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Study on Syntheses and Anti-bacterial Activities of Some New Transition Metal Complexes with Schiff Base Ligand Containing Pyridine and Amide Moieties

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Several new transition metal complexes using Schiff base containing pyridine and amide moieties (*N*, *N*′-bis(*β*-salicylaliminoethyl)-2, 6-pyridinedicarboxylic amide, H_4L) as the ligand have been prepared. Their compositions and structures are corroborated by elemental analysis, IR, UV, ^{1}H NMR, DTA-TG and molar conductivity data. Their anti-bacterial activities have been studied by microcalorimetry. The result shows that the ligand and all complexes are potential anti-bacteria reagent and their inhibitory capacities are concentration-depended. The Mn complex has the strongest inhibitory capacity.

Keywords anti-bacterial activity, transition metal complex, Schiff base, pyridine

Introduction

Both pyridine derivatives and Schiff bases are reported to possess significant antibacterial, anti-fungal, anticancer activities, *etc*.[1-3] Further, amide drugs are also very common in medicine field. This gave a great impetus to the research for potential pharmacologically active Schiff bases containing pyridine and amide moieties. It was also reported that incorporation of transition metal into Schiff bases will augment the biological activity of the ligand and decrease the cytotoxic effects of both the metal ion and ligand on the host.[4] Therefore, research on this kind of complexes has been the interest of many chemists.

Microcalorimetry has been proved to be the efficient method of detecting the bioactivity of inhibitors by recording the metabolic power-time curves of inhibited bacteria to offer all kinds of correlation parameters,[4-6] which has been widely used in detecting the bioactivity of Schiff bases, porphyrin and their metal complexes.[4,7]

In this paper, several new transition metal complexes using Schiff base containing pyridine and amide moieties (*N*,*N*′-bis(*β*-salicylaliminoethyl)-2, 6-pyridinedicarboxylic amide, H_4L) as the ligand have been prepared. Their compositions and structures are corroborated by elemental analysis, IR, UV, ^{1}H NMR, DTA-TG and molar conductivity data. Meanwhile, mircocalorimetry was adopted to study the anti-bacterial activities. The correlations of *k*-*c* and *I*%-*c* (*k* for the metabolic rate constant, *c* for the concentration, *I*% for the inhibition rate) were discussed. The half inhibitory concentrations IC_{50} of all the compounds were found.

Results and discussion

Synthesis of the complexes

In the synthesis of the Ni complex, when the molar ratio of metal/ligand (M/L) was 1∶1, a mixture of two solids with different color was gained, which were quite difficult to be separated because both of them were insoluble in common organic solvent. While, with the molar ratio of M/L being 2∶1, only one product was gained in good yield and purity. This may be because the ligand is easier to form bi-nuclear complex with Ni^{2+} ion.

To change the molar ratio of M/L when synthesizing the other complexes, we only obtained the mono-nuclear products. This indicates that it is difficult for Cd^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+} and Pb^{2+} ions to form bi-nuclear complex because of their larger radius.

Characterization of the complexes

All elemental analyses are consistent with the proposed formula of the complexes. Except the Ni and Mn complexes, which are insoluble in common organic solvent except for DMF and DMSO, the complexes are easily soluble in many organic solvents, such as chloroform, acetone, ethanol, methanol, DMF, DMSO, *etc*. They are all air and light stable. The molar conductivities of all the complexes are less than 50 $s \cdot cm^2 \cdot mol^{-1}$ and in the range for non-electrolyte in the solvent.[8] It is suggested that all complexes are neutral complexes. The proposed structures of the complexes are shown in Figure 1.

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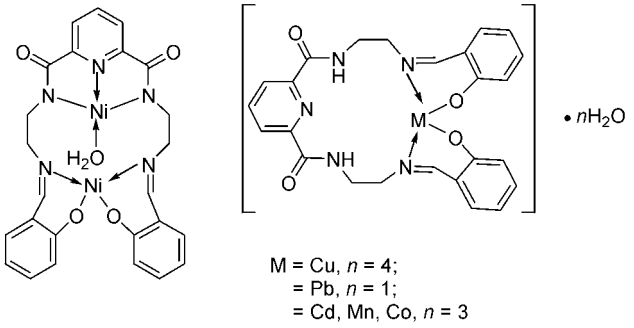

Figure 1 Proposed structures of the complexes.

The ligand H_4L shows a broad weak band in the 2640—2552 cm^{-1} range, which can be assignable to the stretching vibration of the —OH group associated intramolecularly with the N atom of the neighbor —C═N—group (—C═N···HO).[9] This band disappears in all the complexes as result of deprotonation of phenolic OH when the O atoms are coordinated to metal ion, which is further corroborated by the disappearance of phenolic ν(C—OH) at 1281 cm^{-1}.[10] The band at *ca.* 1620 cm^{-1} assigned to ν(C═N), contrast to the free ligand, appears at lower frequencies in all the complexes, indicating that the N atoms of azomethine groups are coordinated to metal ions.[11] In the Ni complex, compared with the ligand, the amide I shifts to lower frequencies, the amide III (1329 cm^{-1}) is strengthened, but amide II totally disappears. These facts suggest that the amide groups are deprotonated when the Ni^{2+} is bound to the two N atoms.[12] While in the other complexes, the characteristic bands of amide groups change little, showing that the amide groups are not coordinated to these metal ions. In the free ligand, the characteristic bands of pyridine ring appear at 1572, 1449 and 1045 cm^{-1}, respectively. However, these bands all shift to higher values in the Ni complex (1599, 1450 and 1149 cm^{-1}) but stand almost no change in the others. This implies that only in Ni complex is the N atom of pyridine coordinated to the metal ion.[13,14] The broad band at 3437 cm^{-1} may be attributed to the $\nu(H_2O)$ in all the complexes. However, only in the Ni complex are two new bands occurring observed at 901 and 644 cm^{-1}, respectively. This implicates that the water in Ni complex is coordination water but it is lattice water in the others.[15] In all complexes, there also appear two new bands in the range of 497—610 cm^{-1} and 446—472 cm^{-1}, which are contributed to ν(N—M) and ν(O—M), respectively.[16]

In the UV spectra of the free ligand, only one strong band (λ 269 nm) is observed, which is assignable to the superim-position of K bands of two conjugated systems  and  . This band appearing at ⩾270 nm in all complexes shifts to longer wave and even more (274 nm) in the Ni complex, which confirms the deprotonation of the ligand on coordination. The UV tonation of the ligand on coordination. The UV spectra also corroborate the coordination of the ligand to all the metal ions with the facts that the ε_1 is evidently enlarged and that a new band at 340—353 nm due to the *d* electron transfer between the ligand and metal ion is observed in all the complexes.[17]

The ^{1}H NMR spectra of the ligand and the investigated complexes exhibit all expected signals with the desired integral values and support the postulated molecular structure. The signals of phenolic O**H** and —CON**H**— can be easily identified because they disappear upon D_2O exchange. In the investigated complexes, compared with the ligand, it is noted that the signal of phenolic O**H** at δ 13.55 totally disappears confirming the deprotonation of the groups on coordination, and that there is a downfield shift in the signal of azomethine protons agreeing with the coordination of the metal ion to the group.[9] Furthermore, in the Ni complex, the signal of pyridine **H** shifts to downfield and the signal of amide **H** vanishes, suggesting the coordination of the N atom of pyridine to Ni^{2+} ion[18] and the deprotonation of amide groups on coordination, respectively.

The thermal behaviors of the Mn and Ni complexes in N_2 surroundings were studied by TG-DTA. The Ni complex starts to lose water until 187 ℃ with the total water-losing rate 3.31%, which suggests that there is a coordination water in the complex. However, the Mn complex loses its water before 80 ℃ and total water-losing rate is 9.37%, indicating that there exist three lattice waters. The two complexes decompose at 325 ℃ and 316 ℃, respectively, and finally transform to metal oxide with the total weight-losing rate 74.49% and 87.27% , respectively.

Antibacterial activities

The plots of growth rate constants vs. concentration (*k*-*c*) and inhibition rate vs. concentration (*I*%-*c)* are shown in Figure 2.

From Figure 2, it is noted that the growth rate constants *k* of *E. coli* inhibited by the ligand and all complexes under different concentrations are less than those in the control experiment, and that all corresponding inhibition rates *I*% are more than zero. These facts demonstrate that the ligand and complexes all have the capacity of inhibiting the metabolic growth of the investigated bacteria to different extent. What is more, with the increment of the concentration, the *k* values decrease progressively, while the corresponding *I*% values increase gradually, which means that the inhibitory capacity of the investigated compounds is reinforced with the increment of concentration. However, the plots of *k* vs. *c* and *I*% vs. *c* for the ligand and the complexes present non-linear shapes, which manifests that the inhibitory capacity of these compounds was concentration-dependent, but the dose-rate constant relationship is not simply linear. In the case of Mn complex, when the concentration is 0—200 μg/mL, the increment of *k* and

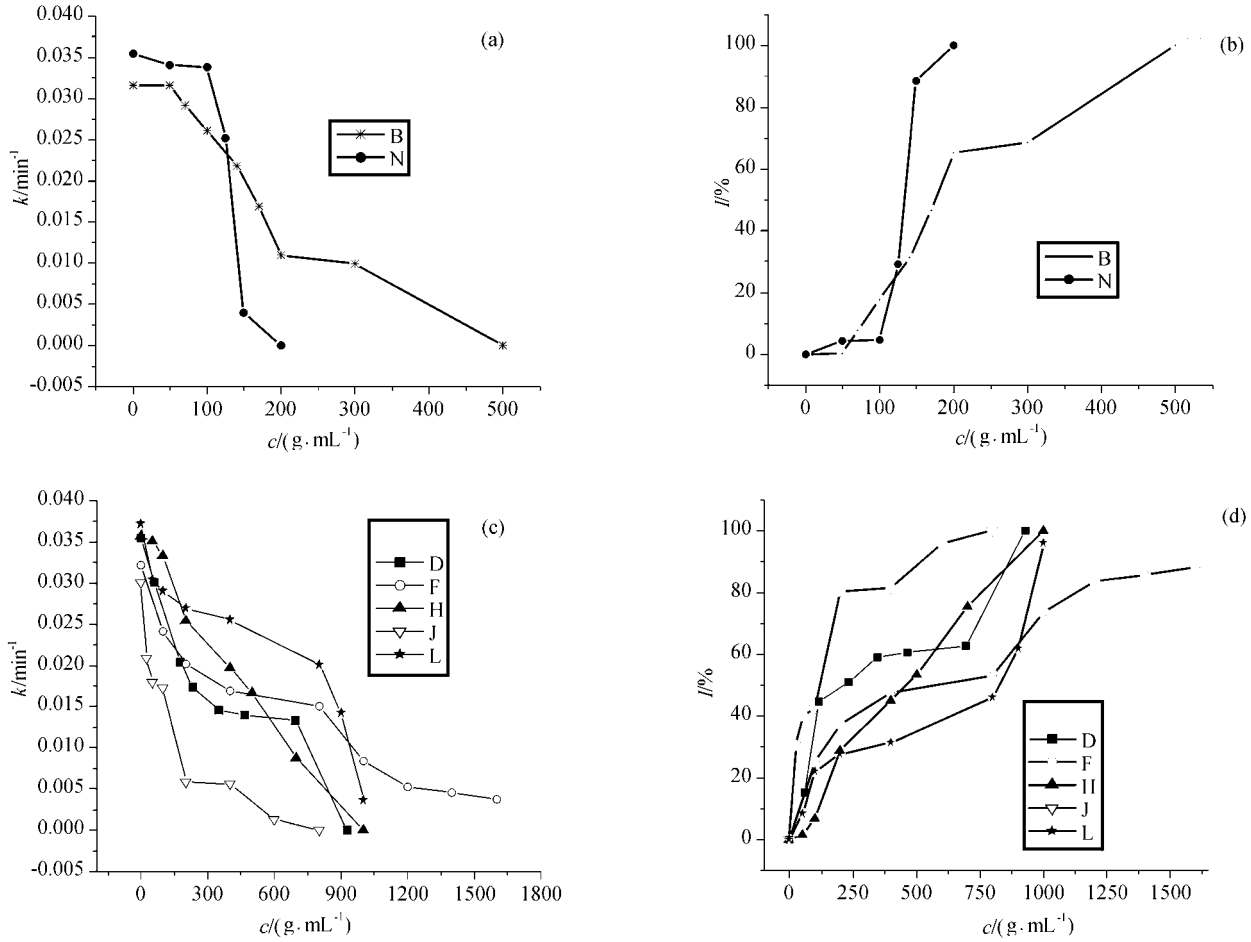

Figure 2 Plots of *k*-*c* and *I*%-*c* for the ligand and complexes (B for the ligand; D, F, H, J, L and N for the Cu, Pb, Co, Mn, Ni and Cd complex, respectively).

the decrement of *I*% are quite evident; while *c* is more than 200 μg/mL, the variation trend turns to be slow. This fact shows that, when the concentration is 0—200 μg/mL, the variation of *c* has a great effect on the inhibitory capacity of the complex; while *c* is more than 200 μg/mL, the effect of *c* slows down. In addition, in the case of Cd complex, when the concentration adds up to a certain value (100 μg/mL), the inhibitory capacity begins to strengthen dramatically with the increment of concentration. The metabolic growth of the bacteria is completely inhibited at 200 μg/mL. The law of gradual increment of *k* and that of gradual decrement are different with one another, indicating that the compounds have different anti-bacterial activity because of the different composition.

The half inhibitory concentration IC_{50} is defined as: the concentration of drugs that reduces the growth rate constants in the log phase to half the control value.[19] In light of this definition, the half inhibitory concentration IC_{50} (μg/mL) are: 226 (H_4L), 216 (Cu complex), 553 (Pb complex), 463 (Co complex), 115 (Mn complex), 838 (Ni complex) and 134 (Cd complex), respectively (see Figure 2). In view that the inhibitory capacity of drugs can be directly reflected on the metabolic power-time curves of the inhibited bacteria, and can be quantitatively characterized by *k* and IC_{50},[19] it is noted that the inhibitory capacity of the ligand is enhanced only when coordinating to metal ions Cu, Cd and Mn, but reduced when coordinating to the other investigated metal ions. Furthermore, the extent varies with different compounds. These facts indicate that different coordination metal ions have different influence on the antibacterial activity of the ligand. This result reflects the difference of the way and mechanism that the complexes interact with bacteria due to the difference in metal ions. It needs further investigation on the interaction mechanism. In all the investigated compounds, the Mn complex has the strongest inhibitory capacity, which is consistent with many reported results.[20]

Experimental

All chemicals and solvents used were purchased as A.R. grade reagents and without further purification before use. The ligand was prepared in our lab previously.[21] *E. coli* (*E. coli* AB91112) was provided by the Institute of Life Science, Wuhan University, China. The LB culture medium contained per 1000 mL (pH = 7.0—7.2): NaCl (5 g), peptone (5 g) and yeast extract (5

g) (oxiod company), sterilized DMF as the solvent. The medium was sterilized at 120 ℃ for 30 min.

Elemental analyses (C, H, N) were conducted by a MOD-1106 elemental autoanalysizer. Melting point was determined by WC-1 Microscope Melting-Pointer. IR spectra in the 4000—400 cm^{-1} range were recorded on a Shimadzu FTIR 3000 instrument. The UV-vis spectra in 200—700 nm were recorded on a Shimadzu UV-160A spectrophotometer. 1D ^1H NMR spectra were acquired at a Variant Mercury-UX 300 MHz spectrophotometer, using TMS as internal standard. Molar conductivities were obtained on a DDSJ-308 type instrument using DMF as solvent at (25 ± 1) ℃. DTA-TG measurements were performed using a Shimadzu DT-40 type instrument in 20—700 ℃. Metal analysis was carried out by titration with EDTA.

A microcalorimeter, LKB-2277 Bioactivity Monitor manufactured by LKB corporation of Sweden was used to obtain the metabolic power-time curves of the bacteria. The microcalorimeter was thermostated at 37 ℃. The baseline stability for the instrument was 0.1 μW/24 h. Two 15 mL of stainless steel ampoule bottles were used. For the details of the structure and performance of the instrument, see Ref. 19.

Preparation of the complexes

$Ni_2L \cdot H_2O$ To 30 mL of ethanol was added H_4L (0.23 g, 0.5 mmol), stirring and heating until H_4L was dissolved completely. Then an ethanolic solution (20 mL) of $Ni(CH_3COO)_2 \cdot 4H_2O$ (0.26 g, 1.04 mmol) was added. The mixture immediately turned orange. With stirring and refluxing for 1 h, orangish-red precipitate began forming. After refluxing for 5 h, the product was filtered off, washed with ethanol and ethyl ether and dried *in vacuo* (0.21 g, yield 71.13%). m.p. >300 ℃; UV-vis (DMF) λ_{max}: 274, 353 nm; ε: 44272, 7120; ^1H NMR (DMSO, 300 MHz) δ: 8.66 (t, J=7.7 Hz, 1H), 8.16 (d, J=9.0 Hz, 2H), 8.06 (s, 2H), 6.77—7.33 (m, 8H), 3.86 (q, 4H), 3.30—3.53 (m, 4H); IR (KBr) v: 3437, 2977, 1667, 1619, 1599, 1149, 466, 497 cm^{-1}; Λ_m: 26 s • cm^2 • mol^{-1}. Anal. calcd for $Ni_2(C_{25}H_{23}N_5O_5)$: C 50.81, H 3.89, N 11.86, Ni 19.88; found C 50.15, H 3.95, N 11.52, Ni 19.53.

Cu, Mn, Cd, Co and Pb complexes were prepared by a procedure analogous to the Ni complex, except the ratio of ligand/metal was 1∶1 and the Co complex was prepared under N_2 protection.

$Cd(H_2L) \cdot 3H_2O$ Yield 32%; color yellow; m.p. >300 ℃; UV-vis (DMF) λ_{max}: 270, 346 nm; ε: 42310, 5367; ^1H NMR (DMSO, 300 MHz) δ: 8.35 (d, J=7.6 Hz, 2H), 8.31 (br, 2H), 8.12 (s, 2H), 7.98 (t, J=8.9 Hz, 1H), 6.66—7.24 (m, 8H), 3.83 (q, 4H), 3.37—3.61 (m, 4H); IR (KBr) v: 3406, 2926, 1670, 1567, 1541, 1569, 1047, 467, 599 cm^{-1}; Λ_m: 30 s •cm^2 •mol^{-1}. Anal. calcd for $Cd(C_{25}H_{29}N_5O_7)$: C 48.15, H 4.65, N 11.23, Cd 17.98; found C 48.62, H 4.31, N 11.54, Cd 17.68.

$Cu(H_2L) \cdot 4H_2O$ Yield 56%; color blackish-green; m.p. >300 ℃; UV-vis (DMF) λ_{max}: 271, 341 nm; ε: 53144, 6886; IR (KBr) v: 3420, 2920, 1673, 1620, 1537, 1572, 1049, 460, 610 cm^{-1}; Λ_m: 36 s • cm^2 • mol^{-1}. Anal. calcd for $Cu(C_{25}H_{31}N_5O_8)$: C 50.63, H 5.23, N 11.81, Cu 10.72; found C 51.17, H 5.51, N 11.26, Cu 10.32.

$Mn(H_2L) \cdot 3H_2O$ Yield 79%; color brown; m.p. >300 ℃; UV-vis (DMF) λ_{max}: 271, 344 nm; ε: 44940, 4985; IR (KBr) v: 3444, 2926, 1673, 1615, 1536, 1571, 1045, 452, 579 cm^{-1}; Λ_m: 29 s • cm^2 • mol^{-1}. Anal. calcd for $Mn(C_{25}H_{29}N_5O_7)$: C 53.00, H 5.12, N 12.37, Mn 9.71; found C 52.68, H 4.97, N 12.58, Mn 10.11.

$Co(H_2L) \cdot 3H_2O$ Yield 34%; color bluish-green; m.p. >300 ℃; UV-vis (DMF) λ_{max}: 271, 342 nm; ε: 42813, 6682; ^1H NMR (DMSO, 300 MHz) δ: 8.36 (d, J=7.5 Hz, 2H), 8.28 (br, 2H), 8.11(s, 2H), 8.01 (t, J=8.9 Hz, 1H), 6.63—7.28 (m, 8H), 3.84 (q, 4H), 3.37—3.61 (m, 4H); IR (KBr) v: 3425, 2921, 1671, 1622, 1542, 1569, 1045, 464, 601 cm^{-1}; Λ_m: 48 s • cm^2 • mol^{-1}. Anal. calcd for $Co(C_{25}H_{29}N_5O_7)$: C 52.64, H 5.13, N 12.28, Co 10.34; found C 53.16, H 5.09, N 12.80, Co 9.87.

$Pb(H_2L) \cdot H_2O$ Yield 46%; color yellow; m.p. >300 ℃; UV-vis (DMF) λ_{max}: 270, 341 nm; ε: 41486, 5776; ^1H NMR (DMSO, 300 MHz) δ: 8.33 (d, J=7.5 Hz, 2H), 8.22 (br, 2H), 8.14 (s, 2H), 7.99 (t, J=8.9 Hz, 1H), 6.76—7.34 (m, 8H), 3.49 (q, 4H), 3.27—3.51 (m, 4H); IR (KBr) v: 3472, 2940, 1669, 1614, 1536, 1568, 1046, 472, 575 cm^{-1}; Λ_m: 47 s • cm^2 • mol^{-1}. Anal. calcd for $Pb(C_{25}H_{25}N_5O_5)$: C 43.99, H 3.67, N 10.26, Pb 30.35; found C 43.46, H 3.24, N 10.71, Pb 30.76.

Test of bioactivities for the ligand and complexes

The experimental principles see Ref. 19. The metabolic power-time curves of bacteria were recorded using ampoule method. The experimental details see Ref. 22. The experimental procedure is shown as follows:

One sealed ampoule contained a reference solution such as the LB culture medium, the other ampoule contained the sample (suspension of bacteria). The sample normally occupied position A in the monitor and reference occupied B. Each ampoule contained 1 mL of sample or reference and 2 mL of air. The system must be cleaned and sterilized and the base line should be stabilized before experiment.

In this type of experiment, the bacteria used were suspended into the LB culture medium. The compound was added from the beginning of the experiment, *i.e*, it was introduced as soon as the bacteria were inoculated in the LB culture medium. The solutions of the compounds were prepared in LB culture medium, and prepared freshly every time.

The temperature of all calorimetric experiments was 37.00 ℃, and the amplifier of the monitor was set at 100 μW or 300 μW.

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