



Synthesis, characterisation and antibacterial activities of some metal(II) complexes of 3-(-1-(2-pyrimidinylimino)methyl-2-naphthol

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ABSTRACT

The Schiff base, 3-(-1-(2-pyrimidinylimino)methyl-2-naphthol with NO chromophores have been synthesised and its Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes isolated. The ligand coordinates to the metal ions in the ratio 2L: 1M, through the azomethine N and naphthol O atoms, resulting in N₂O₂ chromophores around the central metal atom. The % metal, infrared (IR) and electronic spectral measurements are consistent with the adoption of a 4-coordinate square planar/ tetrahedral geometry for the metal complexes. The infrared spectra show lone $\nu_{C=N}$, ν_{M-N} and ν_{M-O} bands for the metal complexes in the range 1649-1655 cm⁻¹, 579-599 cm⁻¹ and 410-497 cm⁻¹ respectively, indicative of the existence of geometric isomerism, and the complexes are in the cis-isomeric forms. Further evidence of coordination is the higher melting points of the complexes in the range 237-301°C in comparison with that of the ligand at 92-94°C. The *in-vitro* antibacterial studies show that the Schiff base and its Zn(II) complex exhibit broad-spectrum antibacterial activities against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Staphylococcus aureus* and *Proteus mirabilis* like ciprofloxacin (30.0-50.5 mm) with inhibitory zones range of 14.0-20.0 and 12.0-17.5 mm respectively in the same assay.

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Introduction

Pyrimidinyl Schiff bases and their metal complexes have a wide range of biological activities such as antimicrobial, anti-HIV, antiulcer and anticancer (1-10). Furthermore, drugs containing pyrimidine moiety are more prominent in treating solid tumours and cancers due to their good anti-proliferation activity and low toxicity e.g. bevacizumab in combination with 5-fluoro uracil is used in treatment of metastatic colorectal cancer (11) while sorafenib and sunitinib, which are small-molecule multikinase inhibitors are used for the treatment of advanced renal-cell carcinoma (12-13). Currently, the drug pazopanib (5-(4-[(2, 3-dimethyl-2H-indazolyl-6-yl)methylamino]-2-pyrimidinyl)amino-2-methylbenzenesulfonamide) is undergoing clinical development for use in treating renal cell cancer and other solid tumours (14). In addition, our group is currently involved in syntheses, physicochemical and bioactivities of various Schiff base chelates containing pyrimidine moiety with the aim of deriving Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) chelates with broad-spectrum antimicrobial activities, which can be further developed into surface cleaning agents, while those with better anticancer activity than Cis-platin are being studied for *in-vivo* anticancer activities (15-19). Recently, we present our findings on synthesis, characterisation, antimicrobial and anticancer properties of the pyrimidinyl Schiff bases, 3-(-1-(4-methyl-6-chloro-2-pyrimidinyl imino)methyl-2-naphthol and 3-(-1-(4,6-dimethyl-2-pyrimidinyl imino) methyl-2-naphthol, 3-(-1-(4-methoxy-6-methyl-2-pyrimidinylimino)methyl-2-naphthol and their Mn(II), Co(II), Ni(II), Cu(II), Zn(II)/Pd(II) complexes (16-19). This work is thus an extension of these former studies, in which there is no substituent on the pyrimidine ring with the sole

aim of deriving metal complexes with better antibacterial activities than the substituted analogs. Their conductance, infrared and electronic properties are also discussed. These complexes and the ligand are new, being reported by us for the first time (20-25).

Experimental

Chemicals

Reagent grade 2-amino-pyrimidine, 2-hydroxy-1-naphthaldehyde, hydrated manganese(II) nitrate, cobalt(II) nitrate, nickel(II) acetate, copper(II) nitrate and zinc(II) nitrate (Aldrich) were used as received. Solvents were purified by standard methods.

Physical measurements

The percentage manganese, cobalt, nickel, copper and zinc were determined titrimetrically (26). The solution spectra were recorded on a Perkin-Elmer λ 20 spectrophotometer equipped with an integrating sphere while infrared spectra were measured as KBr discs on a Bruker-IFS 66V spectrometer in the range 4000-400 cm⁻¹. The melting points (uncorrected) were done using a Stuart scientific melting point apparatus smp3.

Syntheses

Preparation of 3-(-1-(2-pyrimidinylimino)methyl-2-naphthol

The ligand, {[C₁₀H₆(OH)CH:N(C₄H₅N₂)]}, HL, was prepared by refluxing a mixture of 0.012 mol (1.14 g) of 2-amino pyrimidine and 0.012 mol (2.07 g) of 2-hydroxy-1-naphthaldehyde with 6 drops of acetic acid in 60 mL of ethanol for 6 h. The yellow product, formed on cooling to room temperature, was filtered and recrystallized from ethanol and dried *in vacuo* over anhydrous calcium chloride. The yield of the resulting Schiff base was 2.09 g (70%).

Preparation of the Metal(II) Complexes (M = Mn, Co, Cu, Zn).

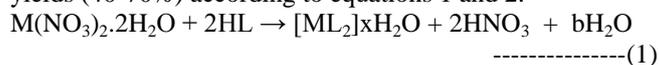
A solution of the metal(II) nitrates (0.69 mmol, 0.12-0.20 g) in 20 mL ethanol was added to a stirring solution of the ligand (1.37 mmol, 0.40 g) in 30 mL ethanol at room temperature (26°C), followed by the gradual addition of triethylamine (1.37 mmol, 0.17 mL). The resulting homogeneous solution was further refluxed for 6 h at 50°C, during which the products formed. These were later filtered, washed with ethanol and dried in *vacuo* over anhydrous CaCl₂. Similar procedure was used to isolate the Ni(II) complex from its acetate.

Antibacterial studies

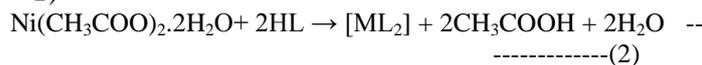
The assay was carried out on the ligand and its metal(II) complexes using Agar diffusion technique. The surface of the agar in a Petri dish is uniformly inoculated with 0.3 mL of 18 hours old culture of *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Staphylococcus aureus*, and *Proteus mirabilis*. Using a sterile cork borer, 5 mm wells were bored into agar. Then 0.06 mL of 10 mg/mL concentration of each metal complex in DMSO was introduced into the wells and the plates were allowed to stand on bench for 30 min before incubation at 37°C for 24 h after which inhibitory zones (in mm) were taken as a measure of antimicrobial activity. The experiments were conducted in duplicates and ciprofloxacin was used as the reference drug.

Results And Discussions

The reaction of the ligand with the metal(II) nitrates (Mn, Co, Cu, and Zn) gave coloured complexes in moderate-good yields (40-70%) according to equations 1 and 2.



(when M = Mn {x = 2, b = 0}, Cu, Zn {x = 1, b = 1}; Co {x = 0, b = 2})



There is a good agreement between experimental and calculated percentages of metal. The ligand melts in the range 92-94 °C while the complexes have high melting points in the range 237-301 °C. This observation further corroborates coordination. The complexes are insoluble in water but have good solubility in other common organic solvents such as MeOH, EtOH, chloroform, DMF, and DMSO. This suggests that the complexes are not polymeric. The % metal, colours, % yields, and melting points of the complexes are presented in Table 1.

Infrared spectra

The relevant infrared data are presented in Table 2. The assignments of the infrared bands are made by comparing the spectra of the compounds with reported literature on similar systems (3, 7, 10, 27). The νOH band of the ligand is seen at 3395 cm⁻¹ and its absence in the complexes is due to the involvement of the naphthol O in bonding to the metal ions. The broad band at 3500 cm⁻¹, in all the metal complexes with the exception of the Co(II) and Ni(II) complexes is assigned to νOH coordinated/hydrated water. The uncoordinated C=N stretching vibration in the ligand is observed as a lone band at 1650 cm⁻¹. It still remains as single band in the metal complexes and suffered bathochromic /hypsochromic shifts to 1655-1649 cm⁻¹ in the Schiff base complexes, thus confirming the involvement of the imine N atom in coordination to metal(II) ion. Furthermore, the observance of a single νC=N band in this metal complexes indicate that they exhibit geometric isomerism and are in the cis-isomeric forms (Figure 1). It has been documented

that metal complexes in cis-isomeric form usually have a single νC=N bands while those in trans-isomeric form have two νC=N bands (28-29). The δC—H vibration of the ligand is observed at 1025 cm⁻¹ and suffers bathochromic shift to 952-951 cm⁻¹ in the complexes due to the pseudo-aromatic nature of the chelates (19). Further evidence of coordination is the observance of bands due to ν(M—O) and ν(M—N) at 497-410 and 599-572 cm⁻¹ respectively in the complexes. These bands are absent in the spectra of the ligands.

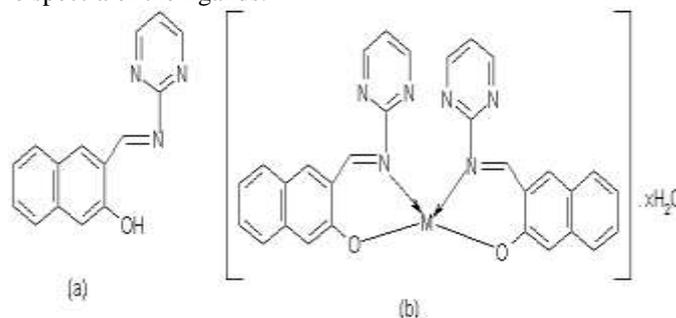


Figure1: Proposed structure for the ligand (a) and its metal(II) complexes (b).

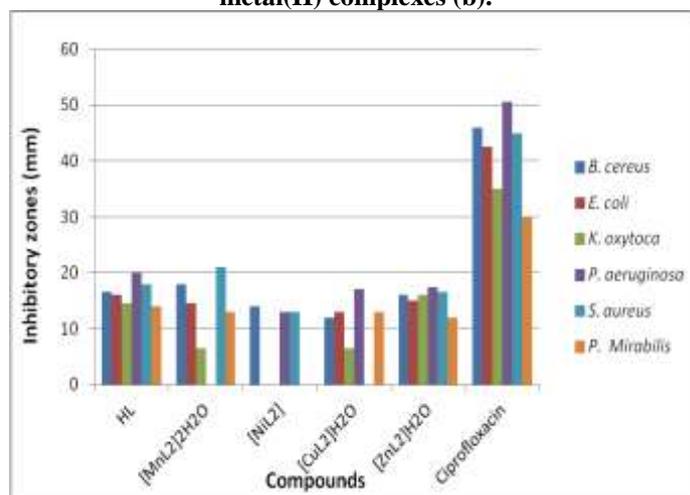


Figure 2: Histogram of the antibacterial activities of the ligand and its complexes

Electronic spectra

The ultraviolet spectra of the compounds in DMSO are characterized by three peaks between 25.04-29.0, 30.3- 38.0 and 42.0 kK with molar absorptivities of 10⁴-10⁶ M⁻¹ cm⁻¹, assigned to n-π*, π-π* and charge transfer transitions respectively. The molar absorptivities of the complexes in the visible region are in the range 10²-10³ M⁻¹cm⁻¹ ruling out octahedral geometry, since octahedral complexes have molar absorptivities in the range 1-50 M⁻¹cm⁻¹ (29). The Mn(II) complex shows a band at 24.63 kK typical of 4-coordinate tetrahedral geometry and is assigned as ⁶A₁ → ⁴E transitions. Similarly, the Co(II) complex, displays a lone band at 24.39 kK assigned to ⁴A₂ → ⁴T₁(P) (ν₃) and the transition, ⁴A₂ → ⁴T₂ (ν₁) in the range 5-7kK is not seen because it is outside the range covered by the instrument (1,6).

Nickel(II) complexes can undergo structural switches from four-coordinate square planar to tetrahedral or to six-coordinate octahedral (27). The observance of a single band at 22.27 kK is indicative of square-planar geometry and is assigned to ¹A_{1g} → ¹A_{2g}, transitions (25). Copper(II) is susceptible to Jahn Teller distortions in a cubic environment, giving rise to an unsymmetrical or multiple bands.

Table 1. Analytical data for the compounds

Compound (Empirical formula)	F. M	Color	% Yield	M.p (°C)	%M (Calc.)
HL (C ₁₅ H ₁₁ N ₃ O)	249.26	Yellow	70	92-94	-
[MnL ₂].2H ₂ O (MnC ₃₀ H ₂₄ N ₆ O ₄)	587.52	Brown	70	290-293	9.14 (9.35)
[CoL ₂] (CoC ₃₀ H ₂₀ N ₆ O ₂)	555.45	Brick Red	50	299-301	11.09 (10.61)
[NiL ₂] (NiC ₃₀ H ₂₂ N ₆ O ₃)	573.25	Orange	70	237-240	10.07 (10.24)
[CuL ₂].2H ₂ O (CuC ₃₀ H ₂₄ N ₆ O ₄)	596.11	Brown	70	279-281	10.55 (10.66)
[ZnL ₂].H ₂ O (CuC ₃₀ H ₂₂ N ₆ O ₃)	579.57	Yellow	60	+252	11.07 (11.22)

+ = Decomposition temperature; F. m = Formula mass; M.p = Melting point

Table 2. Relevant infrared and electronic spectra data of the complexes

Compound	ν OH	ν (C=N) + ν (C=C)	δ C-H	ν (M—N)	ν (M—O)	Electronic spectra(kK)
HL	3395b	1650s 1422s	1025s	-	-	27.45 32.00 42.00
[MnL ₂].2H ₂ O	3500b	1649s 1422s	951s	599m	420m	24.63 25.97 29.0 36.90
[CoL ₂]	-	1649s 1422s	951s	582s	430m	24.39 28.51 36.63
[NiL ₂]	-	1656s 1424s	952s	562m	415m	20.0 22.27 26.32 27.78 36.77
[CuL ₂].H ₂ O	3500b	1655s 1423s	952s	582m	450m	14.26 22.83 26.32 34.97
[ZnL ₂].H ₂ O	3500b	1655s 1423s	952s	595m	410m	20.53 25.04 30.30 36.36

S = strong, m = medium, 1kK = 1000 cm⁻¹

Table 3 Zones of inhibition (in mm) of the compounds against various microbes

Compounds	<i>B. cereus</i>	<i>E. coli</i>	<i>K. oxytoca</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>P. mirabilis</i>
HL	16.5±0.7	16.0±1.4	14.5±2.1	20.0±1.4	18.0±4.2	14.0±1.4
[MnL ₂].2H ₂ O	18.0±1.4	14.5±0.7	6.5±0.7	R	21.0±2.8	13.0±1.4
[CoL ₂]	R	R	R	R	R	R
[NiL ₂]	14.0±1.4	R	R	13.0±2.8	13.0±1.4	R
[CuL ₂].H ₂ O	12.0±1.4	13.0±2.8	6.5±0.7	17.0±2.8	R	13.0±1.4
[ZnL ₂].H ₂ O	16.0±1.4	15.0±2.8	16.0±1.4	17.5±0.7	16.5±0.7	12.0±1.4
+ Ciprofloxacin	46.0±5.7	42.5±3.5	35.0±1.4	50.5±0.7	45.0±7.0	30.0±1.4

R = Resistant, *B. cereus* = *Bacillus cereus*; *E. coli* = *Escherichia coli*, *K. oxytoca* = *Klebsiella oxytoca*,
S. aureus = *Staphylococcus aureus*, *P. mirabilis* = *Proteus mirabilis*

A maximum of three transitions are predicted in the visible spectra of octahedral complexes of copper(II) while regular tetrahedral complex has a single broad band of 10² molar intensity below 10.0 kK while square planar Cu(II) complex has two absorptions between 10.0-20.0kK (30). Consequently, the observance of two bands at 14.26 and 22.83 kK in the Cu(II) complex indicates square planar geometry with the assignment of the bands as ²B_{1g} → ²A_{1g} and ²B_{1g} → ²E_{1g} transitions.

The Zn(II) complex showed M→L CT transitions at 20.53 kK, as no d-d transition is expected and it is tetrahedral because its crystal field stabilization energy is zero (5).

Antibacterial activities

The antibacterial activities of the ligand and its complexes against *B. cereus*, *E. coli*, *S. aureus*, *K. oxytoca*, *P. aeruginosa* and *P. mirabilis* are presented in Table 3 and shown in Figure 2. Generally, the metal free Schiff base ligand is mostly more active than the metal complexes. This is contrary to chelation theory which states that chelation increases antimicrobial

activities mainly because of partial sharing of its positive charge with donor groups of the ligand and possible π -electron delocalisation on the aromatic rings (31), and this suggests that antibacterial activity is mainly independent of the metal ions, with exceptions of the Mn(II), Zn(II) and Co(II) complexes whose activities are higher than that of the ligand against *B. cereus*, *K. oxytoca* and *S. aureus*. Interestingly the Co(II) complex has no activity and on the contrary, the ligand and the Zn(II) complex are active against all the bacteria used with inhibitory zones range of 14.0-20.0 and 12.0-17.5 mm. Thus, proving their potential usefulness as broad-spectrum antibacterial agents.

The inactivity of the Co(II) complex may be attributed to its lipophobic nature. The Ni(II) complex is active against three organisms i.e. *B. cereus*, *P. aeruginosa* and *S. aureus* with inhibitory zones range of 13.0-14.0 mm. Furthermore, Mn(II) and Cu(II) complexes are active against all the bacteria used with the exceptions of *P. aeruginosa* and *S. aureus* with

inhibitory zones range of 6.5-21.0 mm and 6.5-17.0 mm respectively. A look at the antibiotic, Cloxacillin, activities (30.0-50.5 mm) against the various bacterial isolates relative to the metal complexes (6.5-21.0 mm) showed that the activities of the latter are much lower, with optimum activity being about half that of Cloxacillin with $[\text{MnL}_2] \cdot 2\text{H}_2\text{O}$ and $[\text{ZnL}_2] \cdot \text{H}_2\text{O}$ against *S. aureus* and *K. oxytoca* respectively. On comparison this present study with our earlier works on the antimicrobial properties of various substituted pyrimidinyl Schiff bases and their metal complexes, the results showed that the metal complexes with chloro and methyl, methyl and methoxy substituents have more activities than the metal-free Schiff bases whereas the reverse is observed with their unsubstituted (present study) and dimethoxy analogs probably because of their low lipophilic nature (15-17).

Conclusion

The Schiff base, 3-(-1-(2-pyrimidinylimino)methyl-2-naphthol coordinates to the Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) ions using the azomethine N and naphthol O atoms. The assignment of a 4-coordinate square planar/ tetrahedral for the Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) complexes is corroborated by infrared and electronic spectral measurements. The *in-vitro* antibacterial studies show that the Schiff base and its Zn(II) complex exhibit a broad-spectrum antibacterial activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Staphylococcus aureus*, and *Proteus mirabilis* with inhibitory zones range of 14.0-20.0 and 12.0-17.5 mm respectively.

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