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RESEARCH ARTICLE

Synthesis and Pharmacological Activity of Enoxacin Metal Complexes

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Abstract

Aims: The present work comprises the synthesis and Pharmacological activity of metal complexes. **Methods**: Two types of complexes L_1 & L_2 [M(eno)2(M = CuII, ZnII, CoII) were obtained and isolated as solid products. Complexes characterized by analytical means as well as by spectral techniques such as FT-IR, Mass and UV-Vis spectrometry. **Results**: These complexes were also tested for their in vitro antimicrobial activities against some bacterial strains to assess their inhibiting potential and the activities shown by these complexes were compared with standard drugs. **Conclusion**: The results expressed as the minimum inhibitory concentration (MIC), these complex use in the treatment of a patient with infection.

Introduction

Quinolons are discovered in the early 1960s, group of antibacterials has generated considerable clinical and scientific interest[1]. The mechanisms of enoxacin action involve inhibition of bacterial DNA gyrase[2], DNA replication & it has been proposed that metal complex intermediate are involve in this process. The most important structural features necessary for meaningful antibacterial activity of enoxacin include a carboxylic acid attached to the 3-position of the quinolone nucleus and an alkyl group in the 1-position[3]. In addition to this, fluorine attached to the 6-position and a nitrogen heterocycle attached to the 7-position is also required for their activity. This heterocycle in enoxacin is a piperazine derivative.[4] Isosteric replacements of nitrogen for carbon atom at postion 8 (1,8-napthyridines) are consistent and retained the antimicrobial activity.[5]

The coordination of quinolones to metal ions such as Cu(II), Co(II), and Zn(II) appear to be important for the activity of the quinolone antibiotics[6], it has a detrimental effect on their absorption[7]. Early studies by Nakano demonstrated the ability of the quinolone naldixic acid to complex a variety of metal ions.[8] The crystal structures of quinolone complexes.[9, 10] Complexes isolated from acidic media usually contain singly and doubly protonated quinolones that are incapable of bonding to the metal ion and in these cases only electrostatic interaction was observed between the drug and the metal ions[11, 12]. In other cases[13, 14], it was found that neutral quinolones in the zwitterionic state are capable of forming simple complexes. In these complexes the quinolone acts as a bidentate ligands through the ring carbonyl group at position 4 and through one of the oxygen atoms of the carboxylate group at position-3. Quinolones can also act as bridging ligands and thus capable of forming polynuclear complexes. Literature review reveals that there are few studies on the interaction of metal ions with enoxacin.[15]

EXPERIMENTAL

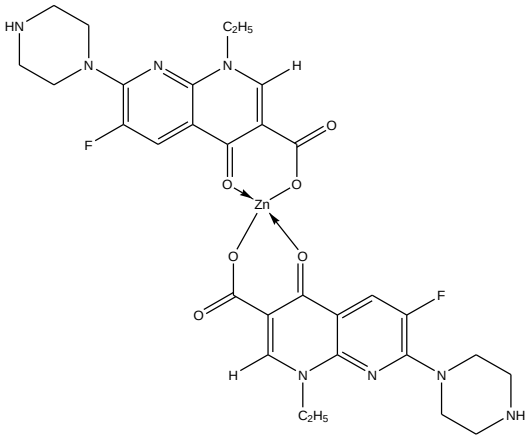
Material & Methods

All chemicals used were of the analytical grade (AR) & of highest purity available, these reagents were received from commercial sources. The solution was proposed with twice distilled water. They included Zinc(II) chloride, Coupper(II) chloride, & Cobalt(II) chloride.

Synthesis of metal complexes Enoxacin (L_1)

Enoxacin (0.270g) was dissolved in10 ml of 0.10m HCL. The solution was add in 0.057g of $Zncl_2$ was added. The solution was stirred and white precipitates appear after one day. The precipitate was filtered and washed with

ethanol. A saturated aqueous solution of the compound was then prepared and kept in an ethanol chamber. After a few weeks, transparent colourless needle shaped crystals appeared and analyzed by X-ray diffraction. The same product was obtained from a saturated aqueous solution of enoxacin hydrochloride at pH = 6, by the addition of $Cu(II)Cl_2$ & $Co(II)Cl_2$ (mole ratio enoxacin: Zn, Co, Cu =2:1). The solution was stirred for one day, though a white precipitate appeared after just half an hour. The precipitate was filtered, washed with ethanol and dried at 60^0C for 5 hours.



[1-ethyl-6-fluro-4-oxo-7-(piperazine-1-y)-1,4-dihydro-1,8-nyphthyridine-3-carboxylic acid].

Physical Characterization The metal complexes of enoxacin & ofloxacin were insoluble in water, acetone, ether, ethylene glycol, 2-propanol, carbon tetrachloride, cyclohexanone, dichloromethane and dimethyl sulfoxide. It decomposed in diluted solutions of all strong acids. All attempts to prepare single crystals were unsuccessful. The complexes were found to be stable at room temperature for two days.[16] The stability was checked by taking melting points of the complexes at an interval of 24 hours and 48 hours. All the samples were stored in room temperature (25°C). No appreciable changes in the melting points were observed, and the estimated error was ±1◦C. Melting points of the metal complexes when compared with the reference drug differ considerably from enoxacin & ofloxacin. The Physical measurements results obtained are tabulated in **Table 1**.

Table 1: Physical Characterization Data for Synthesized Compounds

Compound	Physical state	Mol. formula (Mol. weight)	MP (^{0}C)	Rf[a]	Yield (%)	Elemental analysis Calculated (Found) (%)			
						C	H	N	M
L^1	White crystals	$C_{15}H_{17}FN_4O_3$ (320.319)	168	0.69	54	77.43 (76.80)	6.12 (5.98)	16.43 (13.89)	…… …..
L^2	White crystals	$C_{18}H_{20}FN_3O_4$ (361.368)	173	0.68	61	74.60 (70.20)	5.97 (5.80)	19.73 (19.60)	…… …..
$Cu(L^1)_2$	White crystals	$C_{30}H_{32}CuF_2N_8O_6$ (702.17)	265	0.63	51	51.32 (51.30)	4.59 (4.54)	15.96 (15.93)	9.05 (9.02)
$Cu(L^2)_2$	White crystals	$C_{36}H_{38}F_2N_6O_8Cu$ (783.27)	260	0.67	53	55.13 (55.13)	4.88 (4.88)	10.72 (10.72)	8.10 (8.10)
$Co(L^1)_2$	White solid	$C_{30}H_{32}CoF_2N_8O_6$ (697.56)	320	0.62	65	51.65 (51.62)	4.62 (4.60)	16.6 (16.5)	8.45 (8.43)
$Co(L^2)_2$	White solid	$C_{36}H_{38}F_2N_6O_8Co$ (779.65)	313	0.68	69	55.46 (55.44)	4.91 (4.90)	10.78 (10.76)	7.56 (7.54)
$Zn(L^1)_2$	White crystals	$C_{30}H_{32}F_2N_8O_6Zn$ (704.01)	280	0.61	48	51.18 (51.10)	4.58 (4.54)	15.92 (15.90)	9.29 (9.20)
$Zn(L^2)_2$	White crystals	$C_{36}H_{38}F_2N_6O_8Zn$ (786.11)	285	0.71	51	55.00 (55.00)	4.87 (4.85)	10.69 (10.66)	8.32 (8.30)

Analytical Measurements

The metal: drug ratios were determined by conductometric titration and continuous variation method (jobs plot). The UV-Vis spectra of the drug and metal solutions (in different combination ratios) were recorded on UV-Vis spectrophotometer (Shimadzu 1601 coupled with a P IV–PC and loaded with UVPC version 3.9, software). Thin layer chromatography (TLC) was performed on HSF-254 TLC plate and the samples were visualized under UV lamp. Melting point of the metal complexes was recorded on a Gallenkamp apparatus. The characterization of enoxacin metal complexes was carried out by Fourier Transform Infrared Spectrophotometer (Shimadzu Prestige-21 200 VCE), coupled to a P IV-PC and loaded with IR Resolution software. The disks were placed in the holder directly in the IR laser beam. Spectra were recorded at a resolution of 2cm−1, and 50 scans were accumulated. NMR spectra were recorded on Bruker AMX 500MHz spectrometer in $CDCl_3$ using TMS as an internal standard. Column chromatography was performed on Merck silica gel 60 (particle size 0.06–0.02). The UV/VIS and IR data measurements results obtained are tabulated in **Table 2**.

Table 2: UV/VIS and IR data of ligands and metal complexes.

Compound	IR (cm^{-1})							λ_{max} (nm)		
	$C00^-$ ν(C=0)	$C00^-$ ν(C-0)	ν a ($C00^{-}$)	ν s ($C00^{-}$)	ν (-C=N)	N (M-N)	N (M-N)	Π→Π*	d-d	n→Π*
L^1	1726	1253	--	--	1626	--	--	275	--	308
L^2	1729	1263	--	--	1621	--	--	270	--	312
$Cu(L^1)_2$	--	--	1638	1470	1616	463	370	--	556	376
$Cu(L^2)_2$	--	--	1631	1483	1609	460	373	--	562	379
$Co(L^1)_2$	--	--	1628	1488	1613	466	383	--	632	389
$Co(L^2)_2$	--	--	1630	1486	1604	470	387	--	612	379
$Zn(L^1)_2$	--	--	1625	1480	1615	458	382	--	570	374
$Zn(L^2)_2$	--	--	1631	1483	1609	459	379	--	575	372

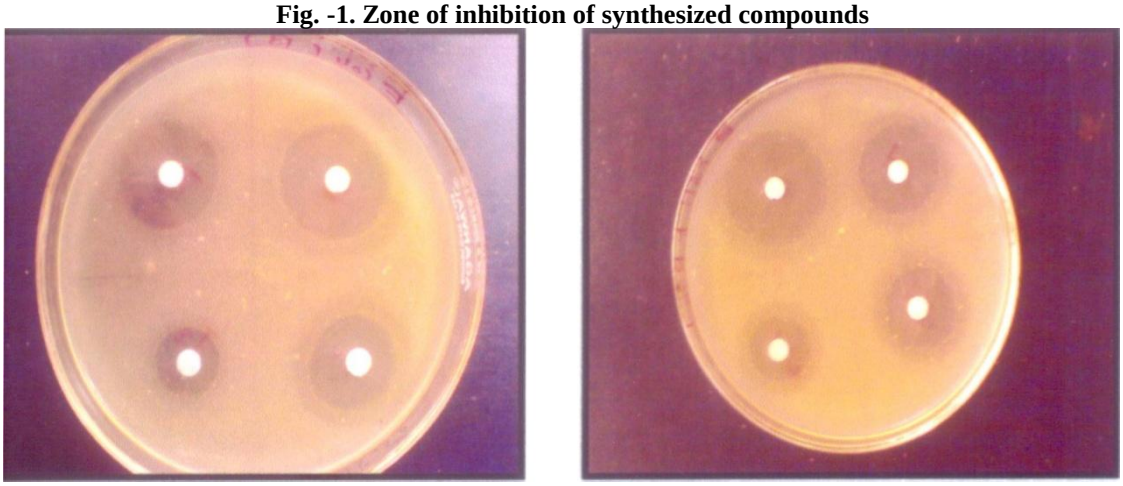
Table 3: MIC (mm) values of ligand and metal complex.

Compound	Microbial species											
	Staphylococcus aureus			Bacillus subtilus			Salmonella typhae			E.coli		
Conc (mg/disc)	5	10	20	5	10	20	5	10	20	5	10	20
L^1	11	14	22	10	13	20	10	14	20	10	14	24
L^2	10	15	21	11	14	22	10	14	20	11	15	23
$Cu(L^1)_2$	11	14	22	9	15	21	10	13	22	10	15	25
$Cu(L^2)_2$	10	15	21	11	13	22	12	14	24	11	16	24
$Co(L^1)_2$	11	14	22	12	14	22	11	16	23	10	17	25
$Co(L^2)_2$	11	15	21	11	16	21	12	17	22	12	15	22
$Zn(L^1)_2$	10	16	24	12	16	24	10	14	22	11	16	23
$Zn(L^2)_2$	11	14	23	12	15	25	10	14	22	11	14	22

Antibacterial activity

The zone of inhibition around the antibiotic discs is related to the susceptibility of the organisms toward enoxacin and its metal complexes. The increased activity of metal chelates can be explained on the basis of the overtone concept and chelation theory. According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which liposolubility is an important factor that controls the antimicrobial activity. Zone of inhibition of synthesized compounds showing antibacterial activity in Fig.-1.

Fig. -1. Zone of inhibition of synthesized compounds



On chelation the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of complexes.[18] The antimicrobial activity of the metal salts was also investigated. It was found that the metal salts did not exhibit antimicrobial activity at the concentration range used to assay the activity of the complexes in our work.

Result & Discussion

The Conductometric titration and jobs plot reveal that the metal: drug complexes are in the ratio 1: 2 (metal = Cu(II), Co(II) or Zn(II). In the IR spectra of enoxacin, two strong absorption peaks at 1690 and 1640 cm−1 are observed due to carboxylic and ring ketonic (C=O) groups, respectively. On comparing the IR spectrum of enoxacin & ofloxacin with its metal complexes, it is found that the band due to carboxylic group at (1690 cm−1) nearly diminishes in the spectra of the complexes indicating the coordination of this moiety to the metal ion.[19] Further the absorption of the ring ketone. Appears at a lower frequency near (1600 cm−1) in the spectra of the complexes which also suggests the binding of enoxacin to the metal ions through the ring carbonyl oxygen atom.[20] On comparing main peaks of enoxacin with its complexes, it is observed that all the signals of the free ligand are present in the ^{1}HNMR spectra of the complexes. The signals for the aliphatic and piperazine protons are practically unchanged since they lie far from the binding site of the ligand.[21] The resonance of the carboxylic proton (COOH) is not detected in the spectra of the complexes which further suggest the coordination of enoxacin through its carboxylate oxygen atoms.[22] The OH proton peak appears near 3.5 ppm, adjacent to the piperazine protons,due to the presence of lattice water. Our studies suggest that enoxacin acts as a monoanionic bidentate ligand and interacts with the metal centre through the 3-carboxylate and 4-oxygen atom. From the results obtained, it is proposed that the Zinc(II), Cobalt(II), and Copper(II) complexes are probably six coordinate with two molecules of enoxacin chelating the central metal atom from four sides and two molecules of water at the vertices of an octahedron. Alternatively, the enoxacin complex of Iron(III) is four coordinate with probably one molecule of the drug and two molecules of water along the edge of a tetrahedron. Despite the crystalline nature of the products, we did not manage to obtain crystals suitable for determination of structures with X-ray crystallography.[23, 24]

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