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Synthesis and microcalorimetric determination of the bioactivities of a new Schiff base and its bismuth(III) complex derived from *o*-vanillin and 2,6-pyridinediamine

Xu Li[1] · Chuan-Hua Li[1] · Jian-Hong Jiang[1] · Hui-Wen Gu[2] · De-Liang Wei[1] · Li-Juan Ye[1] · Ji-Lin Hu[1] · Sheng-Xiong Xiao[1] · Dong-Cai Guo[3] · Xia Li[1] · Hui Zhang[1] · Qiang-Guo Li[1]

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Abstract In this work, a new Schiff base [*N*,*N*′-*bis*(2-hydroxy-3-methoxyphenylmethylidene)-2,6-pyridinediamine, $C_{21}H_{19}N_3O_4$] and its bismuth(III) complex $[Bi(C_{21}H_{17}N_3O_4)]Cl\cdot 2H_2O$ were synthesized with *o*-vanillin and 2,6-pyridinediamine in ethanol and THF solvent, respectively. The compositions and structures of the two synthetic compounds were characterized by elemental analysis, chemical analysis, spectrum analysis (including MS, FT-IR, NMR, and UV–Vis), and thermogravimetric analysis. The thermogenic curves for the growth metabolism of *Helicobacter pylori* (*H. pylori*) and *Schizosaccharomyces pombe* (*S. pombe*) treated by different concentrations of the two synthetic compounds were determined by isothermal heat conduction microcalorimetry at 37.00 and 32.00 °C, respectively. Based on the thermogenic curves, some important thermokinetic parameters including the microbial growth rate constant (k), inhibition ratio (I), and half inhibition concentration (IC_{50}) were calculated. The obtained results revealed that the Schiff base stimulated the growth of *H. pylori*, while the complex inhibited its growth. By contrast, both the Schiff base and the complex inhibited the growth of *S. pombe*, but the inhibitory effect of the complex was stronger than that of the Schiff base.

Keywords Microcalorimetry · Bioactivities · Thermokinetics · Schiff base bismuth(III) complex · *H. pylori* · *S. pombe*

Introduction

Microcalorimetry provides a general analytical tool for the characterization of the microbial growth process. It has been extensively used to investigate drug and the microbial cell interaction and has furnished much useful information [1–6]. One of the most prominent features of the microbial growth process is the production of heat. If the heat is monitored by a microcalorimeter, much useful information (both qualitative and quantitative) may be obtained. Each type of microbial has a unique thermogenic curve, as recorded by the microcalorimeter, under a defined set of growth conditions. Any chemicals that can modify the metabolic growth processes involved in cell will change the thermogenic curve obtained from the microcalorimeter. Based on the thermogenic curves, not only thermodynamic data but also thermokinetic data can be obtained. Many studies have demonstrated that the test results obtained from microcalorimetry are in agreement with those of conventional bioassay methods such as the agar cup

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✉ Hui-Wen Gu
gugo@yangtzeu.edu.cn

✉ Qiang-Guo Li
liqiangguo@163.com

[1] Hunan Provincial Key Laboratory of Xiangnan Rare-Precious Metals Compounds and Applications, College of Chemistry Biology and Environmental Engineering, Xiangnan University, Chenzhou 423043, Hunan Province, People's Republic of China

[2] College of Chemistry and Environmental Engineering, Yangtze University, Jingzhou 434023, Hubei Province, People's Republic of China

[3] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, Hunan Province, People's Republic of China

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method, the cylinder diffusion method, the paper disk diffusion assay, and the broth microdilution method [7, 8]. Owing to its good sensitivity, accuracy, and reproducibility, microcalorimetry is being accepted by more and more researchers, and has been gradually used in new drug discovery [9, 10].

Valen Schiff bases are a type of compounds derived from (modified) *o*-vanillin and various amines via the condensation reaction. At present, the studies of Valen Schiff bases and their metal coordination compounds mainly focused on their functions as catalysts, antioxidants, and luminescent materials, while their antibacterial activities have been less investigated. In fact, on the one hand, the antibacterial activity of *o*-vanillin is better than that of salicylaldehyde [11, 12]. What's more, the antibacterial activities of some Valen Schiff bases are also superior to those of salicylaldehyde Schiff bases [13, 14]. On the other hand, Valen Schiff bases exhibit very strong coordination abilities due to the existence of "O" and "N" atoms in their molecules [15]. Accordingly, more species of metal coordination compounds with plentiful structures can be synthesized from the Valen Schiff bases. In our previous works, some new Valen Schiff bases and their metal coordination compounds were designed and synthesized [15–22]. Their bioactivities on living cells were studied by microcalorimetry [19–22]. The preliminary results indicated that these synthetic compounds really possessed good bioactivities [19–22].

Bismuth is the heaviest stable element locating at the junction of metal and nonmetal elements in the periodic table, which has a ground-state electron configuration [Xe] $4f^{14}5d^{10}6s^{2}6p^{3}$. It exhibits the characteristics of non-toxicity and low radioactivity and is recognized as the only green heavy metal in nature [23]. Although China abounds in bismuth resources, the development of bismuth-refined products is facing a huge challenge [24–26]. Owing to large ionic radius and strong deformability, bismuth(III) could overcome steric hindrance and accept multiple pair of electrons, forming compounds with different coordination numbers. The coordination number of bismuth(III) could be up to 10. At present, the catalytic and biological medicinal properties of bismuth(III) complexes have attracted more and more attention [27–32]. Various bismuth(III) compounds have been used for medicinal purposes for more than 200 years, which have a significant effect on the treatment of a variety of microbial infections such as syphilis, diarrhea, gastritis colitis, ulcer, and *H. pylori* [33, 34]. Meanwhile, some bismuth(III) compounds with good bioactivities can effectively inhibit the growth and reproduction of cancer cells [35–38]. From the standpoint of "Green Chemistry" and "Sustainable Development", design and synthesis of new bismuth(III)-containing compounds will have important scientific values and broad application prospects. In recent years, diaminopyridine-based Valen Schiff bases and their coordination compounds containing 3*d* and 4*f* block metal ions have been widely studied [39–41], However, Valen Schiff bases coordination compounds containing main group metal elements, especially bismuth element, are still less investigated [41].

In this work, a new Schiff base [*N*,*N*′-*bis*(2-hydroxy-3-methoxyphenylmethylidene)-2,6-pyridinediamine, $C_{21}H_{19}N_3O_4$] and its bismuth(III) complex $[Bi(C_{21}H_{17}N_3O_4)]Cl\cdot 2H_2O$ were synthesized via the condensation reaction of 2,6-pyridinediamine and *o*-vanillin in a 1:2 molar ratio. Elemental analysis, chemical analysis, spectrum analysis (including MS, FT-IR, NMR, and UV–Vis), and thermogravimetric analysis were employed to characterize their compositions and structures. Then, *H. pylori* and *S. pombe* cell lines were chosen as ideal biological models to assess the bioactivities of the two synthetic compounds by isothermal heat conduction microcalorimetry. As a basic research, the aim of this study was to provide some useful thermokinetic data for the further development and application of Valen Schiff bases and their bismuth(III) complexes.

Experimental

Chemicals and reagents

o-Vanillin ($C_8H_8O_3$, 99.0 %), 2,6-pyridinediamine ($C_5H_7N_3$, 99.0 %), tetrahydrofuran (THF, 99.0 %), and ethanol (EtOH, 99.5 %) were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO, >99.5 %) and *N*,*N*-dimethyl formamide (DMF, >99.5 %) were bought from Tianjin Guangfu Chemical Research Institutes (Tianjin, China). Bismuth chloride ($BiCl_3$, 99 %) was obtained from Tianjin Jingke Chemical Research Institutes (Tianjin, China). All other chemicals including sodium chloride (NaCl, 99.5 %), silver nitrate ($AgNO_3$, 99.8 %), and EDTA (99.0 %) were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The water used was double-distilled. All chemicals and reagents were used as received without further purification.

Physical measurements

The elemental analysis of C, H, and N was carried out on a PerkinElmer 2400 elemental analyzer. The contents of Bi^{3+} and Cl^{-} in Schiff base bismuth(III) complex were determined by the EDTA titration and Mohr method, respectively. Mass spectra were determined using a Thermo Finnigan MAT95XP high-resolution mass spectrometer with methanol as solvent. FT-IR spectra

(400–4000 cm^{-1}) were measured using a Thermo Nicolet Avatar 360 Fourier transform IR spectrometer with a KBr pellet. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz advance nuclear magnetic resonance spectrometer with DMSO-d_6 as solvent and trimethyl silicane (TMS) as internal standard. All absorption spectra were measured by a Hitachi U-3010 UV–Vis spectrophotometer. The crystallization water content of the complex was determined by a Shimadzu DTG-60 thermogravimetric/differential thermal analyzer (TG/DTA) in flowing air with a heating rate of 10 °C min^{-1} between 20 and 1200 °C. The molar conductance was determined by a DDS-12DW conductivity meter (Shanghai Lida Instrument Factory, China). Microcalorimetric measurements were taken on a 3116-2/3239 TAM Air isothermal heat conduction microcalorimeter (Thermometric AB, Sweden), which was equipped with eight twin calorimetric channels with one side for the samples and the other for a static reference. The principles and structures of the microcalorimeter have been described at length in our previous work [42].

Cell lines and culture conditions

H. pylori (ATCC43504) was provided by Shanghai Beinuo Biological Technology Co., Ltd. The composition of *H. pylori* culture medium (HB8647) was 10.0 g proteose peptone, 10.0 g beef brain infusion, 9.0 g beef heart infusion, 5.0 g NaCl, 2.0 g glucose, 2.5 g Na_2HPO_4, pH = 7.4 ± 0.2, at 25 °C. 3.85 g HB8647 was dissolved in 93 mL distilled water, heated, and stirred until dissolution. Then, the resulting solution was sterilized under high pressure at 121 °C for 30 min. The stock solution of HB8647-HP was prepared by adding 7 mL sterile defibrinated sheep blood and 1 HP bacteriostatic agent to the above HB8647 solution.

S. pombe (ACCC20047) was provided by the Agricultural Culture Collection of China. It was grown in the Edinburgh minimal medium (EMM). 1 L of EEM (natural pH) contained 3 g K_2HPO_4, 2.2 g Na_2HPO_4, 5 g NH_4Cl, 20 g glucose, 20 mL inorganic salt, 1.0 mL vitamin, and 0.1 mL minerals. Before use, the EEM medium was sterilized under high pressure at 121 °C for 30 min.

Synthesis of the Schiff base

The Schiff base, *N*,*N*′-*bis*(2-hydroxy-3-methoxyphenyl-methylidene)-2,6-pyridinediamine, was prepared according to the literature [41, 43]. The synthetic process can be briefly summarized as follows: 1 mmol (0.1091 g) 2,6-pyridinediamine and 2 mmol (0.3043 g) *o*-vanillin were dissolved in 15 mL ethanol, respectively. Then, the ethanol solution of 2,6-pyridinediamine was added dropwise to the ethanol solution of *o*-vanillin at 55 °C. As the reaction progressed, the color of the mixture changed from light green to yellow, and finally became red. The reaction was stirred for 6 h and then allowed to settle overnight, resulting in plenty of nacarat precipitates. Subsequently, the precipitates were filtrated and recrystallized with methanol–methylene chloride mixed solution (1:1, v/v). The product was dried under vacuum at 50 °C until the mass of the crystals was constant, with a yield of 70 %. The synthetic scheme of the Schiff base is outlined in Fig. 1.

Synthesis of the Schiff base bismuth(III) complex

A 50-mL round-bottom flask was used as the reactor. Firstly, 0.5 mmol (0.0189 g) Schiff base was dissolved in a 50-mL round-bottom flask with 20 mL THF, then the solution was heated to keep a constant temperature of 55 °C, and stirred using a magnetic stirrer until the solid was dissolved completely. Subsequently, 0.5 mmol (0.0158 g) $BiCl_3$ was dissolved with 5 mL THF and added dropwise to THF solution of the Schiff base. The mixture was heated with stirring and refluxing. As the reaction progressed, plenty of brown precipitates were generated. After continuously reacted for 3 h, the resulting mixtures were transferred to a clean beaker to settle and cool. The precipitates were filtrated and washed with hot methanol for several times. The filter cake was dried, and finally, dark red powdered solid bismuth(III) complex was obtained, with a yield of 68 %. The synthetic scheme of the Schiff base bismuth(III) complex is outlined in Fig. 2.

Measurement of thermogenic curves of *H. pylori* and *S. pombe* cells treated by the Schiff base and its bismuth(III) complex

The thermogenic curves for the growth metabolism of *H. pylori* and *S. pombe* cells treated by different concentrations of the Schiff base and its bismuth(III) complex were determined by a 3116-2/3239 TAM Air isothermal heat conduction microcalorimeter, respectively. The optimum temperature of growth metabolism of *H. pylori* and *S. pombe* is 37.00 and 32.00 °C, respectively. Steps of the determination were summarized as follows: The microcalorimeter was adjusted to 37.00 °C (or 32.00 °C), and the measurement was taken with the ampoule method. Baselines were taken before each measurement, and the microcalorimeter was calibrated electrically. When the system had gained a stable baseline, 5 mL HB8647-HP (or EMM-sterilized) culture medium was added to the sterilized sample ampoules. *H. pylori* (or *S. pombe*) cell lines were inoculated with an initial density of 1×10^6 cells mL^{-1}. The compounds at different concentrations were added to the cell suspension, respectively. All the ampoules containing the cell suspension of *H. pylori* (or *S. pombe*) were sealed up and put in 8-channel calorimeter block. The data were collected

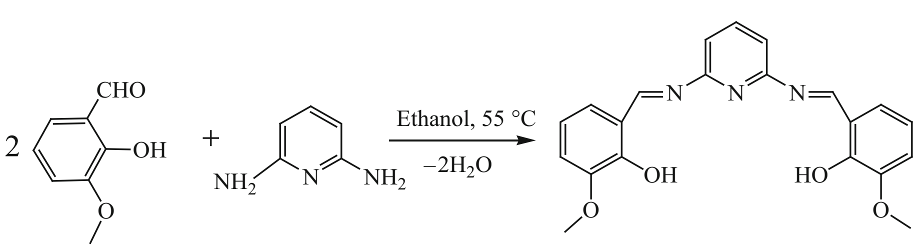

Fig. 1 Synthetic scheme of the Schiff base

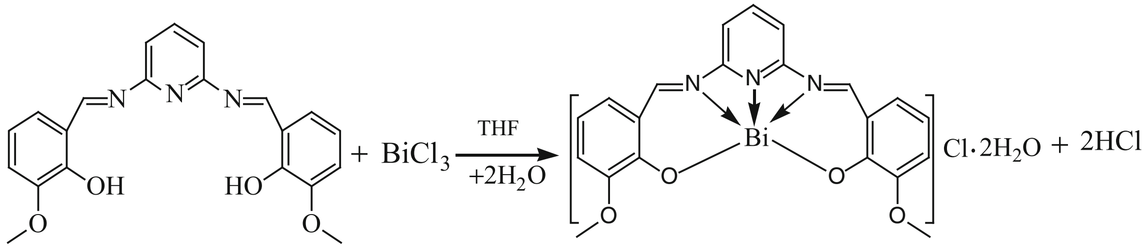

Fig. 2 Synthetic scheme of the Schiff base bismuth(III) complex

continuously by the dedicated software package. All the microcalorimetric experiments were repeated three times, and the obtained results were identical.

Results and discussion

Characterization of the Schiff base and its bismuth(III) complex

The Schiff base was a nacarat powder with a melting point of 219 ± 1 °C. It is very stable in the atmosphere. The powder can be easily soluble in some organic solvents such as dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), *N*,*N*-dimethyl formamide (DMF), and acetone, but insoluble in water, methanol, dichloromethane, trichloromethane, *n*-hexane, benzene, toluene, and ethyl acetate. The molar conductance of Schiff base in DMSO at 25 °C was determined to be 1.29 S cm^2 mol^{-1}, indicating that the Schiff base is a nonelectrolyte and exists as a neutral molecule in DMSO solvent.

The Schiff base bismuth(III) complex was a red brown powder with a melting point of 261 ± 1 °C. The dissolubility experiment indicated that the complex cannot be dissolved in water, methanol, dichloromethane, trichloromethane, *n*-hexane, benzene, toluene, ethyl acetate, acetone, THF, and diethyl ether, while it can be dissolved in DMSO and DMF, especially easily dissolved in the mixed solution of trichloromethane and DMSO (v/v = 1:1). The molar conductance of the complex in DMSO at 25 °C was determined to be 65.45 S cm^2 mol^{-1}, indicating that the complex is an electrolyte in DMSO solvent.

The Schiff base and its bismuth(III) complex were characterized by elemental analysis, chemical analysis, mass spectra, FT-IR, ^{1}H and ^{13}C NMR spectra, UV–Vis spectra, and TG–DTA analysis. The corresponding data for the Schiff base and its bismuth(III) complex are summarized in Tables S1–S6 in the Supplementary Materials, respectively.

It can be seen from Table S1 that the elemental and chemical analysis data are in a good accordance with their theoretical values. Based on these results, we can deduce the compositions of the Schiff base and its bismuth(III) complex were $C_{21}H_{19}N_3O_4$ (abbreviated as H_2L, $L = C_{21}H_{17}N_3O_4$) and $[Bi(C_{21}H_{17}N_3O_4)]Cl \cdot 2H_2O$ (abbreviated as $[Bi(L)]Cl \cdot 2H_2O$), respectively, which will be further characterized by MS, FI-IR, NMR, UV–Vis, and TG–DTA analysis.

The results of mass spectra of the Schiff base and its bismuth(III) complex (see Table S1) demonstrated that the $M^+ = [H_2L]^+ = 377.1$ and $M^+ = [[Bi(L)]Cl \cdot 2H_2O]^+ = 655.5$ for positive EI source, respectively. These experimental results are consistent with their theoretical molecular weight $M[H_2L] = 377.4$ and $M[[Bi(L)]Cl \cdot 2H_2O] = 655.9$, which further confirmed the composition of the Schiff base and the complex.

As can be seen from Table S2, there are four strong absorption peaks appearing at 1604, 1561, 1456, and 1254 cm^{-1} in the FT-IR spectra of the Schiff base H_2L, corresponding to the ν(C=N) stretching vibration of azomethine group [44], ν(C=N) stretching vibration of pyridine group, ν(C–N) stretching vibration of azomethine group [45], and ν(C–O) stretching vibration of phenolic hydroxyl group, respectively. After the complex formation, the peak assigned to ν(C=N) stretching vibration of azomethine group has moved to a high wave number 1611 cm^{-1}, $\Delta\nu = (\nu_{complex} - \nu_{Schiff\ base}) = 7\ cm^{-1}$, which indicated that the nitrogen atoms of azomethine group in the complex involved in the coordination with the bismuth(III) ion Bi^{3+} [40]. What is more, the ν(C–O) phenolic hydroxyl group of the free Schiff base exhibited a strong absorption peak at 1254 cm^{-1}. However, this absorption peak appeared at 1243 cm^{-1} for the complex, which was an indication of C–O–Bi^{3+} bond formation and provided further evidence for coordination via the deprotonated phenolic oxygen atoms. The absorption peak attributed to ν(C=N) stretching vibration of pyridine group disappeared after the complex formation, which could confirm that the nitrogen atom of pyridine group in the complex participated in the coordination. The wide ν(O–H) stretching vibration for the Schiff base and complex appeared at 3376 and 3352 cm^{-1}, respectively. The reason could be due to the hydrogen bonding between the phenolic hydroxyl groups of the free Schiff base or the complex containing water molecules, which was consistent with the results of elemental analysis and the thermogravimetric analysis.

As can be seen from Table S3 and Table S4, the ^{1}H NMR peaks of the complex were very similar to those of the Schiff base except the disappearance of two hydroxyl protons at δ 13.13 ppm, which indicated that the two hydroxyl groups from the Schiff base participated in the coordination to form the complex. This result can be further confirmed by the following NMR analysis. The ^{1}H NMR spectrum of the Schiff base showed a singlet at 9.57 ppm due to the imine protons, multiplet in the range 6.88–8.11 ppm due to the aromatic protons. ^{13}C NMR spectrum of the Schiff base showed at 165.10 (or 164.39) ppm due to the imine carbon, at 56.21 (or 56.10) ppm due to OCH_3 carbon, and at 176.3 ppm due to the pyridine carbon.

As can be seen from Table S5, Schiff base showed a wide and strong absorption peak 382 nm due to the p–π conjunction and n–π^* transition between the lone pair electrons of nitrogen atom in C=N group and the big π bond of benzene ring. Besides, there is another strong absorption peak at 280 nm, which was attributed to the p–π conjugation and n–π^* transition between the lone pair electrons of the phenolic hydroxyl oxygen atom and benzene ring. For the absorption peak at 248 nm, it was resulted from the π–π^* transition of the big conjugated bond in the benzene ring. The experimental results showed that the product is a Schiff base. After the formation of the bismuth(III) complex, the two n-to-π* transition absorption bands have shifted, which indicated that the phenolic hydroxyl oxygen atom and the nitrogen atom of C=N are involved in coordination to the bismuth(III) ion Bi^{3+}. Meanwhile, the absorption bands at 382 and 280 nm have blue-shifted to 343 and 266 nm, respectively. The tentative structure for the complex is shown in Fig. 4. The water molecules may be in the lattice.

As can be seen from Table S6, the thermal decomposition and mass losses of bismuth(III) complex can be divided into two steps. The first step was from 45 to 198 °C with a mass loss of 5.49 %, which was due to the removal of 2 mol H_2O, and consistent with the theoretical value 5.48 %. The composition of the remainder was $[Bi(C_{21}H_{17}N_3O_4)]Cl$. The second step ranges from 198 to 743.5 °C with a mass loss of 64.22 %, which corresponded to the loss of 1 mol of the ligand ($C_{21}H_{17}N_3O_4$) and 1 mol of Cl^-, and was consistent with the theoretical value 62.65 %. The composition of the remainder was Bi_2O_3 (or $BiO_{1.5}$).

Based on the above results of elemental analysis, chemical analysis, mass spectra, FI-IR, ^{1}H and ^{13}C NMR data, UV–Vis spectra, and TG–DTA analysis, we can conclude that the Schiff base and its bismuth(III) complex have a composition of $C_{22}H_{24}N_4O_5$ and $[Bi(C_{21}H_{17}N_3O_4)]Cl\cdot 2H_2O$. The chemical structures and 3D structures of the two compounds are shown in Figs. 3 and Fig. 4.

Thermokinetics of *H. pylori* and *S. pombe* cells treated by the Schiff base and its bismuth(III) complex

The growth rate constant (k) of H. pylori and S. pombe

Microcalorimetry was applied to evaluate the bioactivities of the Schiff base and its bismuth(III) complex on the growth metabolism of *H. pylori* and *S. pombe*. The thermogenic curves (power–time curves) for the growth metabolism of *H. pylori* and *S. pombe* cells treated by different concentrations of Schiff base and its bismuth(III) complex were recorded on a TAM Air isothermal heat conduction microcalorimeter at 37.00 and 32.00 °C, respectively. All microcalorimetric experiments were repeated three times. The measured thermogenic curves for the growth metabolism of *H. pylori* and *S. pombe* are illustrated in Figs. 5 and 6, respectively.

As can be seen from Figs. 5 and 6, the shapes of the thermogenic curves for the growth metabolism of *H. pylori* (or *S. pombe*) are similar to each other except the differences in peak positions and heights caused by different concentrations of Schiff base and bismuth(III) complex. The process of the growth metabolism of *H. pylori* (or *S. pombe*) can be abstracted as a mathematical model as shown in the insert map of Figs. 5a and 6a, respectively. The thermogenic curves could be divided into four phases, i.e., a lag phase (AB), a log phase (BC), a stationary phase (CD), and a decline phase (DE). During the log phase, the thermogenic curves obeyed the following exponential equation:

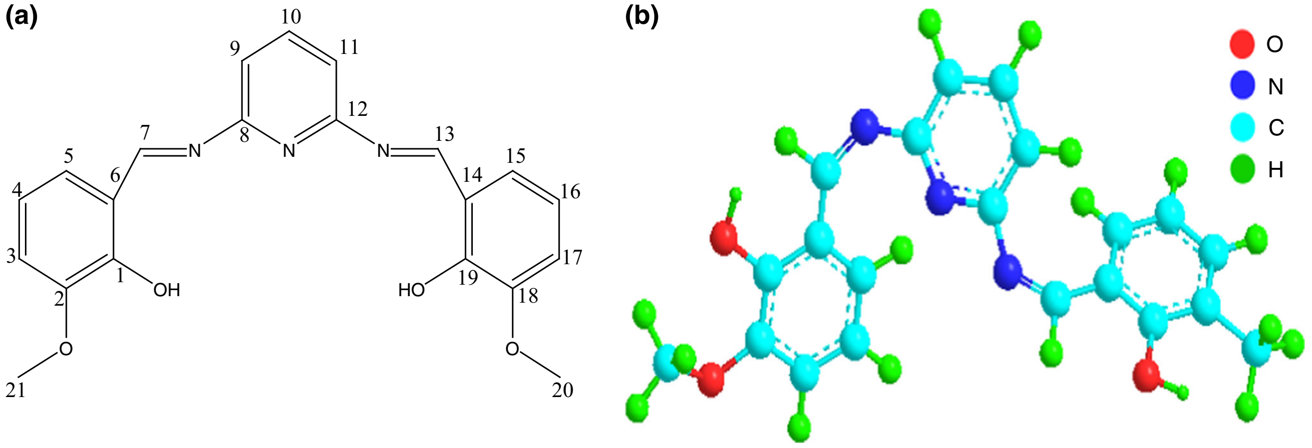

Fig. 3 Chemical structure **a** and 3D structure **b** of the Schiff base

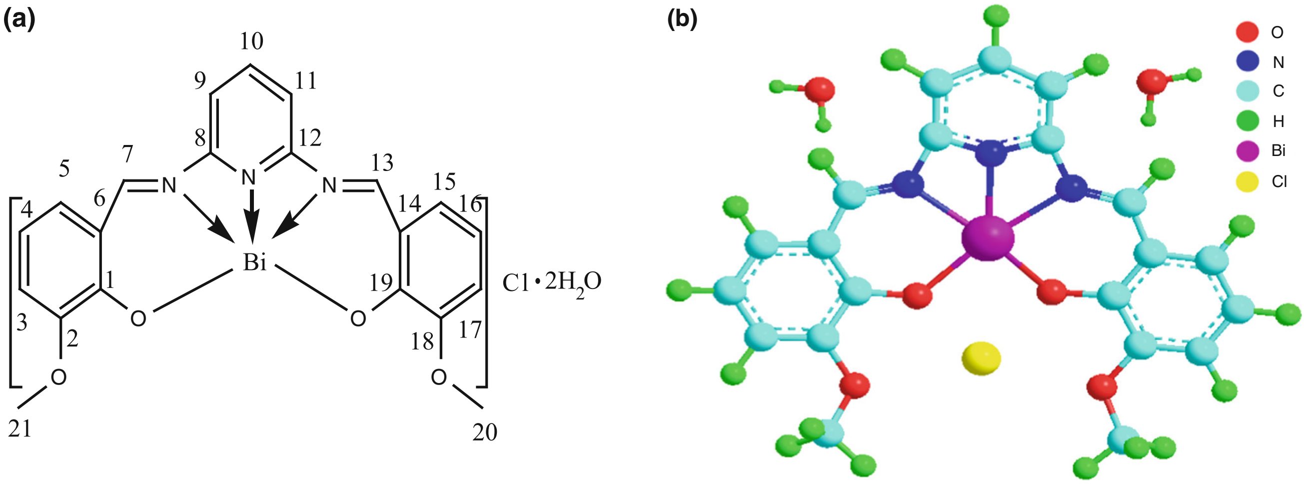

Fig. 4 Chemical structure **a** and 3D structure **b** of the Schiff base bismuth(III) complex

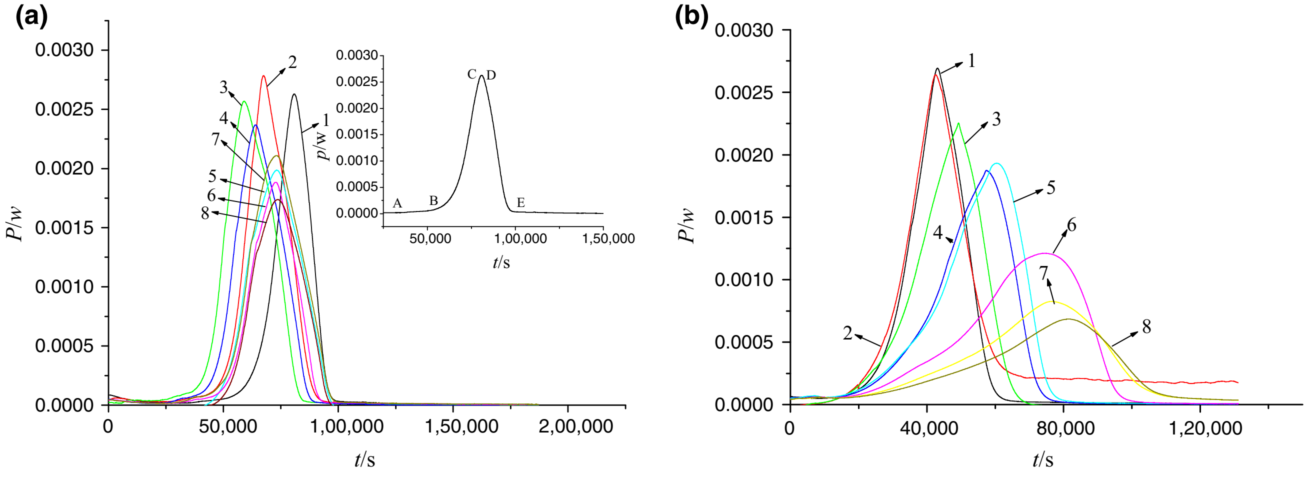

Fig. 5 Thermogenic curves of the growth of *H. pylori* treated by the Schiff base **a** and its bismuth(III) complex **b** at 37.00 °C; **a** concentration $c/(\mathrm{mmol\ L^{-1}})$ of the Schiff base corresponding to curves *1*–*8*: 0.0000, 0.3911, 0.7822, 1.1575, 1.5645, 1.9556, 2.3467, 2.7378; **b** concentration $c/(\mathrm{mmol\ L^{-1}})$ of the complex corresponding to curves *1*–*8*: 0.0000, 0.0830, 0.1245, 0.1660, 0.2074, 0.2489, 0.2904, 0.3319

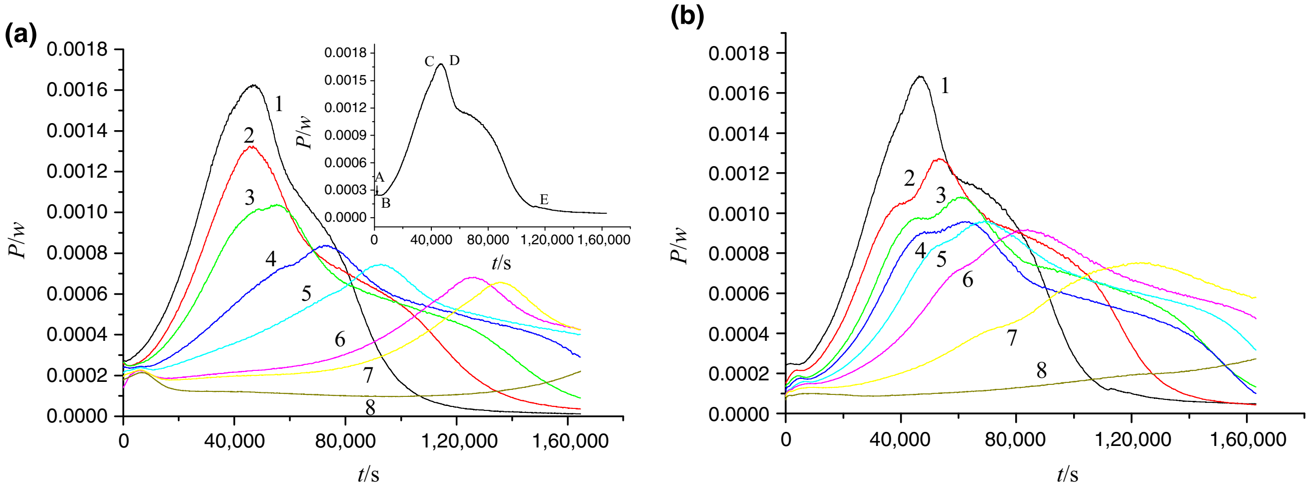

Fig. 6 Thermogenic curves of the growth of *S. pombe* treated by the Schiff base **a** and its bismuth(III) complex **b** at 32.00 °C; **a** concentration $c/(\mathrm{mmol\ L^{-1}})$ of the Schiff base corresponding to curves *1–8*: 0.0000, 0.4059, 0.6089, 0.8118, 1.0148, 1.2178, 1.4207, 1.6237; **b** concentration $c/(\mathrm{mmol\ L^{-1}})$ of the complex corresponding to curves *1–8*: 0.0000, 0.0253, 0.0507, 0.0760, 0.1014, 0.1267, 0.1520, 0.1774

$$n_t = n_0 \exp[k(t - t_0)] \quad (1)$$

where t is the time after the start of logarithmic growth phase, t_0 is the start time of logarithmic growth phase, n_t and n_0 are the germ or cell number at time t and t_0, respectively. If the power output of each germ or cell was one w, then

$$n_t w = n_0 w \exp[k(t - t_0)] \quad (2)$$

If define $P_t = n_t w,\ \ P_0 = n_0 w$, then

$$P_t = P_0 \exp[k(t - t_0)] \quad (3)$$

or

$$\ln P_t = \ln P_0 + kt - kt_0 \quad (4)$$

or

$$\ln P_t = A + kt \quad (5)$$

Eq. (5) is a linear equation, where P_t is the heat output power of the *H. pylori* or *S. pombe* cell at time t, and k is the growth rate constant of the *H. pylori* or *S. pomb* cell at specified conditions, whose size represented growth speed. Using this equation, the growth rate constant k of *H. pylori* and *S. pomb* can be calculated, and the results are shown in Table 1 and Table 2, respectively.

From Fig. 5 and Table 1, it could be seen that the growth rate constant (k) increases with the increase in concentration of the Schiff base, but no rules. With regard to the complex, the growth rate constant (k) decreased with the increase in concentration. These results indicated the free Schiff base stimulated the growth of the *H. pylori*, while the complex inhibited its growth. By contrast, it could be seen from Fig. 6 and Table 2 that the growth rate constant (k) of *S. pombe* decreased with the increase in concentrations of the Schiff base and the complex, which revealed that both the Schiff base and the complex possessed inhibitory effects on the growth metabolism of *S. pombe*.

Inhibition ratio (I) and half inhibition concentration (IC_{50})

The inhibition ratio (I) of the growth metabolism of *H. pylori* and *S. pombe* treated by drugs was defined as follows:

$$I = (k_0 - k_c)/k_0 \times 100\ \% \quad (6)$$

where k_0 is the control rate constant (without any drug inhibition) of *H. pylori* or *S. pombe* and k_c is the growth rate constant of *H. pylori* or *S. pombe* treated by an inhibitor with a concentration of c. When the inhibition ratio (I) was 50 %, the corresponding drug concentration was called as the half inhibition concentration (IC_{50}). The values of (I) and (IC_{50}) are shown in Table 1 and Table 2, respectively.

The results showed that the inhibition ratio I increases with the increase in concentration of the Schiff base, but no rules, for the *H. pylori*, while the inhibition ratio I decreased with the increase in concentration of the complex for the *H. pylori*. In other words, the Schiff base stimulated the growth of the *H. pylori*, but the complex possessed inhibitory effects on the growth of *H. pylori*. The half inhibition concentration (IC_{50}) of the complex was about 0.1812 mmol L^{-1}. Based on this phenomenon, we inferred that the structure factors which govern antimicrobial activities strongly depend on the central metal ion.

By contrast, both the Schiff base and the complex possessed significantly inhibitory effects on the growth

Table 1 Thermokinetic parameters of the growth of *H. pylori* treated by different concentrations of Schiff base and complex at 37.00 °C

Compound	c^{a}/mmol L^{-1}	k^{b}/s^{-1}	I^{c}/%	IC^{d}_{50}/mmol L^{-1}
The Schiff base	0.0000	$1.55 \times 10^{-4} \pm 1.15 \times 10^{-7}$ [e]	0.00	–
	0.3911	$1.82 \times 10^{-4} \pm 2.00 \times 10^{-7}$	−14.99	
	0.7822	$1.98 \times 10^{-4} \pm 2.00 \times 10^{-7}$	−27.74	
	1.1575	$1.87 \times 10^{-4} \pm 1.83 \times 10^{-7}$	−20.75	
	1.5645	$1.86 \times 10^{-4} \pm 3.67 \times 10^{-7}$	−20.22	
	1.9556	$1.71 \times 10^{-4} \pm 1.67 \times 10^{-7}$	−10.54	
	2.3467	$1.92 \times 10^{-4} \pm 4.67 \times 10^{-7}$	−23.55	
	2.7378	$1.58 \times 10^{-4} \pm 2.33 \times 10^{-7}$	−1.61	
The complex	0.0000	$1.56 \times 10^{-4} \pm 1.57 \times 10^{-7}$	0.00	0.1812
	0.0830	$1.37 \times 10^{-4} \pm 1.43 \times 10^{-7}$	13.33	
	0.1245	$1.06 \times 10^{-4} \pm 9.67 \times 10^{-8}$	32.59	
	0.1660	$8.27 \times 10^{-5} \pm 8.50 \times 10^{-8}$	47.51	
	0.2074	$7.05 \times 10^{-5} \pm 5.33 \times 10^{-8}$	55.24	
	0.2489	$4.70 \times 10^{-5} \pm 2.33 \times 10^{-8}$	70.16	
	0.2904	$3.80 \times 10^{-5} \pm 2.17 \times 10^{-8}$	75.87	
	0.3319	$3.45 \times 10^{-5} \pm 2.00 \times 10^{-8}$	78.10	

[a] The concentration; [b] the growth rate constant of *H. pylori*; [c] the inhibition ratio; [d] the half inhibition concentration; [e] mean ± SD., $n = 3$

Table 2 Thermokinetic parameters of the growth of *S. pomb* treated by different concentrations of Schiff base and complex at 32.00 °C

Compound	c^{a}/mmol L^{-1}	k^{b}/s^{-1}	I^{c}/%	IC^{d}_{50}/mmol L^{-1}
The Schiff base	0.0000	$5.42 \times 10^{-5} \pm 2.83 \times 10^{-8}$ [e]	0.00	0.8054
	0.4059	$5.10 \times 10^{-5} \pm 3.51 \times 10^{-8}$	5.90	
	0.6089	$4.22 \times 10^{-5} \pm 2.36 \times 10^{-8}$	22.14	
	0.8118	$2.67 \times 10^{-5} \pm 2.64 \times 10^{-8}$	50.74	
	1.0148	$1.55 \times 10^{-5} \pm 5.92 \times 10^{-9}$	71.40	
	1.2178	$6.02 \times 10^{-6} \pm 6.75 \times 10^{-9}$	88.89	
	1.4207	$3.41 \times 10^{-6} \pm 1.20 \times 10^{-8}$	93.71	
	1.6237	–	100	
The complex	0.0000	$6.32 \times 10^{-5} \pm 5.72 \times 10^{-8}$	0.00	0.1362
	0.0253	$6.15 \times 10^{-5} \pm 5.06 \times 10^{-8}$	2.69	
	0.0507	$5.74 \times 10^{-5} \pm 3.43 \times 10^{-8}$	9.18	
	0.0760	$5.28 \times 10^{-5} \pm 2.85 \times 10^{-8}$	16.45	
	0.1014	$4.43 \times 10^{-5} \pm 2.74 \times 10^{-8}$	29.91	
	0.1267	$3.60 \times 10^{-5} \pm 1.67 \times 10^{-8}$	43.04	
	0.1520	$2.42 \times 10^{-5} \pm 1.62 \times 10^{-8}$	61.71	
	0.1774	$9.32 \times 10^{-6} \pm 9.66 \times 10^{-9}$	85.25	

[a] The concentration; [b] the growth rate constant of *S. pomb*; [c] the inhibition ratio; [d] the half inhibition concentration; [e] mean ± SD., $n = 3$

metabolism of *S. pombe* cell lines. The values of IC_{50} of the Schiff base and the complex on the growth metabolism of *S. pombe* cell lines were found to be 0.8054 and 0.1362 mmol L^{-1}, respectively. The inhibitory ability of the two compounds on the growth metabolism of the *S. pombe* has been observed to decrease according to the following order: the complex > the Schiff base.

Conclusions

In this work, the synthesis and bioactivities of a new Schiff base and its bismuth(III) complex have been reported. The bioactivities of the two synthetic compounds on *H. pylori* and *S. pombe* cell lines were determined by isothermal heat conduction microcalorimetry. The preliminary results showed that the Schiff base stimulated the growth of the *H. pylori*, while the complex inhibited its growth, and the half inhibition concentration (IC_{50}) of the complex was 0.1812 mmol L^{-1}. By contrast, the values of IC_{50} of the two compounds on the growth metabolism of *S. pombe* cell lines were 0.8054 and 0.1362 mmol L^{-1}, respectively, which indicated that the complex possesses stronger inhibitory effect on *S. pombe* than the free Schiff base. The action mechanism of the Schiff base and its bismuth(III) complex on *H. pylori* and *S. pombe* cell lines deserves to be further studied.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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