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ORIGINAL RESEARCH

Anti-inflammatory and selective COX-2 inhibitory activities of metal complexes of Schiff bases derived from aldoses

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Abstract Mn(II)-, Fe(II)-, Co(II)-, Ni(II)-, and Zn(II)-Schiff base complexes containing anthranilic acid and aldoses as part of the base were prepared and characterized by microanalytical, thermogravimetric, and spectroscopic data. The complexes were found to be four-coordinate, anhydrous, and ML_2 type. The spectral and magnetic data indicate a tetrahedral geometry for Mn(II) and Fe(II) complexes, and a planar geometry for Co(II), Ni(II), and Zn(II) complexes. Mn(II) and Zn(II) complexes showed a significant anti-inflammatory activity against kaolin-paw-edema. All the complexes exhibited selective inhibition of COX-2 in two different cell models.

Keywords Aldoses-derived Schiff bases · Schiff base complexes · Metal complexes · Anti-inflammatory activity · COX-2 inhibition

Introduction

Anthranilic acid and its derivatives have been reported to be active as anti-inflammatory agents (Sharma *et al.*, 2002). Mefenamic acid, an important anti-inflammatory drug, is a derivative of anthranilic acid. Their mode of action involves enzyme inhibition (Gaubert *et al.*, 2000) and free radical inactivation (Miche *et al.*, 1997; Daidone *et al.*, 1995). Anthranilic acid contains one $–NH_2$ and one carboxylic group, which can be utilized for derivatization. The $–NH_2$ group can readily be condensed with carbonyl compounds to produce a variety of Schiff base-type compounds. Schiff base formation has been well documented as an intermediate in some biochemical reactions. Horecker *et al.* (Wolform, 1955) discovered that Schiff base formation occurs in vivo between an aldose and a terminal amino group associated with an amino acid of an enzyme. This suggests that the hemiacetal linkage of reducing sugars is labile under physiological conditions and as such the carbonyl group becomes available for Schiff base formation. In our previous paper (Iqbal *et al.*, 1993), we reported the formation of novel Schiff bases from anthranilic acid and some aldoses as their Cu(II) chelates. These chelates were found to be active against kaolin-paw-edema. This study provides a window to a new class of biologically active Schiff base metal complexes. The well-documented role of imino sugars as enzyme inhibitors in treating lysosomal glycosphingolipidoses (Butters *et al.*, 2005) also provides sufficient justification to prepare Schiff bases and from sugars and study their potential as enzyme inhibitors.

Transition metal ions may also inhibit enzymes as they can interact with proteins and, by virtue of having variable oxidation states, can oxidize their sulfhydryl (–SH) groups to disulfide (–S–S–) linkages or reduce the –S–S– to –SH groups. Keeping these facts in mind, we synthesized Mn(II), Fe(II), Co(II), Ni(II), and Zn(II) complexes with Schiff bases derived from anthranilic acid and some aldoses for evaluation as anti-inflammatory agents and COX inhibitors. Therefore, the objectives of this study were set to be (i) to study the complex formation of novel Schiff

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bases derived from anthranilic acid and aldoses with biologically important transition metal ions, (ii) to explore their potential as COX inhibitors and anti-inflammatory agents, and (iii) to discover more safe and effective metal-based drugs.

Materials and methods

Materials

L-Arabinose, D-xylose, D-glucose, D-galactose, lipopolysaccharides (LPS), and arachidonic acid (AA) were obtained from Sigma Chemical Company, and anthranilic acid, $MnCl_2{\cdot}4H_2O$, $FeSO_4{\cdot}H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Ni(CH_3COO)_2{\cdot}4H_2O$, and $Zn(CH_3COO)_2.H_2O$ from E. Merck. Aspirin was a gift from Askari Pharmaceuticals, Lahore. All the solvents were obtained from E. Merck and used without further purification.

Healthy ICR mice (20–25 g) were obtained from the Department of Zoology of GC University, Lahore, and human umbilical vein endothelial cells (ECV304) and RPMI 1640 culture media were from Gibco BRL, Gaithersburg, USA. Fetal bovine serum was from Hangzhou Sijiqing Biomaterial Co Ltd, China. Phosphate buffer solution (PBS) was prepared by mixing KCl (2.68 mM), KH_2PO_4 (1.47 mM), Na_2HPO_4 (5.81 mM), NaCl (136.9 mM), and DMSO (1 %). PGE_2 and 6-keto $PGF_{1\alpha}$ RIA kits were purchased from Chinese Academy of Jiangsu Province Blood Study Institution.

Preparation of complexes

In order to find a suitable method, the preparation was attempted by the following different procedures:

(a) Reaction of metal-anthranilic acid complexes with the sugars: By this procedure a metal-anthranilic acid complex was first prepared which was then allowed to react with a sugar. Anthranilic acid (0.1 mol) was dissolved in ethanol (100 mL). To this the appropriate metal salt (0.05 mol in 100 mL ethanol) was added slowly with constant stirring. The pH of the mixture was adjusted to 6–7 by the use of KOH (1 % in ethanol). The resulting mixture was refluxed for 30 min (approx.). The metal-anthranilic acid complex thus obtained (in solution) was reacted with the appropriate sugar (0.1 mol in a minimum quantity of ethanol or water, as appropriate) under reflux for 30 min (approx.). A dark-brown product was filtered out, washed with ethanol and ether, and dried under vacuum.

(b) Reaction of metal-sugar complexes with anthranilic acid: Some metals are reported to form complexes with sugars (Riahi, 1986); therefore, by this procedure, an attempt was made to prepare metal-sugar complexes for subsequent reaction with anthranilic acid to obtain the desired Schiff base complexes. The appropriate sugar (0.1 mol in a minimum quantity of ethanol or water, as appropriate) was added to the metal salt (0.1 mol in 200 mL ethanol) and the mixture was refluxed for 30 min (approx.). To the resulting reaction mixture, anthranilic acid (0.1 mol) was added under constant stirring, pH was adjusted to 6–7 by the use of KOH (1 % in ethanol) and reflux was continued for further 30 min. A dark-brown product was filtered out, washed with ethanol and ether, and dried under vacuum.

(c) Reaction of anthranilic acid-sugar Schiff base with the metal salts: By this procedure, a Schiff base was prepared first, which was then reacted with the appropriate metal salt to obtain the desired complex. Anthranilic acid (0.1 mol) and the appropriate sugar (0.1 mol) were mixed together in ethanol (200 mL), and KOH (0.1 mol) was added. The mixture was refluxed for 15 min. To the lemon-yellow solution thus obtained, a solution of the appropriate metal salt (0.05 mol in 50 mL ethanol) was added slowly and the reaction mixture was refluxed for further 15 min. The resulting product was isolated by filtration, washed with ethanol and ether, and dried under vacuum. The complexes thus obtained are listed in Table 1.

Characterization

Microanalysis was carried out by the usual techniques. The metal was analyzed using the Hitachi Z-8000 atomic absorption spectrophotometer. Molecular weights were determined mass spectrometrically. Thermal analysis was carried out on a Netzsch simultaneous thermal analyzer in TGA and DTA modes. Conductivity measurements were carried out using the Wescan 212 conductivity meter in *N*,*N*-dimethylformamide (DMF) at room temperature. Infrared spectra were recorded on the Perkin-Elmer 882 IR spectrophotometer using KBr disk and Nujol mull techniques. Electronic absorption spectra were obtained using the Hitachi 220S spectrophotometer in the UV–Visible range using DMF as solvent. Magnetic moments were determined by Guoy's technique using Hg(II)-tetrathiocyanatocobaltate as a calibrant and diamagnetic corrections were calculated from Pascal's constants (Earnshaw, 1968). The ^{1}HNMR spectra of the diamagnetic complexes of nickel and zinc complexes were recorded on a 90 MHz Perkin Elmer machine using deuterated dimethylsulfoxide (DMSO-d6) as the solvent and tetramethylsilane (TMS) as the standard.

Table 1 Microanalytical data of the complexes: %, found (calculated)

Complex, code	Decomp. (°C)	C	H	N	Zn
Bis(*N*-L-arabinoseimineanthranilate)-Mn(II), (arab anth)$_2$Mn, ($C_{12}H_{14}NO_6$)$_2$Mn, MW 591.40	321–333	48.64 (47.73)	4.67 (4.73)	4.66 (4.73)	9.21 (9.29)
Bis(*N*-D-xyloseimineanthranilate)-Mn(II), (xyl anth)$_2$Mn, ($C_{12}H_{14}NO_6$)$_2$Mn, MW 591.40	323–328	48.58 (48.73)	4.98 (4.73)	4.59 (4.73)	9.90 (9.29)
Bis(*N*-D-glucoseimineanthranilate)-Mn(II), (glu anth)$_2$Mn, ($C_{13}H_{16}NO_7$)$_2$Mn, MW 651.38	324–330	47.82 (47.93)	5.05 (4.91)	4.45 (4.30)	8.56 (9.43)
Bis(*N*-D-galactoseimineanthranilate)-Mn(II), (gal anth)$_2$Mn, ($C_{13}H_{16}NO_7$)$_2$Mn, MW 651.38	322–328	47.75 (47.93)	5.02 (4.91)	4.15 (4.30)	8.61 (8.43)
Bis(*N*-L-arabinoseimineanthranilate)-Fe(II), (arab anth)$_2$Fe, ($C_{12}H_{14}NO_6$)$_2$Fe, MW 592.30	322–328	48.75 (48.21)	4.79 (4.76)	4.69 (4.73)	9.55 (9.43)
Bis(*N*-D-xyloseimineanthranilate)-Fe(II), (xyl anth)$_2$Fe, ($C_{12}H_{14}NO_6$)$_2$Fe, MW 592.30	323–328	48.55 (48.66)	4.95 (4.73)	4.58 (4.73)	9.21 (9.43)
Bis(*N*-D-glucoseimineanthranilate)-Fe(II), (glu anth)$_2$Fe, ($C_{13}H_{16}NO_7$)$_2$Fe, MW 652.29	321–328	47.69 (47.86)	5.03 (4.90)	4.40 (4.29)	8.30 (8.56)
Bis(*N*-D-galactoseimineanthranilate)-Fe(II), (gal anth)$_2$Fe, ($C_{13}H_{16}NO_7$)$_2$Fe, MW 652.29	323–328	47.99 (47.86)	4.79 (4.90)	4.41 (4.29)	8.66 (8.56)
Bis(*N*-L-arabinoseimineanthranilate)-Co(II), (arab anth)$_2$Co, ($C_{12}H_{14}NO_6$)$_2$Co, MW 595.34	324–328	48.21 (48.40)	4.76 (4.70)	4.64 (4.70)	9.84 (9.90)
Bis(*N*-D-xyloseimineanthranilate)-Co(II), (xyl anth)$_2$Co, ($C_{12}H_{14}NO_6$)$_2$Co, MW 595.34	323–330	48.30 (48.40)	4.95 (4.70)	4.74 (4.70)	9.68 (9.90)
Bis(*N*-D-glucoseimineanthranilate)-Co(II), (glu anth)$_2$Co, ($C_{13}H_{16}NO_7$)$_2$Co, MW 655.37	322–328	47.65 (47.63)	5.06 (4.88)	4.35 (4.27)	8.78 (8.99)
Bis(*N*-D-galactoseimineanthranilate)-Co(II), (gal anth)$_2$Co, ($C_{13}H_{16}NO_7$)$_2$Co, MW 655.37	321–327	47.73 (47.63)	4.98 (4.88)	4.19 (4.27)	8.87 (8.99)
Bis(*N*-L-arabinoseimineanthranilate)-Ni(II), (arab anth)$_2$Ni, ($C_{12}H_{14}NO_6$)$_2$Ni, MW 595.18	321–329	48.29 (48.03)	4.69 (4.70)	4.73 (4.70)	9.92 (9.87)
Bis(*N*-D-xyloseimineanthranilate)-Ni(II), (xyl anth)$_2$Ni, ($C_{12}H_{14}NO_6$)$_2$Ni, MW 595.18	322–328	48.28 (48.03)	4.99 (4.70)	4.71 (4.70)	9.94 (9.87)
Bis(*N*-D-glucoseimineanthranilate)-Ni(II), (glu anth)$_2$Ni, ($C_{13}H_{16}NO_7$)$_2$Ni, MW 655.15	324–330	47.68 (47.65)	4.96 (4.88)	4.40 (4.27)	8.78 (8.96)
Bis(*N*-D-galactoseimineanthranilate)-Ni(II), (gal anth)$_2$Ni, ($C_{13}H_{16}NO_7$)$_2$Ni, MW 655.15	323–330	47.52 (47.65)	4.74 (4.88)	4.10 (4.27)	8.78 (8.96)
Bis(*N*-L-arabinoseimineanthranilate)-Zn(II), (arab anth)$_2$Zn, ($C_{12}H_{14}NO_6$)$_2$Zn, MW 601.77	320–330	48.79 (47.88)	4.59 (4.65)	4.68 (4.65)	10.90 (10.87)
Bis(*N*-D-xyloseimineanthranilate)-Zn(II), (xyl anth)$_2$Zn, ($C_{12}H_{14}NO_6$)$_2$Zn, MW 601.77	323–329	47.78 (47.88)	4.71 (4.65)	4.70 (4.62)	10.85 (10.87)
Bis(*N*-D-glucoseimineanthranilate)-Zn(II), (glu anth)$_2$Zn, ($C_{13}H_{16}NO_7$)$_2$Zn, MW 659.81	324–329	46.98 (47.17)	5.02 (4.83)	4.15 (4.23)	9.72 (9.88)
Bis(*N*-D-galactoseimineanthranilate)-Zn(II), (gal anth)$_2$Zn, ($C_{13}H_{16}NO_7$)$_2$Zn, MW 659.81	322–331	47.32 (47.17)	4.65 (4.83)	4.19 (4.23)	9.75 (9.88)

Toxicity study

Toxicity (LD_{50} values) was determined as reported earlier (Iqbal *et al.*, 1999) using albino Wistar male rats (185–195 g).

Anti-inflammatory (AI) activity

Kaolin-paw-edema was induced according to a reported method (Lewis *et al.*, 1975) in male Wistar rats (95–110 g) in groups of five. The complexes under investigation were administered orally in 5 % Mulgophen (GAF Co. Manchester) in distilled water (0.2 mL/100 g body weight) 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 as previously (Lewis *et al.*, 1975).

COX-1 and COX-2 inhibitory activities

Inhibition of COX-1 and COX-2 by the complexes under investigation were determined by the use of human

umbilical vein endothelial cells (ECV304) and mouse macrophage models according to a reported method (Yun *et al.*, 2007) with slight modification by replacement of pyridine with DMSO as the solvent for these complexes. The selective inhibition index (SII) of COX-2 was reported as the ratio $IC_{50(COX\text{-}1)}/IC_{50(COX\text{-}2)}$ and compared with that of aspirin taken as standard.

All the procedures involving animals and biological materials were in accordance with the current revision of the Helsinki Declaration. The study was approved by the Ethics Committee of the university.

Results and discussion

Preparation of complexes

The brown products obtained by the experimental methods (a) and (b), on analysis, were found to be mainly a metal in reduced form, whereas well-characterized complexes were obtained by the method (c). Therefore, all the complexes reported in the present work were prepared by following the procedure in the method (c). The ligands could not be isolated due to their instability in free state. The prepared complexes were soluble in coordinating solvents including DMF and DMSO, and insoluble in water and common organic solvents.

Characterization

Microanalyses suggest a ML_2 type composition of the complexes, where M = metallo-element and L = Schiff base ligand. The molecular weights as determined mass spectrometrically confirm this composition. The complexes did not show sharp melting points rather they decomposed above 315 °C. The microanalytical data, molecular weights, and decomposition points of the complexes are given in Table 1. Thermal analysis indicated that the complexes were anhydrous as there was no weight loss in the 100–120 °C range.

Important infrared absorption bands along with their assignments are listed in Table 2. The stretching vibrations at 3,325–3,245 cm^{-1} and near 2,850 cm^{-1} due to NH_2 and CO groups, respectively, were absent in the IR spectra of the complexes. This indicates that the $–NH_2$ of anthranilic acid and –CHO of the sugars have been condensed to produce a –CH=N– (azomethine) group. Two new absorptions at 1,540 cm^{-1} (approx.) and 1,620–1,610 cm^{-1}, indicating the coordination of azomethine group to the metal (Walmsley and Tyree, 1963; Barbieri *et al.*, 1971; Kovacic, 1967), appeared in the IR spectra of the complexes. Two new absorption bands at 400–430 and 300–360 cm^{-1} due to ν(M–N) and ν(M–O), respectively (Ben Saber *et al.*, 2005), were also observed. This indicates coordination of the Schiff base ligands through N and O to the metal ions.

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

Complex	ν(OH)	ν(C=N)	ν(COO)	ν(COO)	δ(CO)	π(CO)	ν(MN)	ν(MO)
(arab anth)$_2$Mn	3,400	1,618	1,544	1,332	755	522	404	342
(xyl anth)$_2$Mn	3,395	1,620	1,545	1,325	745	515	425	330
(glu anth)$_2$Mn	3,390	1,620	1,545	1,318	754	510	420	340
(gal anth)$_2$Mn	3,405	1,620	1,540	1,330	750	510	420	345
(arab anth)$_2$Fe	3,405	1,610	1,540	1,320	740	510	420	320
(xyl anth)$_2$Fe	3,380	1,615	1,540	1,325	740	510	420	340
(glu anth)$_2$Fe	3,395	1,615	1,540	1,320	750	510	400	320
(gal anth)$_2$Fe	3,405	1,616	1,540	1,328	760	570	430	360
(arab anth)$_2$Co	3,400	1,620	1,535	1,325	745	500	400	300
(xyl anth)$_2$Co	3,375	1,615	1,535	1,320	740	510	420	300
(glu anth)$_2$Co	3,385	1,618	1,535	1,320	750	500	430	320
(gal anth)$_2$Co	3,410	1,620	1,545	1,330	730	560	420	350
(arab anth)$_2$Ni	3,405	1,620	1,540	1,325	740	510	420	320
(xyl anth)$_2$Ni	3,380	1,615	1,543	1,320	740	510	420	340
(glu anth)$_2$Ni	3,390	1,618	1,540	1,325	755	530	430	340
(gal anth)$_2$Ni	3,405	1,615	1,540	1,334	730	565	420	340
(arab anth)$_2$Zn	3,405	1,620	1,540	1,320	740	520	425	320
(xyl anth)$_2$Zn	3,385	1,620	1,540	1,325	740	500	420	340
(glu anth)$_2$Zn	3,390	1,620	1,540	1,320	750	510	420	345
(gal anth)$_2$Zn	3,410	1,620	1,535	1,336	730	560	425	345

Imine formation was witnessed by yellowing of the solution and development of an absorption band around 400 nm (Metzler, 1957) in the electronic absorption spectra when the sugar and anthranilic acid solutions were mixed together. On addition of a metal salt, the yellow color was replaced with a different color depending upon the metal salt used. The absorption maxima of the complexes are listed in Table 3. Representative spectra are reproduced in Fig. 1. The intense band around 280 nm is assigned to a phenyl ring π–π^* transition (Bosnich, 1968). The absorption bands in the region 325–370 nm, by analogy with (Sal)(-)pn-Zn (Downing and Urbach, 1969), are attributed to a π–π^* transition originating in the –CH=N– chromophore, while the lower energy bands are due to d–d transitions. The d–d transitions were observed for Mn(II) and Ni(II) complexes at 400–405 nm and 480–500 nm, respectively.

Four-coordinate Mn(II) complexes are usually tetrahedral, alternatively they can possess planar geometry. The intensity of the d–d band ($\varepsilon = 3$ mol^{-1} cm^2) in the spectra of the complexes under investigation is consistent with a tetrahedral environment (Cotton *et al.*, 1962). Thus, a tetrahedral geometry is proposed for the four-coordinate Fe(II) complexes with magnetic moments 5.21–5.36 BM (Greenwood and Earnshaw, 1984a) (Table 4). The Schiff base complexes of Co(II) are usually low spin with magnetic moments in the range of 2.1–2.9 BM (Greenwood and Earnshaw, 1984b) and possess square-planar geometry.

Table 3 Electronic absorption spectra [nm (ε, mol^{-1} cm^2)] in DMF

Complex	Ligand bands		
(arab anth)$_2$Mn	280(40000)	335(7102)	400(3)
(xyl anth)$_2$Mn	282(40002)	337(6998)	405(3)
(glu anth)$_2$Mn	280(40007)	339(7000)	405(3)
(gal anth)$_2$Mn	280(40000)	330(6085)	405(3)
(arab anth)$_2$Fe	285(40019)	337(6999)	
(xyl anth)$_2$Fe	285(40022)	335(7001)	
(glu anth)$_2$Fe	284(40013)	336(7005)	
(gal anth)$_2$Fe	282(40016)	333(6225)	
(arab anth)$_2$Co	287(40034)	335(7003)	
(xyl anth)$_2$Co	285(40015)	335(7009)	
(glu anth)$_2$Co	286(40025)	339(7081)	
(gal anth)$_2$Co	285(40009)	335(6998)	
(arab anth)$_2$Ni	283(40012)	330(7003)	500(59)
(xyl anth)$_2$Ni	282(40008)	335(7001)	490(60)
(glu anth)$_2$Ni	284(40020)	329(6981)	480(58)
(gal anth)$_2$Ni	283(40007)	325(6391)	490(59)
(arab anth)$_2$Zn	285(40017)	325(7000)	
(xyl anth)$_2$Zn	280(40007)	335(6985)	
(glu anth)$_2$Zn	280(40002)	325(7090)	
(gal anth)$_2$Zn	282(40003)	325(6700)	

Electronic absorption spectra of Co(II) complexes provide a clear distinction between planar and tetrahedral structure. The d–d bands of these complexes appear in the NIR region. NIR spectra of the complexes under investigation could not be obtained due to instrumental limitation. Therefore, a planar structure was proposed for the Co(II) complexes on the basis of their magnetic moments (Table 4). The diamagnetic complexes of Ni(II) are planar, whereas the paramagnetic complexes are octahedral or tetrahedral (Greenwood and Earnshaw, 1984c). The planar Ni(II) complexes absorb in the 452–600 nm range with a medium intensity ($\varepsilon = 60$ mol^{-1} cm^2) (Cotton and Wlikinson, 1980). The Ni(II) complexes under investigation absorbed around 500 nm; therefore, a planar structure was proposed for them. The electronic absorption spectra of Zn(II) complexes were also consistent with a planar structure. The magnetic moments of all the paramagnetic complexes reported here are normal values, which suggest the complexes to be mono-nuclear.

Formation of the Schiff base was confirmed by the appearance of an aldimine proton signal around 7.3 ppm with reference to TMS in the ^{1}HNMR spectra of the nickel and zinc complexes. The spectrum of (glu anth)$_2$ Zn is shown in Fig. 2 as the representative, which confirms the

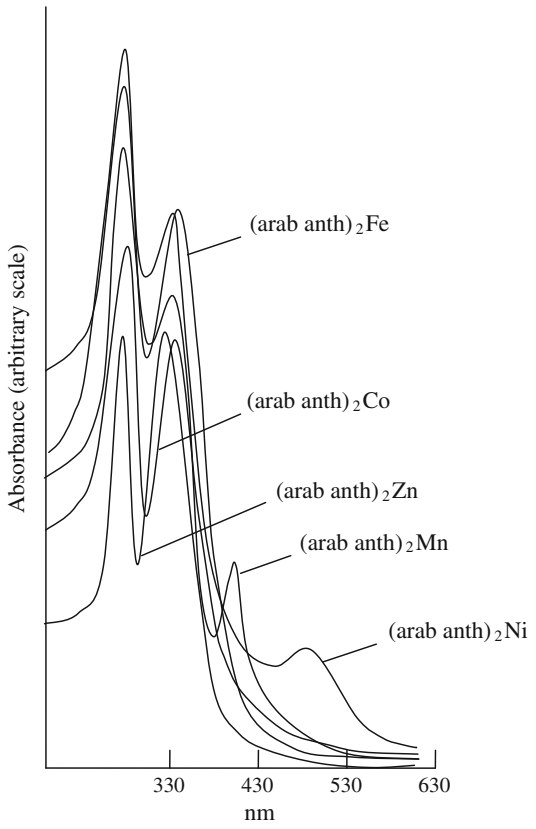

Fig. 1 Electronic absorption spectra of representative complexes

Table 4 Proposed geometry and magnetic moments of the complexes

Complex	Geometry	μ (BM)
(arab anth)$_2$Mn	Tetrahedral	5.84
(xyl anth)$_2$Mn	Tetrahedral	5.75
(glu anth)$_2$Mn	Tetrahedral	5.91
(gal anth)$_2$Mn	Tetrahedral	5.74
(arab anth)$_2$Fe	Tetrahedral	5.32
(xyl anth)$_2$Fe	Tetrahedral	5.21
(glu anth)$_2$Fe	Tetrahedral	5.36
(gal anth)$_2$Fe	Tetrahedral	5.32
(arab anth)$_2$Co	Planar	2.81
(xyl anth)$_2$Co	Planar	2.63
(glu anth)$_2$Co	Planar	2.74
(gal anth)$_2$Co	Planar	2.71
(arab anth)$_2$Ni	Planar	Diamagnetic
(xyl anth)$_2$Ni	Planar	Diamagnetic
(glu anth)$_2$Ni	Planar	Diamagnetic
(gal anth)$_2$Ni	Planar	Diamagnetic
(arab anth)$_2$Zn	Planar	Diamagnetic
(xyl anth)$_2$Zn	Planar	Diamagnetic
(glu anth)$_2$Zn	Planar	Diamagnetic
(gal anth)$_2$Zn	Planar	Diamagnetic

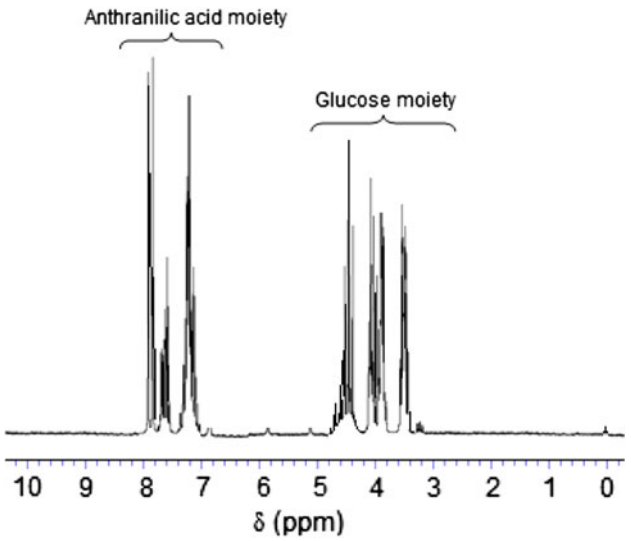

Fig. 2 Proton NMR spectrum of (glu anth)$_2$Zn

presence of the sugar and anthranilc acid moieties in the complexes indicating the Schiff base nature of the ligands.

The lower molar conductance values (1.23–1.19 Ohm^{-1} mol^{-1} cm^2) for the complexes under investigation are indicative of their non-electrolytic nature. The structures proposed on the basis of available data of the complexes are shown in Fig. 3.

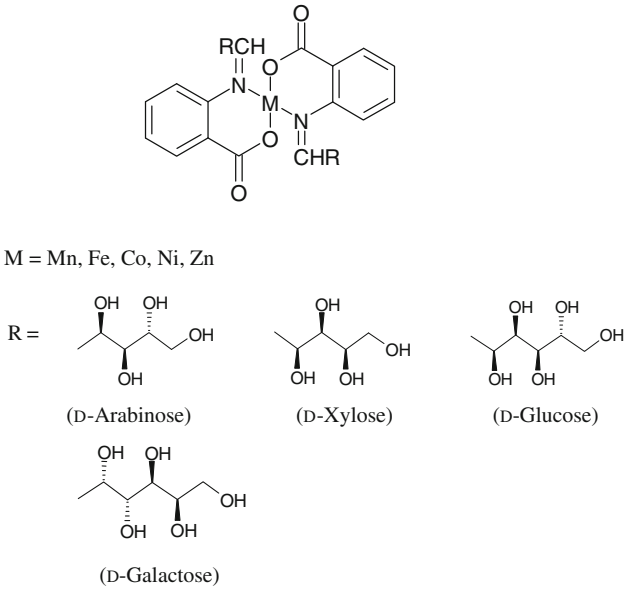

Fig. 3 Proposed structure of the complexes

Toxicity study

The toxicity data (Table 5) indicate that the complexes are less toxic than the Cu(II) complexes. The lower toxicity of the complexes can be attributed to their chelate structure and better biocompatibility due to the presence of sugar moiety. The relative toxicities of the metal complexes as indicated by their LD_{50} values followed the trend Ni > Mn > Co > Fe > Zn, which suggests that the nickel complexes are highly toxic. Therefore, nickel may not be considered as a safe metal in drug design.

Anti-inflammatory activity

The Mn(II) and Zn(II) complexes inhibited kaolin-paw edema significantly (Table 5). The Mn(II) complexes were about two times more active than the Zn(II) complexes at the tested dose.

COX-1 and COX-2 inhibitory activities

All the complexes exhibited selective inhibition of COX-2 comparable with that of aspirin (Table 6) in a dose-dependent manner (Fig. 4), which supports the anti-inflammatory data of the edema test. A precise mechanism of selective inhibition of COX-2 by these complexes cannot be derived from the present data; further work, including drug-DNA interaction study, needs to be done. However, at the onset, it can be postulated that the transition metal complexes having chelate structures are highly stable and as such can survive during transportation to the extent that can interact with the –SH groups of the cell

Table 5 AI effect of the complexes in kaolin-paw edema

Complex	Dose (mg/kg)	% Inhibition of edema	LD_{50} (mg/kg)
(arab anth)$_2$Mn	50	20	2,000
(xyl anth)$_2$Mn	50	19	1,900
(glu anth)$_2$Mn	50	19	1,950
(gal anth)$_2$Mn	50	18	1,970
(arab anth)$_2$Fe	50	5	4,560
(xyl anth)$_2$Fe	50	7	4,290
(glu anth)$_2$Fe	50	6	4,200
(gal anth)$_2$Fe	50	5	4,500
(arab anth)$_2$Co	50	4	2,820
(xyl anth)$_2$Co	50	3	2,850
(glu anth)$_2$Co	50	5	2,900
(gal anth)$_2$Co	50	3	2,800
(arab anth)$_2$Ni	50	2	600
(xyl anth)$_2$Ni	50	4	680
(glu anth)$_2$Ni	50	4	620
(gal anth)$_2$Ni	50	3	650
(arab anth)$_2$Zn	50	10	4,000
(xyl anth)$_2$Zn	50	15	4,200
(glu anth)$_2$Zn	50	16	3,900
(gal anth)$_2$Zn	50	15	4,300

Table 6 COX inhibition data ($n = 3$, ± SE, $p < 0.05$)

Compound	$IC_{50(COX\text{-}1)}$ (mM)	$IC_{50(COX\text{-}2)}$ (mM)	SII for COX-2
Aspirin	0.60 ± 0.10	1.48 ± 0.09	0.41 ± 0.18
(xyl anth)$_2$Mn	0.67 ± 0.09	1.44 ± 0.13	0.47 ± 0.16
(glu anth)$_2$Mn	0.65 ± 0.11	1.53 ± 0.10	0.42 ± 0.18
(gal anth)$_2$Mn	0.63 ± 0.12	1.48 ± 0.15	0.43 ± 0.22
(arab anth)$_2$Fe	0.71 ± 0.13	1.51 ± 0.17	0.47 ± 0.21
(xyl anth)$_2$Fe	0.68 ± 0.11	1.48 ± 0.12	0.46 ± 0.18
(glu anth)$_2$Fe	0.72 ± 0.15	1.39 ± 0.20	0.52 ± 0.25
(gal anth)$_2$Fe	0.68 ± 0.10	1.47 ± 0.18	0.46 ± 0.19
(arab anth)$_2$Co	0.56 ± 0.10	1.44 ± 0.23	0.39 ± 0.24
(xyl anth)$_2$Co	0.59 ± 0.12	1.39 ± 0.19	0.42 ± 0.25
(glu anth)$_2$Co	0.55 ± 0.10	1.41 ± 0.16	0.39 ± 0.21
(gal anth)$_2$Co	0.53 ± 0.13	1.56 ± 0.20	0.34 ± 0.28
(arab anth)$_2$Ni	0.78 ± 0.13	1.48 ± 0.11	0.53 ± 0.18
(xyl anth)$_2$Ni	0.74 ± 0.16	1.49 ± 0.14	0.50 ± 0.24
(glu anth)$_2$Ni	0.77 ± 0.12	1.54 ± 0.16	0.50 ± 0.19
(gal anth)$_2$Ni	0.77 ± 0.13	1.60 ± 0.20	0.48 ± 0.21
(arab anth)$_2$Zn	0.72 ± 0.14	1.59 ± 0.19	0.45 ± 0.23
(xyl anth)$_2$Zn	0.79 ± 0.15	1.63 ± 0.21	0.48 ± 0.23
(glu anth)$_2$Zn	0.74 ± 0.13	1.49 ± 0.18	0.50 ± 0.21
(gal anth)$_2$Zn	0.76 ± 0.15	1.58 ± 0.24	0.48 ± 0.25

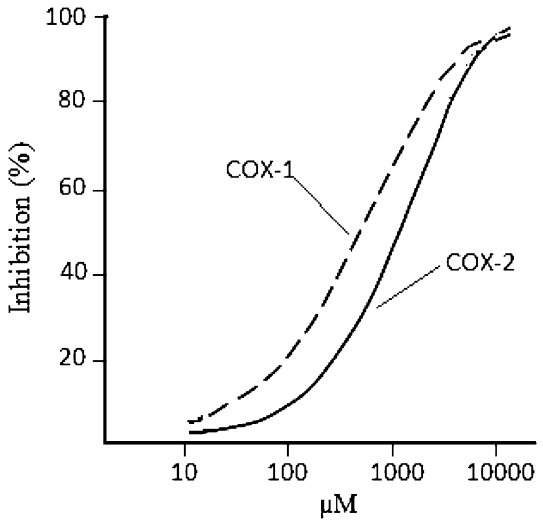

Fig. 4 Typical dose-dependent COX inhibitory patterns (for aspirin)

membrane, where the metal ion will oxidize them to –S–S– linkages resulting in the enzyme inhibition. During this redox cycling, reactive-oxygen species may be generated causing the so-called metal-mediated oxidative stress leading to intracellular alterations and induction of cell deaths. As far as the role of sugar moiety in the Schiff base complexes under investigation is concerned, it can induce trans-glycosylation type reaction in COX-2. The sugar and anthranilic moieties in these complexes are, respectively, hydrophilic and lipophilic in nature and as such may fit appropriately in the hydrophilic side pocket and lipophilic active site of COX-2 for exhibiting a selective inhibition.

Conclusions

This study shows that the Schiff base ligands derived from anthranilic acid and naturally occurring sugars form stable complexes with M(II), Fe(II), Co(II), Ni(II), and Zn(II) as evidenced by microanalytical, magnetic, and spectroscopic data. The Mn(II) and Zn(II) complexes exhibited significant AI activity in rats. All the complexes selectively inhibit COX-2.

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