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Versatile coordinating behaviour of bis(acylhydrazone) ligands derived from imino- and methyl-iminodiacetic acid diethyl ester. Antimicrobial properties of their trinuclear copper(II) complexes

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Abstract The coordinating properties of a new bis(pyridylhydrazone) ligand derived from iminodiacetic acid diethyl ester and 2-pyridinecarboxaldehyde (picolinaldehyde) H_3Imdp and of the bis(salicylhydrazone) H_5Imds and H_4MeImds ligands derived, respectively, from iminodiacetic acid diethyl ester and from methyl-iminodiacetic acid diethyl ester and salicylaldehyde were considered, by means of analytical and spectroscopic methods, towards first row transition metal ions. These ligands showed various coordination modes in complexation with Cu(II), Co(II), Mn(II) and Zn(II) ions. In particular, we have synthesized and characterized, by analytical, ^{1}H NMR and IR techniques, tri-, di- and mononuclear metal complexes of formula Co_3(HImdp)$(NO_3)_4\cdot 2H_2O$, Cu_3(HImdp)$(NO_3)_4\cdot C_2H_5OH\cdot H_2O$, Cu_3(HImdp)Cl_4, Zn_2(H_3Imdp)$(ClO_4)_4\cdot 2H_2O$, Co_3(HImds)$Cl_2\cdot CH_3OH\cdot H_2O$, Zn_2(H_3Imds)$Cl_2\cdot 2H_2O$, Co(H_4Imds)$NO_3\cdot 2H_2O$, Mn(H_4Imds)$Cl\cdot CH_3OH\cdot H_2O$, Cu(H_3Imds)$\cdot CH_3OH\cdot H_2O$ and Cu(H_2MeImds)$\cdot CH_3OH\cdot 3H_2O$. Antibacterial, antifungal and antiprotozoal properties of H_5Imds and H_3Imdp together with three copper(II) trinuclear species of H_5Imds of formula Cu_3(HImds)$(NO_3)_2 2CH_3OH\cdot 2H_2O$, Cu_3(HImds)$(ClO_4)_2 EtOH\cdot 2H_2O$ and Cu_3(HImds)$SO_4\cdot 4H_2O$ are also discussed. The H_5Imds ligand and their trinuclear copper(II) complexes showed good activities versus *Trichomonas vaginalis, Staphylococcus epidermidis* and *Acanthamoeba castellanii.*

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Introduction

Di- and poly-nuclear metal complexes of hydrazonic ligands play a significant role in the study of multi-electron redox reactions, magnetic exchange interactions and intramolecular binding [21, 24]. Acylhydrazone and semicarbazone derivative compounds occupy an important position as ligands in metal coordination chemistry, because this general class of chelating agents shows different stereochemistries and nuclearity in complexation with transition metal ions [4, 16]. In this context, our attention was mainly attracted by bis(acylhydrazone) ligands derived, in particular, from 2,6-diacetylpyridine, whose interest as chelating agents is due to their versatility in complexation with different metal ions giving a wide variety of metal complexes that could range from mono- to di-nuclear [3, 8] and polynuclear species [9, 15]. We are currently interested in the coordinating behaviour of some compartmental bis(acylhydrazones) derived from imino- and methyl-iminodiacetic acid diethyl ester towards first row transition metal ions. To date, we considered the H_5Imds and H_4MeImds bis(salicylhydrazone) ligands (Fig. 1), prepared by the reaction of the bis(hydrazide) of imino- and methyl-iminodiacetic acid diethyl ester with salicylaldehyde as previously described [2, 6], and the new bis(pyridylhydrazone) H_3Imdp (Fig. 2), derived from the bis(hydrazide) of iminodiacetic acid diethyl ester with 2-pyridinecarboxaldehyde (picolinaldehyde). Previously, we reported the synthesis of some linear trinuclear copper(II) complexes of the bis(salicylhydrazone) H_5Imds ligand, together with the X-ray crystal structure of a coordination polymer, of formula $[Cu_6(HImds)_2(dmso)_5(EtOH)(H_2O)_2](ClO_4)_4\cdot 2H_2O$ [2] and the crystal structure of a sulphate-bridged hexanuclear dimer complex $[Cu_3(MeImds)(SO_4)(H_2O)_3]\cdot 5H_2O$ from H_4MeImds bis(salicylhydrazone) [6]. Regarding

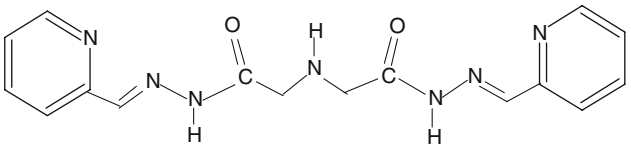

Fig. 1 The compartmental acyclic H_3Imdp ligand

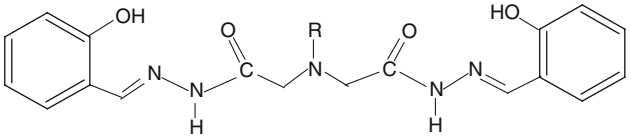

Fig. 2 The compartmental acyclic ligands H_5Imds (R = H) and $H_4MeImds$ (R = methyl)

this, we observed that H_5Imds and $H_4MeImds$ act as aggressive coordinator ligands towards copper(II) ions, giving only linear trinuclear species of general formula $Cu_3(HImds)X_2{\cdot}nH_2O$ (X = nitrate, chloride and perchlorate), $Cu_3(HImds)X{\cdot}nH_2O$ (X = sulphate), $Cu_3(MeImds)X_2{\cdot}nH_2O$ (X = nitrate, chloride and perchlorate) $Cu_3(MeImds)X{\cdot}nH_2O$ (X = sulphate), this independently of the ligand/metal molar ratio. In contrast, we found, as here reported, that the reaction of copper(II) acetate with these ligands in 1:1 molar ratio, gave only mononuclear complexes of formula, respectively, $Cu(H_3Imds){\cdot}CH_3OH{\cdot}H_2O$ and $Cu(H_2MeImds){\cdot}CH_3OH{\cdot}3H_2O$. As a continuation of this work, here, we report preliminary investigations showing the versatile coordinating behaviour, the synthesis and the characterization of H_3Imdp and their trinuclear Cu(II) and Co(II) complexes of formula $Co_3(HImdp)(NO_3)_4{\cdot}2H_2O$, $Cu_3(HImdp)(NO_3)_4.C_2H_5OH{\cdot}H_2O$ and $Cu_3(HImdp)Cl_4$, the dinuclear zinc(II) complex $Zn_2(H_3Imdp)(ClO_4)_4{\cdot}2H_2O$ together with the synthesis and the characterization of dinuclear Zn(II), trinuclear and mononuclear Co(II) and Mn(II) complexes of H_5Imds, respectively, of formula $Zn_2(H_3Imds)Cl_2{\cdot}3H_2O$, $Co_3(HImds)Cl_2CH_3OH{\cdot}H_2O$, $Co(H_4Imds)NO_3{\cdot}2H_2O$, $Mn(H_4Imds)Cl{\cdot}CH_3OH{\cdot}H_2O$, under 3:1 metal/ligand molar ratio conditions, and the above-mentioned Cu(II) mononuclear complexes of H_5Imds and $H_4MeImds$ ligands. All the compounds have been characterized by analytical and IR spectroscopic techniques. It is interesting to note that the Cu_3 core complexes are of interest as potential copper biosite modelling, whereas trinuclear copper clusters are studied as models for the metallo-biosite of the "blue proteins" such as ascorbate oxidase and laccase [1, 11]. In addition, several studies emphasized that some bis-acylhydrazone or semi- and thio-semicarbazone ligands and their metal complexes with Cu(II), Zn(II) or Sn(IV) showed significant pharmacological properties and, in particular, as regards the antimicrobial activity [5, 10, 19]. In this context, preliminary studies concerning the antibacterial, antifungal and antiprotozoal properties of H_5Imds, H_3Imdp ligands together with the trinuclear copper(II) complexes $Cu_3(HImds)(ClO_4)_2EtOH{\cdot}2H_2O$, $Cu_3(HImds)(NO_3)_22CH_3OH{\cdot}2H_2O$ and $Cu_3(HImds)SO_4{\cdot}4H_2O$, previously reported, are here discussed.

Studies concerning the coordinating behaviour of H_3Imdp, H_5Imds and $H_4MeImds$ or derivatives towards other transition metal ions, in order to obtain mono- or poly-nuclear species and hetero-nuclear metal complexes, together with the antimicrobial properties of metal complexes, also reported in this paper, are now in progress.

Experimental

All reactants and solvents were reagent grade and used without further purification. Iminodiacetic acid, methyliminodiacetic acid, salicylaldehyde, 2-pyridinecarboxaldehyde (picolinaldehyde), hydrazine hydrate (98%) and metal salts were purchased from Aldrich. Elemental analyses (C, H and N) were performed on Carlo Erba instruments CHNS-O EA 1108 automatic equipment. All infrared spectra were recorded using KBr discs on a Perkin–Elmer mod. 781 IR spectrophotometer. UV/Vis spectra were recorded on a Beckman DU-65 spectrophotometer. The ^1H NMR and ^{13}C NMR were performed on a Varian 200 XL instrument. The spectra were recorded at 300 K in solution of DMSO-d_6; all chemical shifts are relative to internal tetramethylsilane.

Synthesis of the ligands

H_3Imdp

The bis(hydrazide) was prepared from iminodiacetic acid diethyl ester according to the literature [12]. H_3Imdp was obtained by dropwise addition of an absolute ethanol (50 mL) solution of 2-pyridinecarboxaldehyde (1.52 mL, 16 mmol) to an hot absolute ethanol suspended solution (90 mL) of bis(hydrazide) (1.29 g, 8 mmol). After addition of the 2-pyridinecarboxaldehyde to the suspension of the bis(hydrazide), the resulting solution was completely clear; it was then refluxed for 5 h. After evaporation, *in vacuo*, of the 50% of the solvent, a pale yellow precipitate was obtained by addition of n-hexane (20 mL). After filtration, the title compound was washed with $CHCl_3$ and dried *in vacuo*. (yield 90%). m.p. = 181–183 °C. *Anal. found*: C, 56.7; H, 5.2; N, 28.8%. *Calc. for* $C_{16}H_{17}N_7O_2$ C, 56.6; H, 5.1; N, 29.0%. ^1H NMR [$(CD_3)_2SO$, 25 °C]: δ 3.37 (s, 4H, CH_2), 7.39–8.29 (m, 8H, C_6H_4), 8.57 (s, 2H, CH=N), 11.61 (s, 2H, N–H hydrazonic, exchangeable). ^{13}C NMR [$(CD_3)_2SO$, 25 °C]: δ 169.53 (–NH–C=O), 152.617 (–C=N), 149.59, 144.95, 136.88, 124.53, 119.88 (pyridinics C), 47.80 ($–CH_2–N$).

IR (KBr, cm^{-1}): 3450, 3100 *v*(NH)ms, 3050m (aryl), 1680vs (amide I), 1618m *v*(C=N), 1530m (amide II), 1270s (amide III). UV/Vis (dmso): $\lambda_{max} = 330$ nm.

H_5Imds and H_4MeImds ligands

H_5Imds and H_4MeImds were prepared as described [2, 6].

IR spectra and ^{13}C NMR

H_5Imds; (KBr, cm^{-1}): 3475, 3380, 3190m *v*(OH, NH), 1683vs (amide I), 1615s *v*(C=N), 1520ms (amide II), 1260s (amide III). ^{13}C NMR [$(CD_3)_2SO$, 25 °C]: δ 167.60 (–NH–C=O), 157.46 (–C=N), 147.73, 131.43, 129.67, 119.43, 118.67, 116.47 (aromatics C), 51.16 (–CH_2–N).

H_4MeImds; (KBr, cm^{-1}): 3420, 3200ms *v*(OH, NH), 3050, 1670vs (amide I), 1620s *v*(C=N), 1520m (amide II), 1270s (amide III). ^{13}C NMR [$(CD_3)_2SO$, 25 °C]: δ 167.00 (–NH–C=O), 157.46 (–C=N), 148.33, 132.12, 129.87, 120.07, 118.92, 116.77 (aromatics C), 59.89 (–CH_2–N), 43.27 (–N–CH_3).

Synthesis of the complexes

H_3Imdp complexes

An absolute ethanol solution of copper(II) (nitrate and chloride), or cobalt(II) nitrate hydrate salts was added (3:1, metal/ligand molar ratio) dropwise, under magnetic stirring, to an hot absolute ethanol solution of the ligand. The solution was heated at 70 °C for about 2 h. The Zn(II) perchlorate complex was prepared in the same conditions, but using 2:1 metal/ligand molar ratio. In all cases, a precipitate was formed. The solution was filtered; the precipitate washed with MeOH and dried *in vacuo*.

Cu_3(HImdp)$(NO_3)_4.C_2H_5OH{\cdot}H_2O$, *Anal. found*: C, 25.3; H, 2.6; N, 18.2%. *Calc. for* $Cu_3C_{18}N_{11}H_{23}O_{16}$, C, 25.7; H, 2.8, N, 18.3%. IR (KBr, cm^{-1}): 1615m *v*(C=N–N=C), 1380vs *v*(NO_3).

Cu_3(HImdp)Cl_4, *Anal. found*: C, 28.9; H, 2.6; N, 14.0%. *Calc. for* $Cu_3C_{16}N_7H_{15}O_2$ Cl_4, C, 28.7; H, 2.3; N, 14.6%. IR (KBr, cm^{-1}): 1615m *v*(C=N–N=C).

Co_3(HImdp)$(NO_3)_4{\cdot}2H_2O$, *Anal. found*: C, 23.1; H, 2.2; N, 18.2%, *Calc. for* $Co_3C_{16}H_{19}N_{11}O_{18}$, C, 23.2; H, 2.3; N, 18.6%. IR (KBr, cm^{-1}): 1615m *v*(C=N–N=C), 1385vs *v*(NO_3).

$Zn_2(H_3Imdp)(ClO_4)_4{\cdot}2H_2O$, *Anal. found*: C, 21.4; H, 2.3; N, 18.9%. *Calc. for* $Zn_2C_{16}H_{21}N_7O_{20}Cl_4$, C, 21.3; H, 2.3; N, 18.9%. IR (KBr, cm^{-1}): 1660ms, *v*(amide I), 1610m *v* (C=N), 1100vs *v*(ClO_4). ^1H NMR [$(CD_3)_2SO$, 25 °C] : δ 3.28 (s, 4H, CH_2), 7.47–8.72 (m, 8H, C_6H_4), 8.72 (s, 2H, C=N),12.20 (s, 2H, N–H hydrazonic).

H_5Imds complexes

The preparation of H_5Imds complexes was carried out following the procedure used in a previous work [2]: a methanolic solution of the Co(II) (chloride and nitrate), Zn(II) (chloride) and Mn(II) (chloride) (3:1 metal/ligand molar ratio) was added to a methanol/chloroform (5:1, v/v) solution of the ligand and refluxed for 1.5 h. In all cases, the precipitate formed was isolated, dried and washed with MeOH.

Co_3(HImds)$Cl_2CH_3OH{\cdot}3H_2O$, *Anal. found*: C, 34.4; H, 3.6; N, 10.9%. *Calc. for* $C_{19}H_{25}N_5O_6Cl_2Co_3$, C, 34.2; H, 3.8; N, 10.5%. IR (KBr, cm^{-1}): 1610m *v*(C=N–N=C).

Co(H_4Imds)$NO_3{\cdot}2H_2O$, *Anal. Found*: C, 42.1; H, 4.0; N, 16.7%. *Calc. For* $C_{18}H_{22}N_6O_8Co$, C, 42.5; H, 4.4; N, 16.5%. IR (KBr, cm^{-1}): 3220–2960mb *v*(OH, hydrogen bonded), 1688m and 1660ms (amide I), 1610–1605m *v*(C=N), 1385vs *v*(NO_3).

Mn(H_4Imds)$Cl{\cdot}CH_3OH{\cdot}H_2O$, *Anal. found*: C, 44.5; H, 4.6; N, 13.5%. *Calc. for* $C_{19}H_{24}N_5O_6ClMn$, C, 44.9; H, 4.8; N, 13.8%. IR (KBr, cm^{-1}): 3210–2950mb *v*(OH, hydrogen bonded), 1665–1689vs (amide I), 1610s *v*(C=N).

$Zn_2(H_3Imds)Cl_2{\cdot}2H_2O$, *Anal. found*: C, 35.2; H, 3.7; N, 11.7%. *Calc. for* $C_{18}H_{21}N_5O_6Cl_2Zn_2$, C, 35.7; H, 3.5; N, 11.6%. IR (KBr, cm^{-1}): 1640vs (amide I), 1610ms *v*(C=N).

Synthesis of Cu(H_3Imds)$\cdot CH_3OH{\cdot}H_2O$ and Cu(H_2MeImds)$\cdot CH_3OH{\cdot}3H_2O$ complexes

The mononuclear Cu(H_3Imds)$\cdot CH_3OH{\cdot}H_2O$ and Cu(H_2MeImds)$\cdot CH_3OH{\cdot}3H_2O$, derived, respectively, from H_5Imds and H_4MeImds ligands, were obtained using copper(II) acetate in 1:1 metal/ligand molar ratio, in MeOH/$CHCl_3$ solution for 1 h at 60 °C. In all cases, a green precipitate was formed and characterized as:

Cu(H_3Imds)$\cdot CH_3OH{\cdot}H_2O$, *Anal. found* C, 47.5; H, 4.4; N, 14.8%. *Calc. for* $C_{19}H_{22}N_5O_6Cu$, C, 47.6; H, 4.6; N, 14.6%. IR (KBr, cm^{-1}): 3411m *v*(NH, hydrazonic), 1680ms (amide I), 1610vs *v*(C=N).

Cu(H_2MeImds)$\cdot CH_3OH{\cdot}3H_2O$, *Anal. found* C, 46.0; H, 5.7; N, 13.8%. *Calc. for* C, $C_{20}H_{29}N_5O_8Cu$, C, 45.2; H, 5.5; N, 13.2%. IR (KBr, cm^{-1}): 1665ms (amide I), 1610vs *v*(C=N).

Preparation of H_5Imds copper(II) trinuclear complexes for antimicrobial tests

Cu_3(HImds)$(NO_3)_2 2CH_3OH{\cdot}2H_2O$, and Cu_3(HImds)$SO_4{\cdot}4H_2O$ complexes, utilized for antimicrobial screenings, were prepared as reported [2, 6]. The perchlorate complex of formula Cu_3(HImds)$(ClO_4)_2EtOH{\cdot}2H_2O$ was prepared

as described [2] and used without recrystallization in powder form.

Antimicrobial activity

Bis(acylhydrazones) H_5Imds, H_3Imdp and related copper(II) complexes were dissolved in dimethyl sulfoxide (DMSO), the resulting stock solutions (20 mg mL^{-1}) were stored at +4 °C. In all cases, preliminary tests with DMSO were performed to assure that no microorganism inhibition occurred at used concentrations. Tests were performed in duplicate and were repeated at least three times.

Their antibacterial and antifungal activity was determined as minimum inhibitory concentration (MIC), by using an agar dilution technique [7, 23]. Bacteria included both Gram-positive (*Bacillus* spp., *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* of clinical isolation) and Gram-negative strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae* of clinical isolation). Antifungal assays were performed against *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404, *Pichia guillermondii* and *Rhodotorula glutinis*. Twofold serial dilutions of compound solutions (250–3.9 μg mL^{-1}) were prepared in molten (45 °C) Mueller–Hinton Agar (MHA, Oxoid) for bacteria or potato dextrose agar (PDA, Oxoid) for fungi, and poured in 50-mm-diameter Petri plates. Each plate was inoculated with drops containing 1×10^4 bacteria or 10^3–10^4 conidia or yeast cells. For bacteria, MICs were recorded after 18–24 h of incubation at 35 °C as the lowest concentration which completely inhibit bacterial growth. For fungi, plates were incubated at 25 °C and daily checked for at least 4 days to assess fungal growth.

Antiprotozoal activities were evaluated against *Trichomonas vaginalis* and *Acanthamoeba castellanii*. Our study was performed by using a *T. vaginalis* strain, isolated in Sassari (Italy) from a case of acute vaginal trichomoniasis, axenically grown at 37 °C in Diamond medium supplemented with 10% heat-inactivated fetal calf serum (FCS, GIBCO). Stock solution (2 mg mL^{-1}) of metronidazole (SIGMA), used as reference drug, was prepared in DMSO, and stored in small aliquots at 4 °C. The susceptibility of *T. vaginalis* was determined by the assessment of growth and motility of flagellates exposed to different concentrations of compounds. Tests were performed in sterile tissue-culture microtiter 96-well plates (Corning). Serial twofold dilution of the compounds were made in Diamond medium containing 10% FCS. Control wells received 100 μL of complete Diamond medium. Protozoa, washed twice in phosphate-buffered saline (PBS, pH 7.2), were suspended in the same medium at the density of 2.5×10^5 cells per mL and 100 μL of the calibrate suspension was added to each well. Final concentrations obtained ranged from 250–0.976 μg mL^{-1}. Microtiter plates were incubated aerobically at 37 °C, in a humid atmosphere containing 5% CO_2. At 24, 48, 72 and 96 h of incubation, they were examined with an inverted microscope (OLIMPUS CK) and checked for protozoan motility. The lowest concentration in which no motile flagellates were seen was defined as MIC_{100}, according to Meingassner and Thurner [17].

To study the antiamoebic activity of compounds, we used trophozoites of *Acanthamoeba castellanii*, isolated from a case of amoebic keratitis (in Ancona, Italy), axenically grown at 25 °C in PYG medium [14]. The species identification of this isolate was based on cyst morphology and PCR analysis including primer pair JDP1/JDP2 (targeted to 18S rDNA stretch ASA.S1) [20]. Experiments were performed in sterile 96-well plates. Serial twofold dilution of the compounds, ranging from 100 to 25 μg mL^{-1}, were prepared in PYG medium. Control wells received 100 μl of PYG medium in place of drug dilutions. Amoebae, washed twice in PBS, were suspended in PYG medium at a density of 8×10^3 cells mL^{-1}. One hundred microlitres of the calibrated trophozoite suspension was added to each well, and then the plates were sealed and incubated at 37 °C in a 5% CO_2 atmosphere. At 2, 3, and 6 days of incubation, amoebae were counted with a hemacytometer (Nageotte chamber). Cell viability was determined by the nigrosin dye exclusion method. The lowest drug concentrations that caused complete (100%) inhibition of trophozoite growth (MIC_{100}) at days 2, 3, and 6 was evaluated in comparison with that of rokitamycin (Formenti), used as reference drug (stock solution at 20 mg mL^{-1}, in 95% ethanol).

Results and discussion

The nonadentate, symmetric "tritopic" H_3Imdp, H_5Imds and "ditopic" H_4MeImds ligands belong to the class of compartmental trinucleating ligands and in particular they could be assimilated to one of that "end-off" acyclic ligands that provided speculative models for the type-III site in cuproproteins [13]. The ligands were synthesized by condensation of bis(hydrazide) of the iminodiacetic acid, with 2-pyridinecarboxaldehyde and salicylaldehyde, respectively, in absolute EtOH/$CHCl_3$ solution for H_5Imds [2] and in absolute EtOH as regarding H_3Imdp, and by the reaction of bis(hydrazide) of the methyl-iminodiacetic acid with salicylaldehyde, in MeOH/$CHCl_3$ solution for H_4MeImds ligand [6]. The H_3Imdp compound, is air-stable, obtained in good yield and characterized by IR, ^{1}H NMR ^{13}C NMR, UV/Vis spectroscopy and elemental analysis. The H_3Imdp, H_5Imds and H_4MeImds metal

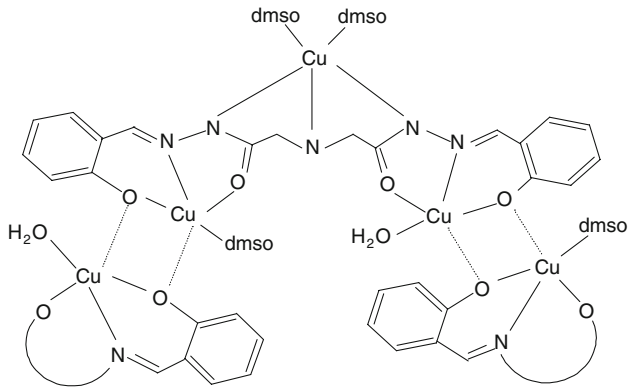

Fig. 3 The repeat unit in $[Cu_6(HImds)_2(dmso)_5(EtOH)(H_2O)_2](ClO_4)_4 \cdot 2H_2O$ [2]

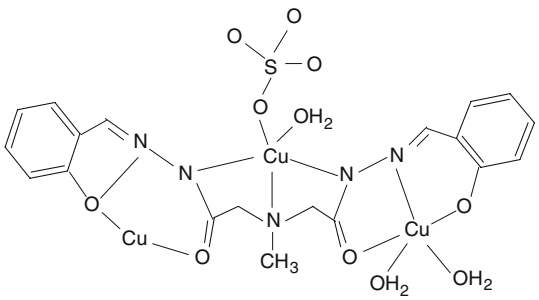

Fig. 4 Trimetallic monomeric unit $[Cu_3(MeImds)(SO_4)(H_2O)_3]$ structure [6]

complexes, showed decomposition above 300 °C without melting, are air-stable, obtained in satisfactory yields and are generally soluble in MeOH, H_2O or in MeOH/H_2O mixture. Unfortunately, the lack of information about the molecular structure of the metal complexes, due to microcrystalline forms not suitable for X-ray analysis, constrains us to suggest plausible structures by means of elemental analysis and spectroscopic methods and in analogy with the coordinating behaviour, unequivocally, established by X-ray structural analysis of the polymeric $[Cu_6(HImds)_2(dmso)_5(EtOH)(H_2O)_2](ClO_4)_4 \cdot 2H_2O$ (Fig. 3) and the dimeric $[Cu_3(MeImds)(SO_4)(H_2O)_3] \cdot 5H_2O$ trimetallic complexes previously described (Fig. 4) [2, 6].

IR spectra

IR spectra of H_3Imdp, H_5Imds and $H_4MeImds$ are mainly characterized by the absorptions due to O–H and N–H stretching in the 3500–3100 cm^{-1} region. Amide I characteristic band of ν(C=O) at 1680 cm^{-1} (H_3Imdp), 1683 cm^{-1} (H_5Imds) and 1670 cm^{-1} ($H_4MeImds$) indicating a keto-form in the solid state. All ligand spectra show the (C=N) absorption band in the appropriate region (1620–1615 cm^{-1}). Hydrazonic chain is represented by two medium-intense bands centred at about 1530 and 1270 cm^{-1} for H_3Imdp, 1520 and 1260 cm^{-1} for H_5Imds, 1520 and 1270 cm^{-1} as regards the $H_4MeImds$, assigned to Amide II and Amide III system, respectively.

H_3Imdp complexes

In spite of the metal ligand/metal molar ratio used in the reactions, only trinuclear metal complexes of formula Cu_3 $(HImdp)(NO_3)_4.C_2H_5OH \cdot H_2O$, $Cu_3(HImdp)Cl_4$ and $Co_3(HImdp)(NO_3)_4 \cdot 2H_2O$ were obtained (Fig. 5).

As mentioned earlier, all the complexes have been, also, characterized in analogy and on the basis of the information obtained by IR and X-ray structural analysis of H_5Imds and $H_4MeImds$ trinuclear copper(II) complexes (Figs. 3, 4). However, with the exception of the absorptions due to anionic groups, the IR spectra of the Co(II) and Cu(II) trinuclear complexes show similar spectroscopic patterns observed in the 4000–600 cm^{-1} region. These similarities agree well with the IR spectra of the trinuclear copper(II) complexes of H_5Imds and $H_4MeImds$. In particular, in the IR spectra of $Cu_3(HImdp)(NO_3)_4.C_2H_5OH \cdot H_2O$, $Cu_3(HImdp)Cl_4$ and $Co_3(HImdp)(NO_3)_4 \cdot 2H_2O$ trinuclear complexes, we found the absence of the amide I band ν(C=O), centred at 1670 cm^{-1} in the free ligand, and the presence of a new band, at about 1615 cm^{-1}, due to the –C=N–N=C– diazinic system, in good agreement with the bi-deprotonated form of H_3Imdp upon coordination. Then, a trimetallic coordination occurs by the central amino atom, pyridinic nitrogen and by the hydrazonic system in its enolic form (Fig. 5).

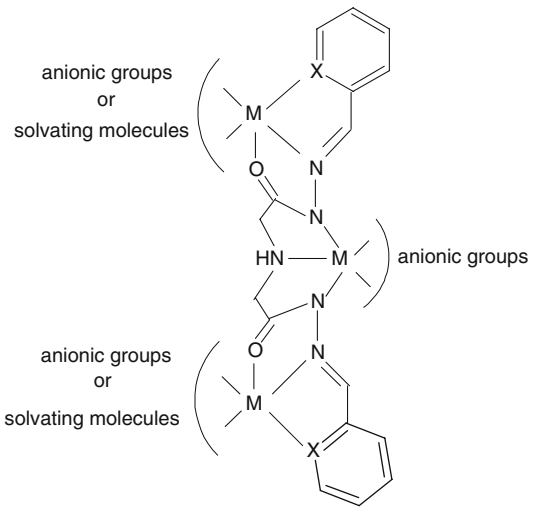

Fig. 5 Proposed coordination mode in $Cu_3(HImdp)(NO_3)_4C_2H_5OH \cdot H_2O$, $Cu_3(HImdp)Cl_4$, $Co_3(HImdp)(NO_3)_4 \cdot 2H_2O$ and $Co_3(HImds)Cl_2$ $CH_3OH \cdot 3H_2O$ trinuclear complexes [X = N (H_3Imdp), OH (H_5Imds); M = Cu(II), Co(II)]

No spectroscopic evidence is attributable to a mono- or bi-dentate ligand behaviour of the nitrate group in the $Cu_3(HImdp)(NO_3)_4.C_2H_5OH{\cdot}H_2O$ complex.

$Zn_2(H_3Imdp)(ClO_4)_4{\cdot}2H_2O$ dinuclear complex was obtained with 1:2 ligand/metal molar ratio. The IR spectrum is mainly characterized by two broad bands in the range 3400–3150 cm^{-1} that could be attributable to solvating water molecules and NH absorptions and by a medium strong broad absorption of amide I ν(C=O) band centred at 1660 cm^{-1} (1680 cm^{-1} in the free ligand). The presence of a weak band at about 1610 cm^{-1} (1618 cm^{-1} in the free ligand), is associated with azomethinic –HC=N– system upon coordination. The ^1H NMR spectrum exhibits an hydrazonic NH (D_2O exchangeable in the free ligand) singlet signal at 12.20 ppm, low-field shifted with respect to the free ligand (11.61 ppm) and a broad multiplet signal in the range 8.85–7.20 ppm associated to a low-field shifted pyridine ring (7.39–8.29 ppm in the free ligand). The inside of multiplet contains a shifted azomethinic –HC=N– singlet signal at 8.72 ppm (7.59 ppm in the free ligand). Therefore, the pyridinic nitrogen, azomethinic nitrogen and carbonylic group are affected by coordination to zinc metal ions. Moreover, the confirmed presence, in the ^1H NMR spectrum, of undeprotonated NH upon coordination, together with the presence of one amide I band ν(C=O) in the IR spectrum, suggests that the ligand is present in keto-form: both carbonylic groups are implicated in the coordination and that "N_2O" donor atom sets are involved in the coordination in the external compartments of the ligand, to form a symmetric Zn_2-complex (Fig. 7).

The IR spectrum of $Zn_2(H_3Imdp)(ClO_4)_4{\cdot}2H_2O$ complex shows no evidence of mono- or bi-dentate ligand behaviour of the perchlorate group.

H_5Imds complexes

The reactions of Co(II) chloride and Co(II) nitrate showed a curious and unexpected behaviour in the complexation with H_5Imds ligand. We obtained, in spite of metal/ligand 3:1 molar ratio used, the trinuclear-chloride and the mononuclear-nitrate complex, respectively. Therefore, a dramatic difference is present in their IR spectra: while the spectrum of $Co_3(HImds)Cl_2CH_3OH{\cdot}H_2O$ (the proposed coordination mode is reported in Fig. 5), shows the characteristic print of the trinuclear complexes described earlier, the IR spectrum of $Co(H_4Imds)NO_3{\cdot}2H_2O$ is mainly characterized by the presence of two strong amide I bands ν(C=O) at 1686 and 1660 cm^{-1} (1683 in the free ligand) that can be attributable, respectively, to one un-coordinated >C=O and to one metal-coordinated >C=O. Two weak shouldered bands present at 1610–1600 cm^{-1} are attributable to ν(C=N) absorptions (1615 cm^{-1} in the free ligand). A new broad band in the range 3220–2960 cm^{-1},

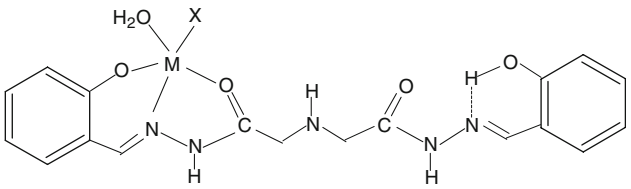

Fig. 6 Mononuclear coordinating fashion proposed for $Mn(H_4Imds)Cl{\cdot}CH_3OH{\cdot}H_2O$ and $Co(H_4Imds)NO_3{\cdot}2H_2O$ complexes (M = Mn(II), Co(II); X = Cl, NO_3)

overlapping the N–H, aliphatic and aromatic νCH bands, could be associated with an intramolecular OH···N=C hydrogen bond between phenolic OH and azomethinic nitrogen [18]. As known, this six-membered hydrogen-bonded species was found in many salicylaldimine systems. While the X-ray structure of H_5Imds ligand has not been determined, the OH···N=C hydrogen bond was evidenced by X-ray molecular structure of $H_4MeImds$ parent ligand [6]. The weak absorptions at 1535 and 1270 cm^{-1} can be attributable, respectively, to the amide II and III bands (1520 and 1260 cm^{-1} in the free ligand). Therefore, "NO_2" coordination occurs by the azomethinic nitrogen, carbonylic group and by the deprotonated phenolic OH, in one of external ligand compartment (Fig. 6). No spectroscopic evidence is attributable to a mono- or bi-dentate ligand behaviour of the nitrate group in the $Co(H_4Imds)NO_3{\cdot}2H_2O$ complex. Therefore, with exception due to NO_3 absorption group (1385 cm^{-1}), marked similarities with the IR spectrum of $Co(H_4Imds)NO_3{\cdot}2H_2O$ complex are shown by the spectrum of the mononuclear $Mn(H_4Imds)Cl{\cdot}CH_3OH{\cdot}H_2O$ complex, also obtained by 3:1 metal/ligand molar ratio (see experimental section). The absorptions of one co-ordinated and one un-coordinated carbonylic groups in $Mn(H_4Imds)Cl{\cdot}CH_3OH{\cdot}H_2O$ IR spectrum are positioned, respectively, at 1670 and 1689 cm^{-1}, together with a new broad band at about 3210–2950 cm^{-1}, overlapped by N–H, aromatics and aliphatic bands, that could be associated with an intramolecular hydrogen bond between the phenolic OH and the azomethinic nitrogen (Fig. 6).

In the case of the dinuclear $Zn_2(H_3Imds)Cl_2{\cdot}2H_2O$ complex, also, obtained with 1:3 ligand/metal molar ratio, the first significant evidence is a lower shift of the amide I band to 1640 cm^{-1} compared to the free ligand (1683 cm^{-1}), this could suggest that both >C=O groups are involved in the coordination of metal ions. Thus, the absence of –C=N–N=C– diazinic system band together with the presence of amide II and amide III bands, respectively, at 1530 and 1270 cm^{-1}, are in agreement with undeprotonated nature of the hydrazonic nitrogens upon coordination. Moreover, the presence of a medium strong band centred at 1610 cm^{-1} (1615 cm^{-1} in the free ligand), attributable to azomethinic nitrogen absorption,

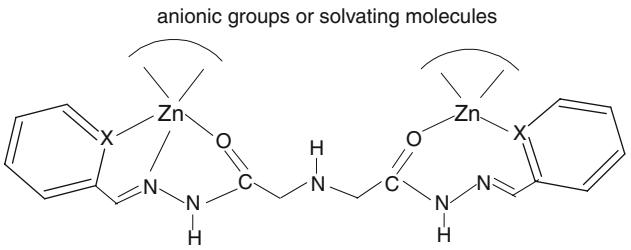

Fig. 7 Proposed coordination mode in $Zn_2(H_3Imds)Cl_2{\cdot}2H_2O$ and $Zn_2(H_3Imdp)(ClO_4)_4{\cdot}2H_2O$ dinuclear complexes. [X = N (H_3Imdp); OH (H_5Imds)]

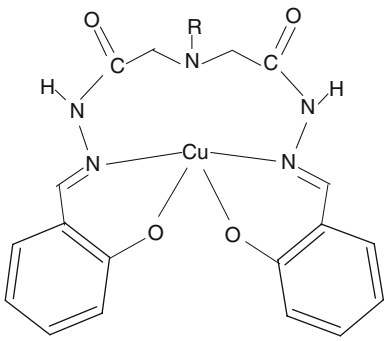

Fig. 8 Plausible mononuclear coordinating mode in $Cu(H_3Imds)\cdot CH_3OH{\cdot}H_2O$, (R = H) and in $Cu(H_2MeImds)\cdot CH_3OH{\cdot}3H_2O$, (R = CH_3)

suggest their involvement in the metal coordination. So, this leads us to conclude that the ligand is present in bi-deprotonated form only through the phenolic OH groups, upon coordination. Therefore, the two zinc ions are coordinated in a dinuclear symmetric fashion in the external compartments of the ligand (Fig. 7). Unfortunately, the assignment of the *v*(NH) and the *v*(OH) bands, in the 3700–3000 cm^{-1} region, are compromised by the presence of a large band due to solvating water molecule absorptions. As mentioned, the reaction between H_5Imds and copper(II) acetate (1:1 molar ratio) does not give the expected trimetallic species, but only the $Cu(H_3Imds)\cdot CH_3OH{\cdot}H_2O$ mononuclear complex. This fact could be, probably, correlated to the different nature of acetate ion with respect to the anionic species of copper salts (nitrate, sulphate, chloride or perchlorate), used in the reactions described earlier, whereas only trinuclear species were obtained regardless of metal/ligand molar ratio. Therefore, related IR spectrum, shows mainly significant evidences as concerning the absence of the symmetric and antisymmetric stretching bands, characteristic of acetate species, and a medium strong band at 1680 cm^{-1} that could be associated to uncoordinated carbonyl groups, suggesting that the ligand is present in keto-form upon coordination. A medium strong band at about 1610 cm^{-1} suggests that the azomethinic nitrogens undergo a negative shift upon coordination. Moreover, the IR spectrum shows a very strong band at about 1530 cm^{-1} associated to amide II absorption. The spectrum is, also, characterized by a medium band at 3411 cm^{-1} that could be associated to hydrazonic *v*(NH) absorption. Thus, as proposed in Fig. 8, the H_5Imds ligand coordinates, in a mononuclear fashion "closed" form, by the azomethinic nitrogens and both deprotonated phenolic groups. As regards the parent $H_4MeImds$ copper(II) complex, the marked similarities in the spectra of the mononuclear $Cu(H_2MeImds)\cdot CH_3OH{\cdot}3H_2O$ and $Cu(H_3Imds)\cdot CH_3OH{\cdot}H_2O$ complexes, taken together with the stoichiometric and preparative similarities, lead us to conclude that the $Cu(H_2MeImds)\cdot CH_3OH{\cdot}3H_2O$ complex has the same structure as that proposed for $Cu(H_3Imds)\cdot CH_3OH{\cdot}H_2O$ (Fig. 8).

Antimicrobial activity

About the biological screening, bis(acylhydrazones) H_5Imds, H_3Imdp and related copper(II) complexes were evaluated in vitro for antibacterial (*Bacillus* spp., *S. aureus, S. epidermidis, C. freundii, E. cloacae, E. coli, K. pneumoniae* and *P. aeruginosa*), antifungal (*C. albicans, P. guillermondii, R. glutinis* and *A. niger*) and antiprotozoal (*T. vaginalis* and *A. castellanii*) activities. The results obtained showed that some of the compounds possess an interesting activity against several tested strains.

With regard to antibacterial activity (Table 1), most of the tested compounds exhibited low activity against Gram-positive and Gram-negative bacteria showing MIC values ranging from 62.5 to 250 μg mL^{-1} with a few exceptions. Compound H_5Imds was more active than H_3Imdp against Gram-positive bacteria (MIC = 7.8 μg mL^{-1} vs. *S. epidermidis*). Similar activity was shown by their copper(II) $Cu_3(HImds)(ClO_4)_2EtOH{\cdot}2H_2O$ and $Cu_3(HImds)SO_4{\cdot}4H_2O$ complexes. Among all compounds tested, only H_5Imds showed a moderate activity against Gram-negative bacteria (MIC = 31.25 μg mL^{-1} vs. *C. freundii*).

Results of antifungal activity evaluation indicated that the tested compounds, in our experimental conditions, were unable to inhibit the growth of *C. albicans, P. guillermondii, R. glutinis* and *A. niger*. Only H_5Imds exerted a very low inhibitory activity against *C. albicans* (Table 2).

About anti-trichomonas assay, the results obtained are reported in Table 3. In all experimental conditions, control cells were viable and motile. The susceptibility of the clinical isolate to metronidazole, used as reference drug, was scanty (MIC_{100} of 50, 12.5 μg mL^{-1}, respectively, at 24 h of incubation and next times). The bis(salicylhydrazone) H_5Imds and the copper(II) complexes tested exhibited a general good activity against *T. vaginalis*. In particular, compound H_5Imds exhibited the best activity (MIC = 1.9 μg mL^{-1}). Therefore, the presence of the phenolic ring instead of pyridinic ring, in the hydrazonic

Table 1 MIC_{100} values (μg mL^{-1}) of bis(acylhydrazones) and copper(II) complexes towards some bacteria evaluated after 18–24 h of incubation at 35 °C

Bacteria	H_5Imds	H_3Imdp	$Cu_3(HImds)\ X_2$ X = NO_3	$Cu_3(HImds)\ X_2$ X = ClO_4	$Cu_3(HImds)\ X$ X = SO_4
Bacillus spp.	125	>250	62.5	62.5	62.5
S. aureus ATCC 25923	62.5	>250	62.5	62.5	62.5
S. aureus ATCC 6538	>250	>250	62.5	62.5	62.5
S. epidermidis	7.8	ND	15.6	7.8	7.8
C. freundii	31.25	ND	>250	>250	>250
E. cloacae	>250	ND	>250	>250	>250
E. coli ATCC 25922	>250	>250	>250	>250	>250
K. pneumoniae	>250	ND	>250	>250	>250
P. aeruginosa ATCC 27853	>250	>250	>250	>250	>250

Table 2 MIC_{100} values of bis(acylhydrazones) and copper(II) complexes towards some fungi obtained after 4 days of incubation at 25 °C

Fungi	H_5Imds	H_3Imdp	$Cu_3(HImds)\ X_2$ X = NO_3	$Cu_3(HImds)\ X_2$ X = ClO_4	$Cu_3(HImds)\ X$ X = SO_4
C. albicans ATCC 10231	125	>250	>250	>250	>250
P. guillermondii	>250	>250	>250	>250	>250
R. glutinis	>250	>250	>250	>250	>250
A. niger ATCC 16404	ND	ND	ND	ND	ND

Table 3 MIC_{100} values (μg mL^{-1}) of bis(acylhydrazones) and copper(II) complexes towards *T. vaginalis* and *A. castellanii* obtained, respectively, after 96 and 144 h of incubation at 37 °C and in humid atmosphere containing 5% CO_2 compared with MIC_{100} values (μg mL^{-1}) of control drugs ([a]metronidazole, [b]rokitamycin) obtained in the same experimental conditions

Protozoa	H_5Imds	H_3Imdp	$Cu_3(HImds)\ X_2$ X = NO_3	$Cu_3(HImds)\ X_2$ X = ClO_4	$Cu_3(HImds)\ X$ X = SO_4	Control drugs
T. vaginalis	1.9	125	15.6	ND	15.6	12.5[a]
A. castellanii	50	>100	25	50	>25	18.75[b]

skeleton, could explain a dramatic increase of the H_5Imds ligand antitrichomonal activity compared to the H_3Imdp ligand.

The investigation on *A. castellanii* indicated that after 6 days of incubation at 37 °C, control cultures produced about 2.5×10^3 amoebae/well, with doubling times of about 42 h. Under the same experimental conditions, cultures of trophozoites treated with the macrolide rokitamycin (75–2.34 μg mL^{-1}), used as reference drug [22], showed significant difference in growth rate and $MICs_{100}$ of 4.68, 18.75 μg mL^{-1}, respectively, at 24 and 144 h of incubation. The lowest concentration of tested compounds that caused complete inhibition of trophozoite growth (MIC_{100}) at 144 h are shown in Table 3. The susceptibility of *A. castellanii* to bis(acylhydrazones) was slight (MIC_{100} >50 μg mL^{-1}), but all the H_5Imds copper(II) complexes showed a moderate activity against trophozoites. The best activity was exhibited by $Cu_3(HImds)(NO_3)_2 2CH_3OH \cdot 2H_2O$ (MIC_{100} 25 μg mL^{-1}) (Fig. 9). Its effect when it was used at the MIC_{100} was amoebistatic, with recovery eventually occurring (data not shown).

Conclusions

The H_5Imds, $H_4MeImds$ and H_3Imdp compartmental ligands exhibit versatile behaviour in complexation with first row transition metal ions, ranging from mono- to di- and trinuclear metal complexes. Further studies aimed to obtain ethero-nuclear, mono- or poly-nuclear metal complexes of H_5Imds, $H_4MeImds$ and H_3Imdp ligands, towards transition metal ions, are now in progress.

In the light of the results obtained from the screening of the antimicrobial activities of bis(acylhydrazones) and copper(II) complexes, a very interesting activity emerged against *T. vaginalis* for compound H_5Imds (MIC_{100} = 1.9 μg mL^{-1}),

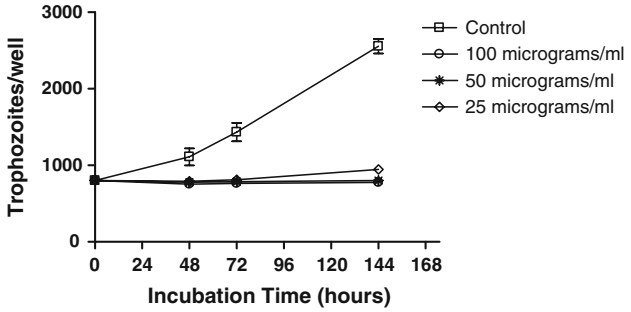

Fig. 9 Growth curves of *A. castellanii* trophozoites incubated in the absence (control) and in the presence of compound $Cu_3(HImds)-(NO_3)_2\ 2CH_3OH{\cdot}2H_2O$ (100–25 μg mL^{-1}) in PYG medium, at 37 °C in a 5% CO_2 atmosphere

which was much more potent than the reference drug (Metronidazole). This observation may promote a further development of our research in this field.

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