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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Effect of derivatization of sulfamethoxazole and trimethoprim with copper and zinc on their medicinal value

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Abstract Cu(II) and Zn(II) complexes of Schiff bases obtained by condensation of sulfamethoxazole with salicylaldehyde/pyridoxal were prepared and characterized by microanalytical, thermogravimetric, magnetic, and spectroscopic data. The Cu (II) and Zn (II) complexes of salicylidenesulfamethoxazole were found to be five coordinate while all others were six coordinate. The electron paramagnetic resonance spectral lines exhibited rhombic distortion from axial symmetry, with $g_{\|} > g_{\perp} > g_e$, in the case of the Cu (II) complexes. The geometry of the complexes appears to be square–pyramidal or octahedral. All the compounds under investigation possess antibacterial activity against the strains tested. The antibacterial activity showed the following trend: Cu (II) complexes > Zn (II) complexes > Schiff base ligands > parent drugs. All the copper complexes were found to be active against kaolin paw edema whereas the parent drugs were inactive.

Keywords Copper complexes · Zinc complexes · Antibacterials · Schiff base complexes · Sulfamethoxazole · Trimethoprim

Introduction

The problem of resistance to antimicrobial activity is being addressed by medicinal chemists and various strategies have been devised and attempted in order to enhance

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the activity or broaden the spectrum of the drugs (DeNap and Hergenrother, 2005). It has been demonstrated that transition elements play a very important role in of various medicinal compounds (Blower, 1999; Iqbal *et al*., 1999; Iqbal *et al*., 2005). In the present work we report new derivatives of sulfamethoxazole and trimethoprim with copper and zinc with greater medicinal value. The drug molecules used in the present study contain $-NH_2$, and other donor groups; construction of molecular models indicates that the structures are suitable for chelate formation.

Materials and methods

Materials

Sulfamethoxazole (GlaxoSmithKline, Pakistan), trimethoprim (GlaxoSmithKline, Pakistan), and all other chemicals (E. Merck, Germany) were used without further purification.

Preparation of Schiff base ligands

Two series of Schiff base ligands were prepared by allowing the drug substances to react with salicylaldehyde and pyridoxal hydrochloride separately. The method of preparation was as follows. The drug substance (2 mmol) dissolved in methanol (25 cm^3) was mixed with salicylaldehyde or pyridoxal hydrochloride (2 mmol) dissolved in methanol (25 cm^3). To this KOH (0.1% in methanol) was added to adjust the pH of the solution at 7–8 and the mixture was refluxed for 30 min (approx.). A clear colored solution was obtained. The Schiff base ligand was isolated by crystallization after volume reduction by evaporation. The crystalline product was dried under vacuum and kept in a desiccator until further use. The compounds thus obtained are listed in Table 1.

Preparation of Schiff base metal complexes

The Schiff base ligand (2 mmol) dissolved in methanol (25 cm^3) was mixed with $Cu(CH_3COO)_2H_2O$ (1 mmol) or $Zn(CH_3COO)_2H_2O$ (1 mmol) dissolved in methanol (25 cm^3). The reaction mixture was refluxed for 2–3 h. The product was isolated after reduction of volume by evaporation. It was filtered off, washed with methanol, and dried under vacuum. The complexes thus obtained are listed in Table 1.

Characterization

Microanalysis was performed by the usual techniques. Copper and zinc were estimated by atomic absorption spectrometry. Molecular masses were determined mass-spectrometrically. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed with a Netzsch simultaneous thermal analyzer. Conductivity measurements were made with the Orion Model 160 conductivity

Table 1 Physical properties and microanalytical data of the Schiff base ligands and their metal complexes

No.	Compound	Colour	Decomposition point (°C)	Elemental analysis: found (calculated)			
				C	H	N	M
1.	Salicylidenesulfamethoxazole [M^+: 357 *m*/*z*]	Pale yellow	197	57.04 (57.13)	4.26 (4.23)	11.62 (11.76)	–
2.	Salicylidenetrimethoprim [M^+: 394 *m*/*z*]	Yellowish-green	190	64.05 (63.95)	5.60 (5.62)	14.19 (14.20)	–
3.	Pyridoxylidenesulfamethoxa-zole [M^+: 402 *m*/*z*]	yellow	193	53.73 (53.72)	4.50 (4.51)	13.95 (13.92)	–
4.	Pyridoxylidenetrimethoprim [M^+: 439 *m*/*z*]	Yellow	199	60.15 (60.12)	5.70 (5.73)	15.89 (15.94)	–
5.	Salicylidenesulfamethoxazole-Cu(II)·H_2O [M^+: 793 *m*/*z*]	Dark yellow	231	51.44 (51.41)	3.83 (3.81)	10.62 (10.58)	8.09 (8.00)
6.	Salicylidenesulfamethoxazole-Zn(II)·H_2O [M^+: 795 *m*/*z*]	Light yellow	292	51.19 (51.29)	3.83 (3.80)	10.60 (10.56)	8.19 (8.22)
7.	Salicylidenetrimethoprim-Cu(II)·$2H_2O$ [M^+: 885 *m*/*z*]	Yellowish-green	230	56.89 (56.91)	5.19 (5.23)	12.67 (12.64)	7.22 (7.17)
8.	Salicylidenetrimethoprim-Zn(II)·$2H_2O$ [M^+: 887 *m*/*z*]	Light yellow	250	56.69 (56.79)	5.29 (5.22)	12.66 (12.61)	7.32(7.36)
9.	Pyridoxylidenesulfamethoxazole-Cu(II)·$2H_2O$ [M^+: 901 *m*/*z*]	Dark yellow	220	47.84 (47.91)	4.16 (4.24)	12.39 (12.42)	6.99 (7.04)
10.	Pyridoxylidenesulfamethoxazole-Zn(II)·$2H_2O$ [M^+: 903 *m*/*z*]	Light yellow	226	47.84 (47.82)	4.18 (4.24)	12.37 (12.39)	7.18 (7.23)
11.	Pyridoxylidenetrimethoprim-Cu(II)·$2H_2O$ [M^+: 975 *m*/*z*]	Yellowish-green	215	54.06 (54.12)	5.28 (5.37)	14.34 (14.34)	6.56 (6.51)
12.	Pyridoxylidenetrimethoprim-Zn(II)·$2H_2O$ [M^+: 977 *m*/*z*]	Light yellow	210	53.97 (54.02)	5.29 (5.36)	14.34 (14.32)	6.68 (6.69)

meter using dimethylformamide (DMF) as solvent at room temperature. The magnetic moments of the copper complexes were determined by Gouy's technique using Hg(II)-tetrathiocyanatocobaltate as calibrant; diamagnetic corrections were calculated from Pascal's constants (Earnshaw, 1968). Infrared spectra were recorded with a Fourier-transform infrared (FT-IR) (Midac) spectrophotometer using KBr disc and Nujol mull techniques. Electronic absorption spectra were obtained with a Hitachi Model 121-0032 spectrophotometer using methanol (for the ligands) and DMF (for the complexes) as solvents. Electron paramagnetic resonance (EPR) spectra were recorded as a powder and in solution (DMF), at room temperature, on a Jeol JES-FE 1XG instrument, in the X-band, operating at a microwave frequency of 9.44 GHz. The g values were determined by use of the Kneubühl approximation (Kneubühl, 1960). The spectra were calibrated using the α,α-diphenyl-p-picrylhydrazyl radical ($g = 2.0036$) as a field marker. Proton nuclear magnetic resonance (NMR) of the zinc complexes were recorded on a 90 MHz Perkin Elmer machine using dimethyl sulfoxide (DMSO) -d_6 as the solvent and tetramethylsilane (TMS) as the standard.

Antibacterial activity

Antibacterial study of the complexes under investigation was performed using standard strains of *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739), and *P. aeruginosa* (ATCC 9027). Minimum inhibitory concentration (MIC) was determined by the standard dilution technique (Anhalt and Washington, 1985) by use of tryptic soy broth (Difco), in which the complexes dissolved. The tubes were incubated at 37°C for 24 h.

Anti-inflammatory activity

Kaolin paw oedema was induced, by a method reported elsewhere (Lewis *et al*., 1975), in male Wistar rats, weighing 105–115 g, in groups of five. The complexes under investigation were administered orally in 5% mulgophen (GAF, Manchester) in distilled water (0.2 mL per 100 g) 1 h before the kaolin. The rats were dosed on a weight of drug (mg) per body weight (kg) of animal basis. Edema was evaluated 4 h after subplantar administration of kaolin in 0.9% w/v sodium chloride solution. Inhibition of edema was evaluated by comparing the swelling obtained in treated animals with that in controls and was expressed as percentage inhibition. Statistical significance was evaluated by using Student's t-test.

Toxicity study

Experiments were performed on albino Wistar male rats, weighing 160–180 g. Animals were kept at constant temperature (25 ± 0.5°C) and relative humidity (20–40 percent). Conventional laboratory diet and water were freely available. The complexes under investigation were administered orally in 0.15% agar suspension (50 $cm^3\ kg^{-1}$) to four groups of ten rats. After treatment, the animals were monitored every hour for several hours and then every day for 14 days.

Results and discussion

The complexes under investigation were isolated by refluxing the copper(II) acetate monohydrate and zinc(II) acetate dihydrate with the previously prepared Schiff base ligands in methanol. The isolated complexes were fairly soluble in DMF and DMSO and insoluble in other common organic solvents. Microanalytical data (Table 1) depicted a ML_2 composition of the complexes, in which M is Cu (II) or Zn (II) and L the ligand. The purity of the complexes was ensured by effective washing with methanol in which the free ligands and the metal salts were soluble. The complexes decomposed between 210 and 292°C and did not show sharp melting points. Karl–Fischer titration and thermal analysis (TGA and DTA) indicated the presence of water molecules in the complexes. There was a weight loss equivalent to one water molecule in Cu (II) and Zn (II) complexes of salicylidenesulfamethoxazole, and two water molecules in other complexes around 120°C, showing that the water was coordinated. From 210 to 500°C a sharp decrease in weight indicated a loss of one of the Schiff base ligands from the complexes. Thus the thermal decomposition of complexes may be represented by the following equations:

$$ML_2 \cdot xH_2O \rightarrow ML_2 + xH_2O$$

$$ML_2 \rightarrow ML + L$$

The molecular masses determined mass-spectrometrically (Table 1) confirmed the proposed composition.

The bonding of the ligands to metal ions was investigated by comparing the FT-IR spectra of the complexes with those of the free ligands. The FT-IR spectra of the complexes contained all the absorption bands due to the ligands and some new absorption bands indicative of coordination of the ligands with metal ions through N and O. Some important absorption bands and their assignments are listed in Table 2.

The spectra of these complexes exhibited a broad band around 3380–3482 cm^{-1}, which was assigned to water molecules, ν(OH), associated with the complexes.

Table 2 Observed infrared frequencies (cm^{-1}) and assignments

Comp. no.	ν(OH)	ν(C=N)	$\rho_r(H_2O)$	$\rho_w(H_2O)$	ν(MN)	ν(MO)
1	3520	1625	885, 850	535	–	–
2	3480	1625	890, 845	545	–	–
3	3505	1620	889, 855	530	–	–
4	3490	1632	890, 850	538	–	–
5	3380	1620	875, 841	535	455	345
6	3385	1630	865, 840	530	445	340
7	3475	1625	885, 850	545	442	345
8	3482	1620	880, 845	542	450	345
9	3470	1640	890, 855	545	448	345
10	3465	1635	885, 860	550	435	330
11	3470	1635	880, 855	538	445	342
12	3463	1633	875, 850	545	448	345

Table 3 Physical data of the ligands and complexes

Comp. no.	Conductance (μS cm^{-1})	Geometry	μ (BM)	nm (ε, cm^{-1} mol^{-1})	
				Ligand bands	d–d bands
1	3.4	–	–	210 (7375), 270 (4473)	–
2	12.3	–	–	210 (15261), 90 (3261)	–
3	18.3	–	–	210 (20175), 252 (19627)	–
4	28.6	–	–	221 (21200), 290 (13055)	–
5	2.9	Square-pyramidal	1.97	214 (2565), 272 (1556)	652 (105)
6	6.2	Square-pyramidal	Diamagnetic	210 (2555), 271 (1538)	–
7	9.1	Octahedral	2.13	212 (2563), 293 (1141)	662 (485)
8	4.1	Octahedral	Diamagnetic	210 (2560), 292 (1140)	–
9	6.8	Octahedral	2.12	213 (6814), 254 (6630)	661 (469)
10	9.9	Octahedral	Diamagnetic	211 (6810), 253 (6622)	–
11	8.3	Octahedral	2.20	220 (7221), 294 (4447)	672 (508)
12	13.4	Octahedral	Diamagnetic	223 (7218), 291 (4442)	–

Coordinated water exhibited, in addition to these modes, $\rho_r(H_2O)$, rocking between 840–890 cm^{-1}, $\rho_w(H_2O)$, and wagging between 530–550 cm^{-1} (Nakamoto 1986). FT-IR spectra of all the ligands contained a band at 1620–1640 cm^{-1}, ν(C=N), which shifted slightly to a higher value in all the complexes, suggesting that the ligands are coordinated to the metal ion through –C=N– (Ben-saber *et al.*, 2005). New absorption bands, ν(MN) and ν(MO), appeared at 435–455 cm^{-1} and 330–345 cm^{-1}, respectively, in the spectra of the complexes, indicating coordination of the ligands through N and O. In the electronic absorption spectra of the complexes (Table 3) there is an intense band at 253–294 nm which is assigned to a π–π* transition originating in the phenyl ring. The low-energy broad absorption band in the range of 652–672 nm in the spectra of the copper complexes arises from a d–d transition (Jianmin and Yugeng, 1991). Formation of the Schiff base was confirmed by extinction of the aldehyde proton signal at 9.2 ppm and the appearance of the aldimine proton signal at 7.6 ppm with reference to TMS in the ^{1}H NMR spectra. The coordination of imino group to the metal ion was confirmed by a change in chemical shift of the aldimine proton by a change proton to 7.3 ppm on complexation with zinc. Lower molar conductance values (2.9–13.4 μS cm^{-1}) for the complexes (Table 3) indicate the nonelectrolytic nature of the complexes.

The copper complexes showed normal values of the magnetic moments, i.e., 1.97–2.20 BM (Table 3), indicating their mononuclear nature. The EPR parameters of the copper complexes are given in Table 4. There was a general correspondence between the powder and solution spectra; however, the $g_{\|}$ region was well resolved in solution. The spectra were indicative of rhombic distortion from axial symmetry. Owing to coordination of two different kinds of atoms (O and N) with the metal ion, the spectra were characteristic of magnetically dilute systems with Cu (II) ions in the $d_{x^2-y^2}$ ground state ($g_{\|} > g_{\perp} > g_e$). Hyperfines as a result of nitrogen (I = 1)

Table 4 EPR parameters of the copper complexes

Comp. no.	Solid		Solution		
	$g_{\parallel}$	$g_{\perp}$	$g_{\parallel}$	$g_{\perp}$	$\lvert A_{\parallel(Cu)}\rvert$
5	2.20	2.05	2.284	2.017 2.026	16.00
7	2.21	2.06	2.273	2.023	16.01
9	2.16	2.03	2.291	2.019	16.41
11	2.18	2.06	2.302	2.020	16.50

were visible on the main absorption line, $g_{\perp}$ confirming the coordination through N. Based on the experimental evidence thus obtained the complexes were characterized as five or six coordinated with the fifth or sixth position occupied by one or two water molecules, respectively. Zinc is known to form four-, five- or six-coordinate complexes. The coordination number six is favored when oxygen donors are present. In the complexes 8, 10, and 12 there are two water molecules which occupy the fifth and sixth positions in the octahedron. The hydrated complexes have significance in the enzymatic systems as the substrates can bind to zinc by substituting the coordinated water molecules in the complexes. Six-coordinate complexes of zinc (II) derived from indomethacin (Zhou *et al*., 2003) and other ligands (Iqbal *et al*., 2005; Niklas *et al*., 2003; Zhang and Janiak, 2001) have also been reported.

The proposed structures of the complexes under investigation, on the basis of the above experimental evidence, are shown in Figs. 1–4. Construction of molecular

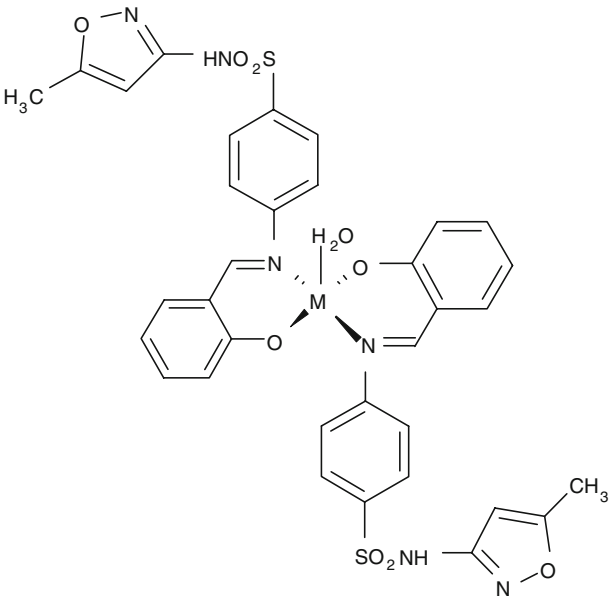

Fig. 1 Proposed structure of metal complexes of salicylidenesulfamethoxazole. M: Cu(II) or Zn(II)

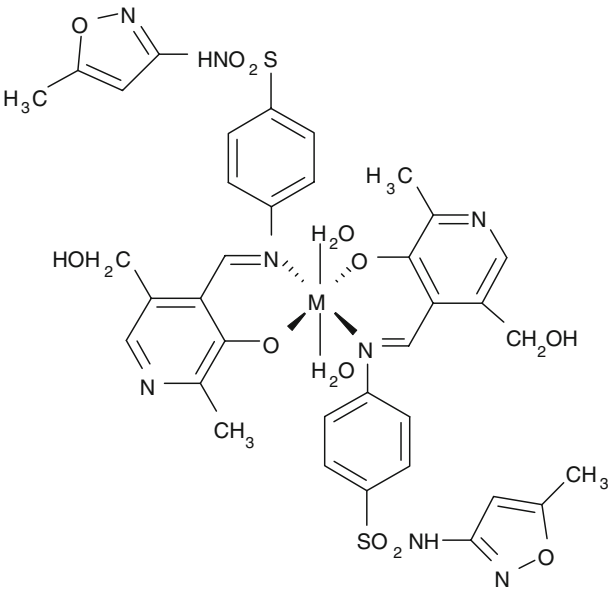

Fig. 2 Proposed structure of metal complexes of pyridoxylidenesulfamethoxazole. M: Cu(II) or Zn(II)

models indicates that salicylidenetrimethoprim and pyridoxylidenetrimethoprim can coordinate to the metal ions through different ways, as shown in Figs. 3a–c and 4a–c. The hydrogens associated with the 2-amino group of trimethoprim are more acidic than those with the 4-amino group due to the greater electron-withdrawing effect of the pyrimidine ring. As the reaction was carried out at an alkaline pH the Schiff base formation, with equimolar quantities of salicylaldehyde and trimethoprim, the 2-amino group is more likely. This was confirmed by comparing the ^{1}H NMR spectra of trimethoprim (2-N$\underline{H_2}$ at 6.16 ppm; 4-N$\underline{H_2}$ at 5.80 ppm) and the ligand (extinction of 2-N$\underline{H_2}$ signal at 6.16 ppm). On this basis the structure 3(b) is not possible. In structure 3(c) the chelate ring is eight-membered which would be less stable than the six-membered ring in structure 3(a). Moreover, the presence of salicylaldehyde moiety on the azomethine group in structure 3(a) enhances the electron density on the nitrogen associated with it, thereby rendering it a preferred donor. Thus the structure 3(a) appears to be the most probable one. Similarly, structure 4(a) is proposed as the most probable one amongst 4a–c. These structures were supported by the observed change of chemical shift to a lower value of aldimine proton in the ^{1}H NMR spectra of the zinc complexes due to coordination. Unsuccessful attempts to isolate crystals suitable for X-ray analysis prevented further structure elucidation.

Antibacterial activity

The results of antibacterial study are given in Table 5. A cursory view of the data indicates the following trend in activity of the substances under investigation against

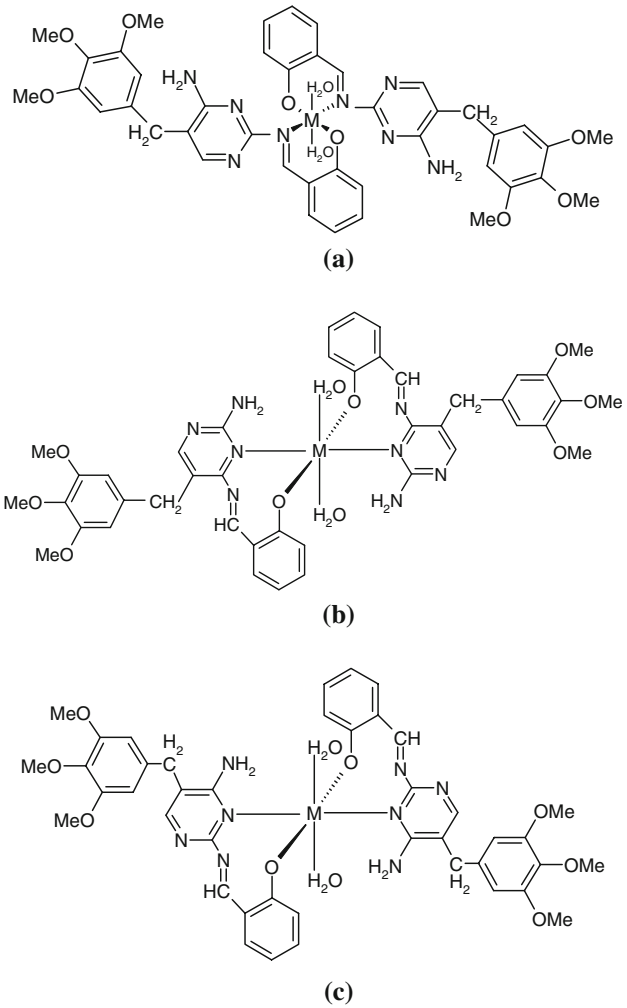

Fig. 3 A possible structure of metal complexes of salicylidenetrimethoprim. M: Cu(II) or Zn(II)

E. coli and *S. aureus*: Cu(II) complexes > Zn(II) complexes > Schiff base ligands > parent drugs. The Cu(II) complexes were found to be ten times more active than the parent drugs, up to ten times more active than the Schiff base ligands, and slightly more active than the zinc complexes against *E. coli*. On the other hand, the Cu (II) complexes were found to be up to 12 times more active than the parent drugs, up to 9 times more active than the Schiff base ligands, and slightly more active than the zinc complexes against *S. aureus*. All the Schiff base ligands under study were slightly more active than the parent drugs against *E. coli* and *S. aureus*. The enhanced activity of the complexes may be attributed to the facilitation provided by the metal ion for binding of the drug with the substrate through coordination.

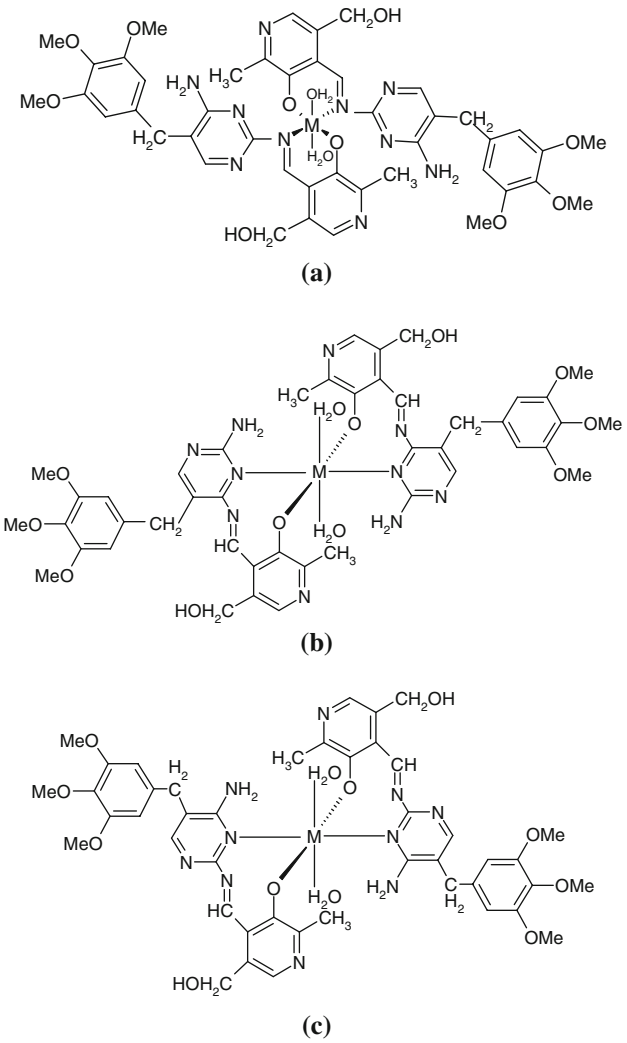

Fig. 4 A possible structure of metal complexes of pyridoxylidenetrimethoprim. M: Cu(II) or Zn(II)

Anti-inflammatory activity

The results of the paw edema test are summarized in Table 5. The copper complexes were found to be active whereas the zinc complexes and the parent drugs had no significant activity. The results are in line with previous findings (Iqbal *et al.*, 1999, 2005).

Toxicity study

The LD_{50} values (quantities resulting in the death of half the rats) are given in Table 5. Toxicity was reduced (lower LD_{50} values) by complexation.

Table 5 Minimum inhibitory concentrations, LD_{50} values, and anti-inflammatory activity data

Comp. no.	Dose (mg kg^{-1})	Inhibition of oedema (%)	LD_{50}^{a} (g kg^{-1})	MICs (μg cm^{-3})		
				E. coli	*S. aureus*	*P. aeruginosa*
Sulfame-thoxazole	50	2†	3662	100	65.00	>300
Trimethoprim	50	2†	6980	0.5	0.50	>300
1	50	3†	3700	75.5	45.00	>300
2	50	2†	7000	0.35	0.40	>300
3	50	4†	3805	70.3	41.00	>300
4	50	4†	7020	0.4	0.43	>300
5	50	39†	3600	8.3	5.00	>200
6	50	5†	3700	9.0	5.50	>200
7	50	35†	6905	0.04	0.10	>200
8	50	4†	6900	0.05	0.15	>200
9	50	47†	3599	8.8	5.30	>200
10	50	5†	3613	9.3	5.80	>200
11	50	36†	6900	0.05	0.15	>200
12	50	3†	6950	0.06	0.20	>200

† $p < 0.05$ compared with control

[a] Quantity resulting in the death of the half the number of rats

Conclusion

These results show that the antibacterial activity and toxicity of the drugs under investigation is improved after derivatization. The parent drugs having no anti-inflammatory activity became active on complexation with copper. These observations, in line with other studies, suggest that the metal-based drugs possess a great potential as therapeutic agents.

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