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Cu(II), Ga(III) and In(III) complexes of 2-acetylpyridine *N*(4)-phenylthiosemicarbazone: synthesis, spectral characterization and biological activities†

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In this paper, synthesis and characterization of metal complexes [Cu₂(L)₃](ClO₄) (1), [Ga(L)₂](NO₃)·2H₂O (2) and [In(L)₂](NO₃)·H₂O (3) (HL = 2-acetylpyridine *N*(4)-phenylthiosemicarbazone) was carried out, including elemental analysis, spectral analysis (IR, UV-vis, NMR), and X-ray crystallography. Complex 1 contains one S-bridged binuclear [Cu₂(L)₃]⁺ unit, where two Cu atoms display diverse coordination geometries: one being square planar geometry and the other octahedral geometry. Both 2 and 3 are mononuclear complexes, and the metal centers in 2 and 3 are chelated by two NNS tridentate ligands possessing a distorted octahedral geometry. Biological studies show that all the complexes possess a wide spectrum of modest to effective antibacterial activities and remarkable cytotoxicities against HepG2 cells, and 1, in particular, with an IC₅₀ value of 0.19 ± 0.06 μM, is 113-fold and 28-fold more cytotoxic than HL and the antitumor drug mitoxantrone, respectively. In addition, 3 exhibits excellent photoluminescence properties. Upon the addition of 1 equiv of In³⁺ ions, a remarkable fluorescence intensity of HL and fluorescent color change (from transparent to light-green) could be observed with 365 nm light, indicating that this ligand may be used as a promising colorimetric and fluorescent probe for In³⁺ detection.

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1. Introduction

Thiosemicarbazones are a significant family of Schiff bases not only in terms of their coordination capacity, but their analytical, biological and pharmacological properties.^{1–6} In the past several years, many metal compounds have been derived from thiosemicarbazones. For example, Yang *et al.* proposed the use of a metal pro-drug whose design is based on the natural HSA IIA subdomain and the known cancer cell to improve the anti-cancer activity and selectivity of drugs.^{7–11} In addition, as versatile ligands, they also have the ability to provide diverse binding sites for metal ions to form stable metal complexes, which allows them to be used in biological and environmental samples for the selective extraction and detec-

tion of certain vital metal ions.^{2,4} For example, some fluorescence and colorimetric sensors based on thiosemicarbazones have been designed to identify metal ions which are reactive and toxic in biology *e.g.*, Cu²⁺, Hg²⁺ and In³⁺.^{5,6,12,13} Among thiosemicarbazones with excellent biological activities,^{2,14–16} the most outstanding representative of this family is 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine), which is a potent inhibitor of ribonucleotide reductase and employed in phase I and II clinical tests for treatment of a variety of malignancies at present.^{17,18} Though the mechanisms involved in the biological activities of thiosemicarbazones are controversial in many ways to date, it is well-accepted that the biological activities are also intensely connected to their ability to form complexes with metals.^{3,14} Lipophilicity, which dominates the rate of molecules entering cells, may be improved through complexing.^{19,20} Moreover, in many cases the metal complex may display higher bioactivities than free ligands and certain side effects likely decline *via* complexing.^{21–23} Hence the synthesis and application of thiosemicarbazones and their complexes have attracted continuous interest and become one of the most promising research areas. Copper(II) is a biologically essential ion whose redox potential permits its participation in electron transfer reactions of cellular processes.²⁴ Cu(II)–thiosemicarbazone

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complexes have drawn more attention owing to their diversity of structures and fascinating biological activities, especially their anticancer activity.^{25–27} In recent years, they have been also expanded into other fields such as therapy for neurodegenerative diseases and radiopharmaceuticals.^{27–29} Gallium(III) is the second metal ion applied for tumor therapy after platinum(II).³⁰ Complexation of gallium(III) with thiosemicarbazones could enhance the bioactivity of Ga(III), and was considered as an important strategy to develop cytotoxic drugs.^{31–37} Indium(III) is an Auger electron emitter, which enables its complexes to be potential dual imaging-therapeutic agents.³⁷ Nevertheless, reports on indium(III) thiosemicarbazone complexes are quite rare.^{1,37–40}

As a continuation of our study on heterocyclic thiosemicarbazones as well as their complexes,^{16,40–44} we showed three metal complex derivatives of 2-acetylpyridine *N*(4)-phenylthiosemicarbazone (HL, Scheme 1) in this paper, namely, [Cu₂(L)₃]ClO₄ (1), [Ga(L)₂]NO₃·2H₂O (2) and [In(L)₂]NO₃·H₂O (3), and also discussed a series of characterizations and biological activities of these complexes. An investigation of the luminescence characteristics of these compounds has been performed. Moreover, the antibacterial activity and cytotoxic activity of HL and 1–3 have been investigated against eight selected kinds of bacteria and the HepG2 cell line, respectively.

2. Results and discussion

2.1. Chemistry

The ligand HL was acquired on the basis of the method reported in the literature⁴⁵ and verified through analyzing the IR spectra. The structures of all the compounds obtained were confirmed by X-ray crystallography. Fig. 1–3 depict the structure of molecules as well as the atomic number scheme and the packed unit cell, respectively.

2.1.1. Crystal structure of 1. Complex 1 is composed of one S-bridged dinuclear [Cu₂(L)₃]⁺ cation and one free perchlorate counteranion (Fig. 1). In the [Cu₂(L)₃]⁺ cation, two copper atoms are located in different coordination environments. Cu1 is four-coordinated with one ligand as monobasic tridentate (N3, N4, S1) forming two 5-membered fused chelate rings, and the sulfur atom (S2) of another ligand occupies the fourth site. The coordination geometry around the central Cu1 atom could be depicted as slightly distorted square planar with a parameter $\tau = 0.178$ [$\tau = [360 - (\alpha + \beta)]/141$, where $\alpha = \text{N3–Cu1–S2} = 175.4(3)^\circ$, $\beta = \text{N4–Cu1–S1} = 159.5(3)^\circ$ and $\tau = 0$ and 1 for regular square planar and tetrahedral geometries, respectively].⁴⁶ The Cu2 atom exhibits a

Scheme 1 2-Acetylpyridine *N*(4)-phenylthiosemicarbazone, HL.

Fig. 1 Molecular structure of 1, showing the binuclear [Cu₂(L)₃]⁺ unit. For clarity, the hydrogen atoms are omitted.

contorted N₄S₂ octahedral geometry. The basal plane is composed of two nitrogen atoms (N7, N8) and the sulfur atom (S2) from the same ligand together with an imine nitrogen atom (N11) from the second ligand, whereas the axial sites are occupied by a sulfur atom (S3) and pyridine nitrogen (N12) from the second ligand. The bond length of Cu–N ranges from 1.951(10) to 2.226(10) Å, while the Cu–S distances are in the range of 2.274(4)–2.708(4) Å. The Cu1...Cu2 distance inside the dimeric unit is 3.540(24) Å, similar to those in μ_2 -thiolate bridged Cu(II) dimers.^{47–57}

Noticeably, three deprotonated ligands in the [Cu₂(L)₃]⁺ cation display two kinds of coordination modes: two ligands coordinate to two Cu atoms in an expected N₂S tridentate manner, while the last one serves as a tetradentate donor with two N atoms (pyridine N atom, imine N atom) adopting a normal chelating mode and S bridging mode (μ_2) linking two metal centers. The C–S distances range between 1.738(13) and 1.767(12) Å, being within the normal range of a C–S single bond, indicating that the ligands are coordinated in their deprotonated thiolate form.⁵⁸ Such a structure of the [Cu₂(L)₃] dimeric unit is unique and more interesting since the stoichiometry of metal:thiosemicarbazone ligand is 2:3 and two copper(II) ions show different chelating modes with bridging S atoms to form a square-planar/octahedral complex (4:6-coordination), as compared with those of reported Cu(II)–thiosemicarbazone dimers,^{47–57} where the stoichiometry of metal:thiosemicarbazone is 1:1 and two Cu centers both have the same 5-coordinated square-pyramidal topology. In addition, the crystal structure is stabilized by the linking of the different components of intermolecular hydrogen bonds of 1 (Fig. S1 in the ESI†). The hydrogen bond contains the terminal nitrogen atom N5 and sulfur atom S2 with N5...S2 equal to 3.501(11) Å, and the angle N5–H5A...S2 being 150.7° (symmetry code: $x + 1, y + 2, z$), respectively.

2.1.2. Crystal structures of 2 and 3. Complexes 2 and 3 have similar structures (Fig. 2), thus only 2 was described in detail. As depicted in Fig. 2a, 2 consists of one [Ga(L)₂]⁺ cation, one nitrate ion and two water molecules. Two tridentate

Fig. 2 (a) Molecular structure of **2**. For clarity, the hydrogen atoms are omitted. (b) Molecular structure of **3**. Hydrogen atoms are omitted.

(N, N, S) anionic ligands put the Ga(III) ion in a distorted octahedral polyhedron as found in similar Ga(III) 2-acetylpyridine thiosemicarbazones complexes.^{37,59–62} The pyridine nitrogen atoms are mutually in a *cis* position to each other and *trans* to sulfur atoms, whereas the imine nitrogen atoms are in a *trans* position to each other. The N8–Ga–S2, N4–Ga–S1 and N3–Ga–N7 angles, 155.70(13)°, 155.44(12)° and 176.75(15)°, deviate from the ideal value of 180° obviously, indicating distortion from the geometry of a conventional octahedron. The bond lengths between gallium and pyridine nitrogen (2.104(4) and 2.105(4) Å) are slightly longer than those of gallium and imine nitrogen (2.062(4) and 2.065(4) Å), which may be attributed to the fact that the imine nitrogen is a stronger base compared with the pyridine nitrogen.⁵⁸ Two measured C–S bond distances (1.749(5) and 1.708(5) Å) suggest that thiosemicarbazone moieties adopt a form of tautomeric thiol and serve as negative ligands.⁵⁸

In the crystal packing of **2**, two kinds of intermolecular hydrogen bonds occur containing the terminal nitrogen atoms N1 and N5, O1W of the water molecule and O1 of the nitrate ion (Fig. S2†). The separation for N1...O1W is 3.157(9) Å with the N1–H1A...O1W angle being 169.4° and the separation for N5...O1 (symmetry code: $x - 1, y, z$) is 3.014(9) Å with the N5–H5A...O1 angles being 167.2°, respectively. Unlike those of **2**, three kinds of intermolecular hydrogen bonds are formed involving N1 and N5 of the ligand and N9 and O1 of

the nitrate ion in **3**, and the lattice water molecule is not involved in the hydrogen bond formation (Fig. S2†). The N1 and N5 atoms from the ligand serve as hydrogen bond donors, whereas the oxygen atom O1 and nitrogen atom N9 of the nitrate ion serve as acceptors with N1...O1 equal to 2.880(7) Å and the angle N1–H1A...O1 being 158.8° (symmetry code: $-x, y - 1/2, -z + 1/2$), N5...O1 equal to 3.037(8) Å and the angle N5–H5A...O1 being 156.9° and N(5)...N(9) equal to 3.428(9) Å and the angle N5–H5B...N(9) being 170.2°, respectively. These hydrogen bond interactions link [In(L)₂]⁺ units in the crystal cell giving rise to a 1D zigzag chain along the *b* axis and further stabilize the structure of **3** (Fig. S2†).

2.2. IR spectra

Infrared characteristic absorption peaks of the complexes are usually different from those of the free ligand and can offer important information on ligand bonding. For thiosemicarbazones and their complexes, the bonding vibration of $\nu(\text{C}=\text{N})$, $\nu(\text{N}=\text{N})$, and $\nu(\text{C}=\text{S})$ has been given more attention in the IR spectrum. In the IR spectrum of the ligand, the $\nu(\text{C}=\text{N})$ band appears at 1581 cm⁻¹ and moves to 1596–1599 cm⁻¹ in complexes **1–3**, indicating that the imine nitrogen participated in the coordination.^{31,63} Meanwhile, the increases in the wavenumber of $\nu(\text{N}=\text{N})$ from 1068 cm⁻¹ in HL to 1156, 1160 and 1157 cm⁻¹ in **1–3** may be attributed to the enhancement in bond strength, further proving the coordination of imine nitrogen. The strong band at 801 cm⁻¹ in HL is due to $\nu(\text{C}=\text{S})$ of the thione form in the solid state, and this band moves to lower wavenumbers at 746, 759, and 764 cm⁻¹ in **1–3**, respectively. The stretching band at 1263 cm⁻¹ [$\nu(\text{CS}) + \nu(\text{CN})$] shifted to 1252, 1252 and 1253 cm⁻¹ for **1–3**, respectively. These characteristic shifts indicate the thione–thiol tautomerism of the coordinated thiosemicarbazone.^{63–65}

2.3. UV-vis spectra

The UV-vis absorption behaviors of these compounds are determined in methanol solution. As depicted in Fig. 3, the ligand HL gives only one band at 318 nm in the ultraviolet region, which can be attributable to the π – π^* transition; and complexes **1–3** exhibit one weak band in the ultraviolet region (311, 304, and 303 nm for **1–3**, respectively) and one

Fig. 3 UV-vis absorption spectra of the HL and **1–3** in methanol solution at room temperature.

strong band in the visible region (406, 405 and 403 nm for 1–3, respectively). Therefore, the bands in the range 303–311 nm for the three complexes, which are only blue-shifts of 7–15 nm relative to the ligand, might be attributed to the intra ligand transition. Meanwhile, the intense bands at 406, 405, and 403 nm for 1–3 are obviously different from that of the ligand, and accordingly may be assigned to the LMCT transitions. Such a characteristic provides obvious evidence for the ligand chelating the metal center.

2.4. Fluorescence properties

The fluorescence behaviors of HL and 1–3 were investigated at room temperature in 1×10^{-5} M methanol solutions. Fluorescence experiments were carried out with 260 nm as the excitation wavelength for HL, 2 and 3, and 234 nm as the excitation wavelength for 1, respectively. As shown in Fig. 4, free ligand HL and 1–3 all exhibit two similar peaks: one strong peak at ca. 306 nm and one weak peak at ca. 586 nm, which indicates that these emissions should be ascribed to intra ligand fluorescence emission. In addition, 3 displays a noticeable enhancement in emission intensity while 2 and 1 show a slight change or decrease in emission intensity compared with the free ligand. These observations indicate that the differences between the central metal ion and its coordination environment may have significant effects on the emission intensity.⁶⁶

Based on the better fluorescence behavior of 3, fluorescence titration experiments for the binding of HL (1.0×10^{-5} M) with In^{3+} were further carried out in methanol solution. Fig. 5a shows the fluorescence spectral changes of HL with increasing amount of In^{3+} . Upon the addition of 0.125–1 equiv. of In^{3+} , the emission intensity of HL increases efficiently. The highest peaks at 306 nm and 586 nm are at least four times as intense as the corresponding band in HL solution without In^{3+} . Moreover, the fluorescence response of HL with In^{3+} can also be observable by the naked eye using a UV lamp (365 nm) (Fig. 5b), indicating that this ligand may be used as a promising colorimetric and fluorescent probe for In^{3+} detection.

Fig. 4 Fluorescence properties of HL and 1–3 in methanol solution at room temperature.

Fig. 5 (a) Fluorescence behavior of HL in the presence and absence of In^{3+} (methanol solvent) at $\lambda_{\text{ex}} = 260$ nm and a concentration of 1.0×10^{-5} M HL. Inset: changes in the emission intensity at 306 nm and 586 nm with increasing amount of In^{3+} . (b) Observation of the visual changes for HL in the presence of different amounts of In^{3+} under a UV lamp (365 nm).

2.5. Biological activity studies

The biological activities of the obtained compounds were detected on the basis of antibacterial activity and cytotoxic activity in comparison to HL.

2.5.1. Antibacterial activity. The antibacterial activity of HL, compounds 1–3, standard antibiotic Kan (kanamycin sulfate) and the solvent (10% dimethylsulfoxide (DMSO) in phosphate-buffered saline (PBS)) was tested by using four strains of Gram positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Sarcina lutea*) and four strains of Gram negative bacteria (*Escherichia coli*, *Agrobacterium tumefaciens*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*). The data concerning the zone of inhibition (zoi) and minimum inhibitory concentrations (MICs) are listed in Tables 1 and 2, respectively. The results reveal that HL and 1–3 possess a wide spectrum of modest to effective

Table 1 Diameter of the inhibition zone (mm) of the tested compounds (1 mg mL^{-1})

Microorganism	Diameter of inhibition zone					Kan
	HL	1	2	3	Solvent	
<i>B. subtilis</i>	8	12	21	10	— ^a	27
<i>B. cereus</i>	8	8	10	8	—	15
<i>S. aureus</i>	12	8	25	16	—	19
<i>S. lutea</i>	8	10	—	12	—	16
<i>A. tumefaciens</i>	8	8	11	10	—	32
<i>E. coli</i>	8	8	13	8	—	17
<i>P. aeruginosa</i>	—	8	17	10	—	16
<i>S. typhimurium</i>	8	—	12	8	—	18

^a No inhibition or $d < 8$ mm. Solvent: 10% DMSO in PBS.

Table 2 Minimum inhibitory concentration ($\mu\text{g mL}^{-1}$) of the tested compounds

Microorganism	MIC ($\mu\text{g mL}^{-1}$)				Solvent	Kan
	HL	1	2	3		
<i>B. subtilis</i>	1000	62.5	125	500	— ^a	15.63
<i>B. cereus</i>	1000	125	250	1000	—	250
<i>S. aureus</i>	250	125	62.5	125	—	15.63
<i>S. lutea</i>	1000	250	—	125	—	1000
<i>A. tumefaciens</i>	1000	500	250	500	—	15.63
<i>E. coli</i>	1000	500	250	1000	—	250
<i>P. aeruginosa</i>	—	125	62.5	125	—	250
<i>S. typhimurium</i>	1000	—	250	1000	—	500

^a No inhibition or MIC > 1000 $\mu\text{g mL}^{-1}$. Solvent: 10% DMSO in PBS.

antibacterial activities ranging from 8 to 25 mm zoi against almost all the tested microorganisms. At the same time, it can be seen from Tables 1 and 2 that, in general, compounds 1–3 show much higher antibacterial activity with increased zoi and decreased MICs as compared to HL. For instance, 1–3 show zoi ranging 10–21 mm and MICs ranging 62.5–500 $\mu\text{g mL}^{-1}$ against *B. subtilis*, while HL shows lower activity with 8 mm zoi and 1000 $\mu\text{g mL}^{-1}$ MICs against *B. subtilis*. And in the case of *P. aeruginosa*, 1–3 display activity in the range 8–17 mm zoi and 62.5–250 $\mu\text{g mL}^{-1}$ MICs; however, HL is found to be inactive under the same experimental conditions. Particularly, of the three compounds, 2 exhibits the maximum activity against *P. aeruginosa* with 17 mm zoi and the lowest MIC value of 62.5 $\mu\text{g mL}^{-1}$, better than the positive control antibiotic Kan (zoi: 16 mm; MIC: 250 $\mu\text{g mL}^{-1}$). A possible explanation for the increased activity of the obtained compounds relative to the ligand is that they have a better membrane penetrating ability endowed by their increased lipophilicity upon complexation.⁶⁷ These results suggest that the coordination of thiosemicarbazones to metals has a synergistic effect on the antibacterial activity and the antibacterial activity is also closely relevant to the type of metal ion.

2.5.2. Cytotoxic activity. Human hepatocellular carcinoma HepG2 cells were applied to evaluate the anti-proliferative activities of these compounds. Mitoxantrone (a kind of anti-tumor drug) was employed as the reference compound for comparison. IC_{50} values (compound concentration that induces death of 50% of cells) in micromolar units are calculated and represented in Fig. 6. It can be clearly observed that compounds 1–3 have shown a significant inhibitory potency against the proliferation of HepG2 cells at a low concentration ($\text{IC}_{50} = 0.19 \pm 0.06 \mu\text{M}$, $3.25 \pm 0.78 \mu\text{M}$, and $3.33 \pm 0.21 \mu\text{M}$ for 1–3, respectively). More importantly, the cytotoxic effects of the three compounds are significantly improved compared with HL ($\text{IC}_{50} = 21.55 \pm 2.41 \mu\text{M}$) and even greater than that of mitoxantrone ($5.3 \pm 2.38 \mu\text{M}$). Particularly, 1 is the most active compound in this study and is 113-fold and 28-fold more cytotoxic than HL and mitoxantrone, respectively. In general, the compounds studied in this paper were endowed with important biological activity and would be good candidates as anti-cancer chemotherapeutic agents.

Fig. 6 Cytotoxic effects of these compounds against HepG2 cells. All data are presented as the mean $\pm$ SD from three separate measurements.

3. Conclusions

In summary, the copper(II), gallium(III) and indium(III) complexes based on 2-acetylpyridine *N*(4)-phenylthiosemicarbazone have been synthesized and characterized. These compounds display a wide spectrum of modest to effective antibacterial activities and possess considerable cytotoxicity against the HepG2 cancer cell line, higher than mitoxantrone. In^{3+} determination using 2-acetylpyridine *N*(4)-phenylthiosemicarbazone has been carried out by fluorescence spectrometry, and the result shows that this ligand may be used as a promising colorimetric and fluorescent probe for In^{3+} detection. The results of this study are of great significance to broaden the applications of thiosemicarbazone derivatives in the fields of fluorescent and colorimetric probes.

4. Experimental

4.1. Materials and chemicals

All reagents and solvents used in this study were of reagent grade and used without further purification. Elemental analyses (C, H and N) were performed on a Perkin-Elmer 240 analyzer. The IR spectrum was recorded on a Nicolet FT-IR 360 spectrometer using KBr pellets in the range of 4000–400 cm^{-1} . The UV-vis absorption spectra were collected using a TU-1900 spectrometer. The fluorescence emission spectra were collected using a HITACHI F-7000 model instrument at room temperature. The ^1H NMR spectra were recorded using a Bruker AV-400 spectrometer in DMSO-d_6 .

4.2. Synthesis

4.2.1. Synthesis of HL.⁴⁵

4.2.2. Synthesis of the complexes

General synthetic strategy for compounds 1–3. The metal salt was dissolved in methanol solution in a separate beaker ($\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.074 g, 0.2 mmol) for 1; $\text{Ga}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (0.073 g, 0.2 mmol) for 2; $\text{In}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (0.078 g, 0.2 mmol) for 3). The solution of metal salt was added dropwise to a

reaction mixture of 2-acetylpyridine *N*(4)-phenylthiosemicarbazone (HL) and NaOAc under continuous stirring (HL (0.081 g, 0.3 mmol), NaOAc (0.025 g, 0.3 mmol) for 1; HL (0.108 g, 0.4 mmol), NaOAc (0.032 g, 0.4 mmol) for 2 and 3, respectively). Then the mixture was stirred for 1 h and filtered after cooling to room temperature. The crude product was further recrystallized using methanol solution and dried over P_4O_{10} *in vacuo*. By slowly evaporating the methanol solution, single crystals for X-ray studies were acquired.

$[Cu_2(L)_3]ClO_4$ (1). Yield: 61%. Anal. calcd for $C_{42}H_{40}ClCu_2N_{12}O_4S_3$ (1): C, 48.71; H, 3.89; N, 16.23. Found: C, 48.79; H, 3.72; N, 16.15. IR [KBr, ν (cm^{-1})]: 3296 (NH), 1599 (C=N), 1156 (N-N), 746 (C=S).

$[Ga(L)_2]NO_3 \cdot 2H_2O$ (2). Yield: 68%. Anal. calcd for $C_{28}H_{30}GaN_9O_5S_2$ (2): C, 47.60; H, 4.28; N, 17.84. Found: C, 47.73; H, 4.35; N, 17.91. 1H NMR(DMSO- d_6 , δ ppm): 10.20 (s, 1H, NH), 8.31 (d, J = 7.6 Hz, 1H, Py), 8.24 (t, J = 7.8 Hz, 1H, Py), 8.04 (d, J = 4.8 Hz, 1H, Py), 7.73 (d, J = 8.4 Hz, 2H, Ph), 7.61 (t, J = 6.4 Hz, 1H, Ph), 7.32 (t, J = 8.0 Hz, 2H, Ph), 7.04 (d, J = 7.4 Hz, 1H, Py), 2.44 (s, 3H, CH_3); IR [KBr, ν (cm^{-1})]: 3246 (NH), 1598 (C=N), 1160 (N-N), 759 (C=S).

$[In(L)_2]NO_3 \cdot H_2O$ (3). Yield: 71%. Anal. calcd for $C_{28}H_{28}InN_9O_4S_2$ (3): C, 45.85; H, 3.85; N, 17.19. Found: C, 45.91; H, 3.81; N, 17.26. 1H NMR(DMSO- d_6 , δ ppm): 10.23 (s, 1H, NH), 8.31–8.29 (m, 2H, Py), 8.13 (d, J = 4.8 Hz, 1H, Py), 7.83 (d, J = 7.6 Hz, 2H, Ph), 7.74–7.71 (m, 1H, Ph), 7.40 (t, J = 8 Hz, 2H, Ph), 7.10 (t, J = 7.4 Hz, 1H, Py), 2.87 (s, 3H, CH_3); IR [KBr, ν (cm^{-1})]: 3254 (NH), 1596 (C=N), 1157 (N-N), 764 (C=S).

4.3. Crystallography

Single crystals of 1 ($0.62 \times 0.27 \times 0.10$ mm), 2 ($0.42 \times 0.40 \times 0.24$ mm), and 3 ($0.68 \times 0.52 \times 0.26$ mm) were selected for collecting crystallographic data. A Siemens SMART-CCD diffractometer with graphite-monochromated Mo $K\alpha$ radiation (λ = 0.71073 Å) was used to collect the crystallographic data. The crystal structures of these complexes were worked out by direct methods and refined by full-matrix least squares on F^2 with anisotropic displacement parameters for all non-hydrogen atoms using SHELXTL.⁶⁸ The hydrogen atoms were added at idealized geometrical positions.

4.4. Fluorescence measurements

The preparation of HL, $In(NO_3)_3 \cdot 5H_2O$, and 1–3 (1×10^{-4} mol L^{-1}) solutions was performed in methanol. Then the solutions of HL and 1–3 were diluted to 1×10^{-5} mol L^{-1} . HL (1 mL, 10^{-4} mol L^{-1}) and appropriate amounts of In^{3+} (0, 125, 250, 500, 750, 1000 μ L) were put in a volumetric flask (10 mL), and then diluted to 10 mL with methanol for a concentration gradient experiment. The excitation wavelength used in this experiment was 234 nm for 1 and 260 nm for HL, 2 and 3, respectively and the emission wavelength was acquired from 250 nm to 800 nm.

4.5. Biological experiments

4.5.1. Antibacterial activity. The antibacterial activity was evaluated against the standard strains of several representative Gram positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Sarcina lutea*) and Gram negative bacteria (*Escherichia coli*, *Agrobacterium tumefaciens*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*). All germs were obtained from the China General Microbiological Culture Collection Center (CGMCC). The basic culture medium for the organisms was prepared using a Muller Hinton Agar. The sensitivity of the different bacterial strains to the different compounds was measured in terms of zone of inhibition by the disk diffusion method.

Minimum inhibitory concentrations (MICs) were measured through an agar dilution method. The ultimate concentration of all nutrients in MHA was set at 10^6 CFU mL^{-1} and applied for inoculation in the MIC experiment. Serial dilutions of the tested compounds dissolved in PBS containing 10% DMSO were set at concentrations of 0–2000 μ g mL^{-1} . Each plate was inoculated with 0.1 ml of bacterial culture medium. An empty disk, only including 10% DMSO in the center at each plate, served as negative control and kanamycin sulfate (Kan) as positive control. All inoculated plates were cultivated at 37 °C for 18–20 h. The MICs were determined as the minimum concentration of agents in the plate for which no marked growth occurred at the macro level. All results and data were obtained from at least three independent experiments.

4.5.2. Cytotoxicity test. The cell cytotoxicity was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. A 96-well plate at a cell density of 1×10^4 cells per well was applied to plate cells in a CO_2 incubator. The nutrient solution with MTT was changed and displaced with a fresh medium after 24 h. The tested compounds were dissolved in dimethylsulfoxide (DMSO) at 0.01 M. Before the test, the solution was diluted to different concentrations with PBS, and the terminal concentration of DMSO is less than 1%. After a culture cycle of 24 h, the cells were allowed to grow in a 100 μ L solution containing 10 μ L of 5 mg mL^{-1} MTT for 4 h at 37 °C. The nutrient solution with MTT was changed, and each well was displaced with 100 μ L DMSO to dissolve the formazan. A microplate reader (Bio-Tek ELX800, USA) was used to measure the absorbance at 570 nm. The inhibitory rates of the compounds in different concentrations were calculated, and the IC_{50} value was measured.

Conflicts of interest

The authors declare no competing interests.

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