iskander and L. S. Refaat, *Polyhedron*, 1996, **15**, 349–357.

30 U. El-Ayaan, M. M. Youssef and S. Al-Shihry, *J. Mol. Struct.*, 2009, **936**, 213–219.

31 (*a*) R. Diemer, U. Dittes, B. Nuber, V. Seifried, W. Opferkuch and B. K. Keppler, *Met.-Based Drugs*, 1995, **2**, 271–292; (*b*) K. Nomiya, K. Sekino, M. Ishikawa, A. Honda, M. Yokoyama, N. C. Kasuga, H. Yokoyama, S. Nakano and K. Onodera, *J. Inorg. Biochem.*, 2004, **98**, 601–615; (*c*) L. P. Battaglia, A. Bonamartini Corrradi, C. Pelizzi, G. Pelosi and P. Tarasconi, *J. Chem. Soc., Dalton Trans.*, 1990, 3857–3860; (*d*) M. X. Li, L. Z. Zhang, M. Yang, J. Y. Niu and J. Zhou, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 2418–2423.




This journal is © The Royal Society of Chemistry 2012

View Online

32 A. I. Matesanz, C. Joie and P. Souza, *Dalton Trans.*, 2010, **39**, 7059–7065.
33 P. Chellan, N. Shunmoogam-Gounden, D. T. Hendricks, J. Gut, P. J. Rosenthal, C. Lategan, P. J. Smith, K. Chibale and G. S. Smith, *Eur. J. Inorg. Chem.*, 2010, 3520–3528.
34 U. Dittes, E. Vogel and B. K. Keppler, *Coord. Chem. Rev.*, 1997, **163**, 345–364.
35 R. Diemer, U. Dittes, B. Nuber, V. Seifried, W. Opferkuch and B. K. Keppler, *Met.-Based Drugs*, 1995, **2**, 271–292.
36 S. Hassfjell, K. O. Kongshaug and C. Rømming, *Dalton Trans.*, 2003, 1433–1437.
37 L. P. Battaglia, A. B. Corradi, C. Pelosi and P. Tarasconi, *J. Chem. Soc., Dalton Trans.*, 1989, 671–675.
38 L. P. Battaglia, A. B. Corradi, G. Pelizzi, G. Pelosi and P. Tarasconi, *J. Chem. Soc., Dalton Trans.*, 1990, 3857–3860.
39 O. Anjaneyulu, T. K. Prasad and K. C. K. Swamy, *Dalton Trans.*, 2010, **39**, 1935–1940.
40 A. Mishra, N. K. Kaushik, A. K. Verma and R. Gupta, *Eur. J. Med. Chem.*, 2008, **43**, 2189–2196.
41 S. A. Khan, K. Saleem and Z. Khan, *Eur. J. Med. Chem.*, 2007, **42**, 103–108.
42 R. Yanardag, T. B. Demirci, B. Ülküseven, S. Bolkent, S. Tunali and S. Bolkent, *Eur. J. Med. Chem.*, 2009, **44**, 818–826.
43 P. F. Iqbal, A. R. Bhat and A. Azam, *Eur. J. Med. Chem.*, 2009, **44**, 2252–2259.
44 M. X. Li, C. L. Chen, D. Zhang, J. Y. Niu and B. S. Ji, *Eur. J. Med. Chem.*, 2010, **45**, 3169–3177.
45 S. Elmore, *Toxicol. Pathol.*, 2007, **35**, 495–516.
46 *SMART and SAINT, Area Detector Control and Integration Software*, Siemens Analytical X-ray Systems Inc., Madison, WI, 1996.
47 G. M. Sheldrick, *SHELXTL V5.1, Software Reference Manual*, Bruker AXS Inc., Madison, WI, 1997.
48 G. M. Sheldrick, *SHELXS 97, Program for Crystal Structure Solution*, University of Göttingen, Göttingen, 1997.

Downloaded by Wuhan University on 15 October 2012
Published on 29 August 2012 on http://pubs.rsc.org | doi:10.1039/C2DT31256E

This journal is © The Royal Society of Chemistry 2012 