

Convenient approach to the direct synthesis of some mixed-ligand tris-chelates of Mn^{II} containing neutral N,N-donors and to study their antimicrobial activities

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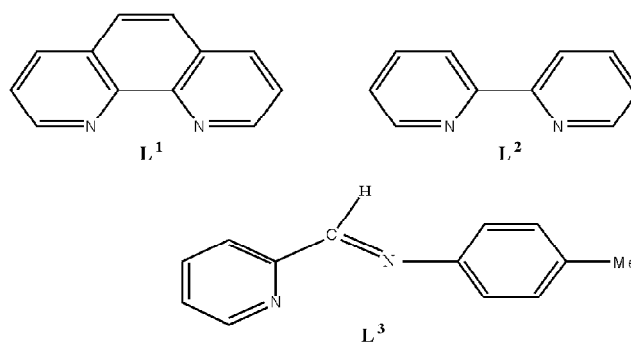
Four new mixed-ligand tris-chelates of Mn^{II}, containing 1,10-phenanthroline; 2,2'-bipyridine and N-*p*-tolylpyridine-2-alimine as neutral N,N-donor ligands, have been synthesized by silver(I) assisted trans-metallation reactions. The complexes were characterized by the analysis of different physicochemical data. Electron spray ionization mass spectrum (ESIMS) of one of their representatives has been reported which authenticates its formulation. The synthesized complexes showed good antimicrobial activities towards some Gram-positive and Gram-negative bacterial strains in aqueous medium.

Keywords: Silver(I) assisted synthesis, manganese(II), mixed-ligand complexes, neutral N,N-donors, antimicrobial activities.

Introduction

Transition metal complexes have attracted much attention in modern drugs due to their antimicrobial properties¹. In this respect, the coordination compounds of Mn are important in inorganic biochemistry² due to their interesting magnetic properties and catalytic activities³. Although a good number of mixed-ligand transition metal complexes are known in the literature⁴, to our knowledge, facile synthetic strategy and antimicrobial studies of mixed-ligand tris-chelates of Mn^{II} containing neutral N,N-donors are not known. It is to be noted here that synthetic routes⁵ of tris(2,2'-bipyridine)-manganese(II) perchlorate and tris(1,10-phenanthroline)-manganese(II) perchlorate are not only time consuming but also cumbersome.

In this respect, a general synthetic route for the syntheses of hexa-coordinated mixed-ligand tris-chelates of Mn^{II} with 1,10-phenanthroline (L¹); 2,2'-bipyridine (L²) and N-*p*-tolylpyridine-2-alimine (L³) have been described. The controlled syntheses of the complexes with compositions [MnL¹₂L²](ClO₄)₂, [MnL²₂L¹](ClO₄)₂, [MnL¹₂L³](ClO₄)₂ and [MnL²₂L³](ClO₄)₂ have been achieved following the silver(I) assisted metal exchange reaction strategy^{4,6}. Promising results have been obtained against the *in vitro* antimicrobial



activity of different sets of microorganisms for these complexes.

Experimental

Materials: Manganese(II) chloride, silver nitrate, 2,2'-bipyridine and 1,10-phenanthroline were purchased from Merck Emplura and pyridine-2-carboxaldehyde was obtained from Merck, Germany. The starting manganese(II) complexes, *cis*-MnL₂Cl₂ (L = L¹ and L²), ligand L³ and the silver complexes of L¹ to L³ were synthesized by earlier procedures⁴. The bacterial strains were obtained from the Department of Microbiology, University of Kalyani and from the Department of Physiology, Krishnath College, Berhampore. All other chemicals and solvents used for the preparative work were of reagent grade and were used as received.

Physical measurements: FTIR spectra (KBr) were recorded using a Perkin-Elmer BX-1 FTIR spectrophotometer. ESIMS measurements were carried out using a Waters Q-TOF Mass Spectrometer. EI make 2373 UV-Vis spectrophotometer was used to record the electronic spectra. Solution electrical conductivity measurements were performed using a Systronics Direct Reading Conductivity Meter 304. The magnetic susceptibilities of the samples were measured on a PAR 155 vibrating sample magnetometer fitted with a Walker scientific L75 FBAL magnet. Microanalyses (CHN) were done by using Perkin-Elmer Series II 2400 elemental analyzer.

Preparation of complexes: CAUTION! Perchlorate salts of metal complexes are potentially explosive. Although no detonation tendencies have been observed, caution is advised and handling of only small quantities is recommended.

The tris-chelated complexes were prepared by using a general procedure. A representative example is described below:

$[\text{MnL}^1_2\text{L}^2](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$: *cis*- $\text{MnL}^1_2\text{Cl}_2$ (0.15 g, 0.28 mmol) and $[\text{AgL}^2_2]\text{NO}_3$ (0.277 g, 0.574 mmol) were refluxed for 45 min in 30 ml ethanol. The colour of the solution appeared pale yellow. The mixture was then brought to room temperature and was filtered through a G-4 sintered glass funnel to remove precipitated AgCl. The filtrate was then concentrated to about 10 ml and few drops of conc. NaClO_4 solution was added. Pale yellow amorphous compound was precipitated. The product was then isolated by filtration, recrystallized thrice from ethanol-diethyl ether and dried in vacuum. Yield: 80%. Anal. Calcd. (%) for $\text{MnC}_{34}\text{H}_{26}\text{N}_6\text{O}_9\text{Cl}_2$: C, 51.78; H, 3.30; N, 10.66. Found: C, 52.11; H, 3.46; N, 10.93; μ_{eff} (298 K): 5.76 BM; $\Lambda_{\text{M}} = 220 \Omega^{-1} \text{cm}^2 \text{M}^{-1}$ ($1 \times 10^{-3} \text{M}$ in MeOH); ν/cm^{-1} (KBr): $\nu_{\text{C=N}}$ 1620, $\nu_{\text{C=C}}$ 1591, $\nu_{\text{C-H}}$ (out-of-plane bending in pyridine ring) 772, $\nu_{\text{ClO}_4^-}$ 1100, 625; χ_{max} , nm (CH_3CN) (ϵ , $\text{dm}^3 \text{M}^{-1} \text{cm}^{-1}$): 300 (3400), 230 (3650), 345 sh (850).

$[\text{MnL}^1\text{L}^2_2](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$: Colour, yellow; yield: 76%. Anal. Calcd. (%) for $\text{MnC}_{32}\text{H}_{26}\text{N}_6\text{O}_9\text{Cl}_2$: C, 50.26; H, 3.40; N, 11.0. Found: C, 50.65; H, 3.32; N, 10.78; $\Lambda_{\text{M}} = 240 \Omega^{-1} \text{cm}^2 \text{M}^{-1}$ ($1 \times 10^{-3} \text{M}$ in MeOH); ν/cm^{-1} (KBr): $\nu_{\text{C=N}}$ 1624, $\nu_{\text{C=C}}$ 1590, $\nu_{\text{C-H}}$ (out-of-plane bending in pyridine ring) 775, $\nu_{\text{ClO}_4^-}$ 1081, 622; χ_{max} , nm (CH_3CN) (ϵ , $\text{dm}^3 \text{M}^{-1} \text{cm}^{-1}$): 290 (3200), 225 (3450), 330 sh (800).

$[\text{MnL}^1_2\text{L}^3](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$: Colour, brownish-yellow; yield: 70%. Anal. Calcd. (%) for $\text{MnC}_{37}\text{H}_{30}\text{N}_6\text{O}_9\text{Cl}_2$: C, 53.62; H, 3.62; N, 10.14. Found: C, 53.23; H, 3.48; N, 10.31; μ_{eff} (298 K): 5.62 BM; $\Lambda_{\text{M}} = 225 \Omega^{-1} \text{cm}^2 \text{M}^{-1}$ ($1 \times 10^{-3} \text{M}$ in MeOH); ν/cm^{-1} (KBr): $\nu_{\text{C=N}}$ 1622, $\nu_{\text{C=C}}$ 1578, $\nu_{\text{C-H}}$ (out-of-plane bending in pyridine ring) 777, $\nu_{\text{ClO}_4^-}$ 1120, 620; χ_{max} , nm (CH_3CN); (ϵ , $\text{dm}^3 \text{M}^{-1} \text{cm}^{-1}$): 295 (3190), 225 (3430), 340 sh (780).

$[\text{MnL}^2_2\text{L}^3](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$: Colour, dirty-yellow; yield: 72%. Anal. Calcd. (%) for $\text{MnC}_{33}\text{H}_{30}\text{N}_6\text{O}_9\text{Cl}_2$: C, 50.77; H, 3.84; N, 10.77. Found: C, 50.51; H, 3.90; N, 10.59; $\Lambda_{\text{M}} = 240 \Omega^{-1} \text{cm}^2 \text{M}^{-1}$ ($1 \times 10^{-3} \text{M}$ in MeOH); ν/cm^{-1} (KBr): $\nu_{\text{C=N}}$ 1620, $\nu_{\text{C=C}}$ 1585, $\nu_{\text{C-H}}$ (out-of-plane bending in pyridine ring) 770, $\nu_{\text{ClO}_4^-}$ 1100, 630; χ_{max} , nm (CH_3CN); (ϵ , $\text{dm}^3 \text{M}^{-1} \text{cm}^{-1}$): 286 (3170), 235 (3520), 360 sh (750).

Antimicrobial assay:

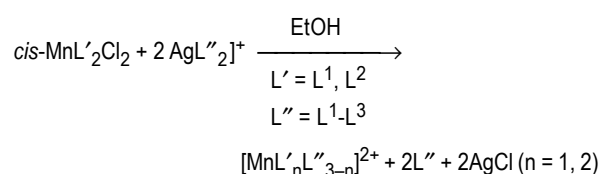
The antimicrobial activities of the complexes were determined using Agar well diffusion method⁷ with further modifications in our laboratory. The bacterial strains studied were two Gram-positive *Bacillus subtilis*, *Staphylococcus aureus* (clinical isolate and methicillin resistant), and one Gram-negative *Escherichia coli* (environmental isolate). Amoxicillin, a clinically recommended antibacterial agent was used as the reference antimicrobial agent⁸. The compounds were dissolved into autoclaved sterile distilled water. All the bacterial strains were cultured in Luria-Bertani broth (LB) and Mueller Hinton medium for *in vitro* antimicrobial analysis and kept aerobically at 37°C for 18 h in incubator. 0.1 ml of diluted inoculum (approximately 10^8 CFU/mL of each strain) was spread on the Luria-Bertani Broth/Agar (Modified) plate. Wells of 2 mm diameter were made in the Luria-Bertani Broth/Agar plate and 50 μL of these compounds were added to the wells and all plates were kept aerobically at 37°C for 24–48 h in incubator for *in vitro* antimicrobial analysis.

The compounds having antimicrobial activity were further tested to determine minimum inhibitory concentrations (MIC) as per NCCLS protocol⁹. All the compounds with antimicrobial activity were diluted for the final concentrations of 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240 and 255 $\mu\text{g}/\text{ml}$. 100 μL of (10^6 CFU/ml) each of the strains mentioned earlier was transferred into tubes with Luria-Bertani broth (LB) supplemented with each of the said compounds. Then the tubes were incubated aerobically

at 37°C for 24–48 h. Media control, organism control and compound control were maintained for each strain. The lowest concentration (highest dilution) of the compound that produces no growth (no turbidity) after comparing with the control tubes in 48 h was regarded as MIC. Beside this, growth inhibition zone diameter of MIC (mm) of the synthetic compounds against the selected bacterial strains was also determined using Agar well diffusion method⁷ which was previously described.

Results and discussion

Syntheses: The complexes were synthesized by the silver(I) assisted trans-metallation reactions. Each of the three bidentate ligands (L¹-L³) are known to form stable compounds¹⁰ with silver(I). The syntheses of the complexes are described by Scheme 1:



Scheme 1

By the selection of an appropriate combination of reactants, the mixed-ligand complexes of desired compositions can be prepared very easily. All the tris-chelates described above are obtained as their perchlorate salts in high yields (*ca.* 75%). The compounds were characterized based on their analytical data.

Conductance and magnetic moments: The conductivity values of the complexes in 10⁻³ M methanolic solution are in the range 220–240 Ω⁻¹ cm² M⁻¹, suggesting 1:2 electrolytic nature. Room temperature magnetic moment values (5.62 BM for [MnL¹₂L³](ClO₄)₂ and 5.76 BM for [MnL¹₂L²](ClO₄)₂) of two representatives indicate the complexes are high spin paramagnetic.

FTIR: FTIR spectra of the complexes have similar features and consist of characteristic absorptions of the coordinated ligands⁴. Some selected frequencies are: (i) absorptions at *ca.* 1580–1620 cm⁻¹ characterizes ν_{C=N} and ν_{C=C}; (ii) absorptions at *ca.* 1425 cm⁻¹ characterizes ν_{C-C}; (iii) absorptions at *ca.* 720–845 cm⁻¹ are assigned to C-H out-of-

plane bending vibrations, (iv) the presence of ionic ClO₄⁻ was confirmed by the two characteristic absorptions: a broad absorption at about 1100 cm⁻¹ and a sharp band at about 620 cm⁻¹, (v) a broad transition at *ca.* 3450 cm⁻¹ was due to the presence of water of crystallization. The coordination of L¹, L² and L³ to the manganese through the nitrogen atoms is indicated by the ν_{C=C} and ν_{C=N} values, shifted at lower frequencies^{4,5}. IR spectrum of [MnL¹L²](ClO₄)₂.H₂O, is provided as electronic supplementary information.

Electronic spectrum: The UV-Vis spectroscopic data are quite similar for the complexes. The absorption bands in the UV region at *ca.* 220–360 nm, are assigned to ligand based π→π* or n→π* transitions. Due to high spin d⁵ configuration of Mn²⁺, no d-d transition is expected in the visible region of the spectrum. UV-Vis spectra of [MnL²L³](ClO₄)₂, as representative, is provided as electronic supplementary information.

ESI mass spectrum: The ESIMS measurement of one of the complex, [MnL¹L²](ClO₄)₂, have been done and it strongly suggests its formulation. Fig. 1 displays the positive ion mass spectrum of the compound (M.W. 769.94). The multiply charged ions were generated from loss of negative counter ions, which is denoted by [M-nX]²⁺, where M and X represent the molecule and ClO₄⁻ respectively. The low intensity peak at *ca.* 670.92 is assigned to [M-X]⁺, which confirms the proposed molecular formula. The peak detected at *ca.* 514.47 is due to [M-L²-X]⁺ and that at *ca.* 207.70 is due to [M-L²-2X]²⁺, derived from ligand dissociation. Other weak peaks are of unclear origin and these may be due to fragmentation or unknown combination of fragmented ions¹¹.

Antimicrobial screening: The four synthesized metal compounds exhibited active antimicrobial properties against three bacterial strains, with IZ values in the range 6–12 mm, in aqueous medium. Although the antibacterial properties of these organic ligands against these microorganisms have already been reported in the literature¹², we did not find any such significant properties in aqueous medium probably because of their water insolubility. The complexes thus can be applied for the treatment of infections caused by these organisms in mammals, as water is the main component in these systems and the mixed-ligand complexes have an advantage in that the respective bioactivities of the uncoor-

Table 1. MIC ($\mu\text{g/mL}$) values of the synthetic compounds against selected bacterial strains

Strains	Synthesized compounds				Amoxicillin
	1	2	3	4	
<i>Bacillus subtilis</i>	120	135	120	150	225
<i>Staphylococcus aureus</i>	105	90	105	105	205
<i>Escherichia coli</i>	135	105	120	Nil	195

Table 2. Growth inhibition zone diameter of MIC (mm) of the synthetic compounds against the selected bacterial strains

Strains	Synthesized compounds				Amoxicillin
	1	2	3	4	
<i>Bacillus subtilis</i>	9.67 \pm 0.17	8.67 \pm 0.06	8.83 \pm 0.13	8.13 \pm 0.41	6.83 \pm 0.17
<i>Staphylococcus aureus</i>	10.17 \pm 0.31	11.5 \pm 0.57	9.67 \pm 0.31	10.33 \pm 0.21	7 \pm 0.1
<i>Escherichia coli</i>	8.17 \pm 0.06	10.83 \pm 0.27	8.5 \pm 0.17	–	7.31 \pm 0.1

1 = [MnL¹L²](ClO₄)₂.H₂O; 2 = [MnL¹L²]₂(ClO₄)₂.H₂O; 3 = [MnL¹L²L³](ClO₄)₂.H₂O; 4 = [MnL²L³]₂(ClO₄)₂.H₂O

minated ligands are combined to make them more potent antimicrobial agents.

The activity of the compounds can be explained by Overtone's concept and chelation theory¹³. According to this concept of cell permeability, the lipid membranes that surround the cell favour the passage of only lipid soluble material and lipid solubility is an important factor that controls antimicrobial activity. On coordination, the polarity of the metal ion is reduced due to overlap of the metal-ligand orbitals and the partial sharing of the positive charge of the metal ion with the donor atoms of the ligand and possible π -electron delocalization over the whole chelate ring. Such chelation can increase the lipophilicity of the metal complexes enabling them to penetrate the bacterial cell membrane effectively. The antibacterial activities of the compounds were further studied by determining the minimum inhibitory concentration (MIC). In Tables 1 and 2, the MIC values and inhibition zone diameter for these compounds are given. The MIC values of the metal complexes are quite low compared with the reference, amoxicillin, indicating they are more effective.

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References

- (a) M. A. Zoroddu, S. Zanetti, R. Pogni and R. Basori, *Inorg. Biochem.*, 1996, **63**, 291; (b) M. Geraghty, J. F. Cronin, M. Devereux and Mccann, *Biometals*, 2000, **3**, 1; (c) H. B. Tiabi, M. Akkurt, M. B. Filali, M. Daoudi, B. Bennani and H. C. Zahid, *Enzyme Inhibition and Medicinal Chemistry*, 2009, **24**, 457; (d) K. P. Pragnesh and M. N. Patel, *Nano-Metal Chemistry*, 2005, **34**, 1277; (e) J. N. L. Nagababu, P. Latha, S. H. Pallavi and S. Satyanarayana, *Canadian Journal of Microbiology*, 2006, **52**, 1247.
- V. L. Pecoraro, W. Y. Hsieh, A. Sigel, H. Sigel and D. Marcel, "Metal ions in biological systems: manganese and its role in biological processes", New York, 2000, **37**, 431.
- (a) E. M. McGarrigle and D. G. Gilheany, *Chem. Rev.*, 2005, **105**, 1563; (b) S. Mandal, A. K. Raut, M. Fleck, G. Pilet and J. Ribas, *Inorg. Chim. Acta*, 2010, **363**, 2250.
- (a) P. Majumdar, A. K. Ghosh, L. R. Falvello, S. M. Peng and S. Goswami, *Inorg. Chem.*, 1998, **37**, 1651; (b) W. Kharmawphlang, S. Choudhury, A. K. Deb and S. Goswami, *Inorg. Chem.*, 1995, **34**, 3826; (c) M. Kakoti, A. K. Deb and S. Goswami, *Inorg. Chem.*, 1992, **31**, 1302.
- I. Kani, O. Atlier and K. Guven, *J. Chem. Sci., Indian Acad. Sci.*, 2016, **128**, 523.
- (a) A. K. Deb, M. Kakoti and S. Goswami, *J. Chem. Soc., Dalton Trans.*, 1991, 3249; (b) S. Choudhury, A. K. Deb, W. Kharmawphlang and S. Goswami, *J. Chem. Soc., Dalton Trans.*, 1994, 1305.
- (a) A. W. Bauer, W. M. M. Kirby, J. C. Sherris and M. Turck, *Am. J. Clin. Pathol.*, 1966, **45**, 493; (b) I. Wiegand, K. Hilpert and R. E. Hancock, *Nature Protocols*, 2008, **3**, 163.
- N. C. Saha, R. Pradhan, M. Das, N. Khatun, D. Mitra, A. Samanta, M. Z. Alexandra, A. M. Z. Slawin, A. D. Jana, J. Klanke and E. Rentschler, *J. Coord. Chem.*, 2014, **67**, 286.
- National Committee for Clinical Laboratory Standards (NCCLS), Approved Standard M7 A3, 3rd ed., NCCLS, Villanova.

10. (a) A. K. Deb, S. Choudhury and S. Goswami, *Polyhedron*, 1990, **9**, 2251; (b) D. P. Murtha and R. A. Walton, *Inorg. Chem.*, 1973, **12**, 368; (c) W. Heiber and F. Muhlbauer, *Ber. Dtsch. Chem. Ges.*, 1928, **61**, 2149.
11. (a) P. Majumdar, *J. Indian Chem. Soc.*, 2016, **93**, 19; (b) M. Haga, M. Md. Ali and R. Arakawa, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 76.
12. M. O. Agwara, P. T. Ndifon, N. B. Ndosiri, A. G. Paboudam, D. M. Yufanyi and A. Mohamadou, *Bull. Chem. Soc. Ethiop.*, 2010, **24**, 383.
13. (a) P. Shakru, N. J. P. Subhashini, S. Kumar and K. Shivaraj, *J. Chem. Pharm. Res.*, 2010, **2**, 38; (b) B. G. Tweedy, *Phytopathology*, 1964, **55**, 910.