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Sparfloxacin–metal complexes as antifungal agents – Their synthesis, characterization and antimicrobial activities

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ABSTRACT

Metal complexes with the third-generation quinolone antibacterial agent sparfloxacin (SPFX) or 5-amino-1-cyclopropyl-7-(cis-3,5-dimethyl-1-piperazinyl)-6,8,di-fluoro-1-4-dihydro-4-oxo-3-quinocarboxylic acid have been synthesized and characterized with physicochemical and spectroscopic techniques such as TLC, IR, NMR and elemental analyses. In these complexes, sparfloxacin acts as bidentate deprotonated ligands bound to the metal through the pyridone oxygen and one carboxylate oxygen. The antimicrobial activity of these complexes has been evaluated against four Gram-positive and seven Gram-negative bacteria. Antifungal activity against five different fungi has been evaluated and compared with reference drug sparfloxacin. Fe^{2+}–SPFX and Cd^{2+}–SPFX complexes showed remarkable potency as compared to the parent drug.

1. Introduction

Quinolones, the sequence of synthetic antibacterial agents are used for the treatment of a wide variety of infectious diseases [1]. During recent years much attention has been devoted to the syntheses of new 4-quinolone-3-carboxylates and their testing for antibacterial activities [2,3]. Despite many advances in the fluoroquinolones field, still continuous study for novel quinolones with better activity profile, pharmacokinetics, and tolerability is needed. Sparfloxacin (SPFX) (Fig. 1) is an orally active synthetically broad spectrum third-generation quinolone, characterized by good to excellent activity against Gram-positive cocci (notably *Streptococcus pneumonias*) and selected activity against anaerobes and atypical pathogens. It is also moderately active against some (*Bacteroides fragilis* group) L. mono-cytogenes resistant [4,5].

The absorption of the quinolone drugs is lowered, when they are consumed simultaneously with essential or trace metals [6,7]. The proposed mechanism of the interaction is chelation between the 4-oxo and adjacent carboxyl group of quinolone and metal cations. Mostly the quinolone acts as a bidentate ligand [8–17].

Literature survey reveals that concurrent administration of magnesium and aluminium containing antacid with ciprofloxacin resulted in a nearly complete loss of activity of the drug [18] and patients who orally administrated fluoroquinolones should avoid mixtures containing multivalent cations, because quinolones were chelate bonded to these metals and in consequence formed metal complex in the gastric system [19].

Metals are considered essential to a human body being an integral part of an organic structure in performing physiologically important and vital functions, in the body [20].

It seems that the role of metal ions is imperative for the way of function of fluoroquinolones. The synthesis and characterization of new metal complexes with fluoroquinolones are of great importance for better understanding of the drug–metal ion interactions [21]. It was suggested that the reactions of metal ions with fluoroquinolones were essential for the activity of these antimicrobial agents, and the metal ions (magnesium, copper, and iron) may bridge the binding of the quinolone to DNA gyrase or of bacterial DNA directly [22,23].

Different studies suggested that the chelate structure may be affected by the presence of a 5-substituent at quinolone ring, that the C-4 carbonyl oxygen and the acidic proton on the C-3 carboxylic acid in sparfloxacin (SPFX) are weaker than the corresponding hydrogen bond in the 5-unsubstituted pefloxacin. In accord with this data, SPFX should suffer less disruption of absorption upon metal chelation or antacid co-administration than other quinolone agents [24].

A thorough survey of the literature has revealed a limited number of structurally characterized metal sparfloxacinato complexes. More specifically, the crystal structure, the interaction with

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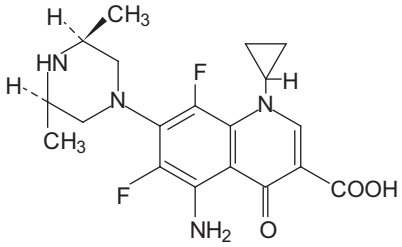

Fig. 1. Structure of SPFX.

calf-thymus (CT) DNA and the antibacterial activity of $Cu(sf)_2$ [25] and $Ni(sf)_2(py)_2$ [26] have been reported as well as the crystal structures and the antiproliferative properties of complexes $Cu_2(sf)_4$ and $[Cu(Hsf)(phen)\text{-}(H2O)]^{2+}$ [27] and antibacterial activity have been tested against 3–4 microorganism only [28]. Paper on cobalt (II)– sparfloxacin complex with its structure, antimicrobial activity and DNA-binding properties has been published earlier [29].

Here, we present the synthesis of a series of sparfloxacin–metal complexes with essential and trace elements in an attempt to find the mode of coordination as well as biological properties of the resultant complexes. More specifically, the complexes have been synthesized and characterized with elemental analysis and diverse spectroscopic techniques (TLC, IR, UV–Vis and NMR techniques) and elemental analyses. The biological activity of the complexes has been evaluated by determining zone of inhibition against four Gram-positive and seven Gram-negative bacteria. Antifungal activity against five different fungi has been evaluated and compared with reference drug sparfloxacin.

2. Experimental

2.1. Materials and reagents

Sparfloxacin was a kind gift by Abbott Pharmaceuticals (Karachi) while solvents and chemicals of analytical grade were purchased from the market. Metal salts ($MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$, $CrCl_3{\cdot}6H_2O$, $MnCl_2{\cdot}H_2O$, $FeCl_3{\cdot}6H_2O$ or $FeSO_4{\cdot}7H_2O$, $CoCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $ZnCl_2$, $CdCl_2{\cdot}H_2O$) were of pious grade from E. Merck. All solutions were prepared fresh before work.

2.2. Instruments

The melting points were taken on an electro thermal melting point apparatus (Gallenkamp) in open capillary tubes and are uncorrected. TLC spots were detected by UV lamp. Infrared spectra were recorded as KBr pellets on Shimadzu 470 instrument. ^1H NMR spectra were obtained by using Bruker/XWIN NMR spectrometer with TMS as internal standard. Complexes were dissolved in $CDCl_3$, D_2O or MeOH for NMR. An elemental analysis is done by Carlo Erba Strumentazione Elemental analyzer-MOD 1106 instrument.

2.3. Synthesis of complexes

The complexes were synthesized by refluxing sparfloxacin and metal salts. Sparfloxacin was dissolved in methanol and to this, metal solution in methanol was added in the ratio of 1:2 [M:L], with continuous stirring, refluxed on water bath at 80 °C for 4 h and the volume was reduced by evaporation. The precipitated complexes were filtered, washed with water and methanol and dried under reduced pressure at room temperature. Purity of all synthesized complexes was checked by TLC on precoated silica gel plates utilizing methanol/ethyl acetate as eluting solvent in different ratios (1:1/1:2 v/v) and spots were detected either in UV lamp or in iodine chamber. Moreover, their melting points and solubility were noted.

2.4. Antibacterial activity

The formed complexes of sparfloxacin were screened for their antimicrobial activity against a series of Gram-positive (*Bacillus subtilis*, *Micrococcus luteus, Staphylococcus aureus,* and *Streptococcus features*) and Gram-negative (*Salmonella typhi, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Citrobacter* and *Shigella flexneri) organisms* by the conventional cylinder-plate method [30]. The solutions for soaking discs were made of different dilutions including 5, 10, 20, and 40 ppm by simple dilution method using water/methanol (99:1 v/v) as solvent. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with methanol at the same dilutions as used in the experiment.

Nutrient agar was prepared and then autoclaved at 121 °C for 15 min, cooled and then poured in Petri dishes. Streaking was done with the help of sterile cotton swab, soaked discs of complex solutions were placed in them and the dishes incubated for 24 h at 37 °C. Finally the zones of inhibition were carefully measured with the help of Vernier's caliper.

2.5. Antifungal activity

Same procedure was repeated for antifungal activity (as done for antibacterial activity) against series of fungi *Candida albican, Fusarium solani, Trichophyton rubrum, Aspergillus purasiticus, Aspergillus effuses and Saccharomyces cerevisiae.* Dilutions were made in same manner for soaking discs as before. Sabraoud dextrose agar was then prepared and autoclaved at 121 °C for 15 min, cooled and then poured in Petri dishes. Streaking was done in same way as done for antibacterial activity and dishes then incubated for 48 h at 37 °C. Finally the zones of inhibition were carefully measured.

3. Results and discussion

Only a few complexes of sparfloxacin have been made earlier with entirely different methods such as amperiometric and potentiometric techniques and so we were encouraged to prepare a series of metal complexes with a different method and to evaluate their spectroscopic and antimicrobial properties.

Metal complexes of sparfloxacin were prepared by refluxing metal salt solutions in methanol with sparfloxacin in 1:2 ratios and then crystallizing them at room temperature. Solubility and melting points were noted; these were then characterized by elemental analyses (as shown in Table 1), infrared spectroscopy and

Table 1
Elemental analyses for the complexes.

Metal complexes	Found (calcd.) % C	Found (calcd.) % H	Found (calcd.) % N
$[Mg(SPFX)_2H_2O]{\cdot}2Cl_2{\cdot}H_2O$	45.936(46.91)	4.850(5.18)	11.243(11.52)
$[Ca(SPFX)_2H_2O]{\cdot}2Cl_2$	46.481(47.02)	4.795(4.98)	11.103(11.54)
$[Cr(SPFX)_2H_2O]{\cdot}2Cl_2{\cdot}2H_2O$	47.552(48.16)	5.517(5.53)	11.526(11.82)
$[Mn(SPFX)_2H_2O]{\cdot}2Cl_2{\cdot}H_2O$	45.641(45.48)	4.887(5.02)	10.888(11.17)
$[Fe(SPFX)_2H_2O]{\cdot}SO_4{\cdot}2H_2O$	46.029(46.73)	5.068(5.37)	11.357(11.47)
$[Fe(SPFX)_2H_2O]{\cdot}2Cl_3{\cdot}H_2O$	42.355(42.44)	4.445(4.69)	10.671(10.42)
$[Co(SPFX)_2H_2O]{\cdot}3Cl_2$	43.721(43.04)	4.158(4.56)	11.001(10.57)
$[Ni(SPFX)_2H_2O]{\cdot}2Cl_2$	45.900(46.13)	4.614(4.89)	11.232(11.33)
$[Cu(SPFX)_2H_2O]{\cdot}2Cl_2{\cdot}H_2O$	44.747(45.09)	4.660(4.98)	10.582(11.07)
$[Zn(SPFX)_2H_2O]{\cdot}2Cl_2$	45.548(45.82)	4.827(4.86)	10.863(11.25)
$[Cd(SPFX)_2H_2O]{\cdot}2Cl_2$	43.584(43.76)	4.581(4.64)	10.402(10.74)
Sparfloxacin	58.160(57.097)	5.650(5.531)	14.280(14.061)