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New copper(I) complexes bearing lomefloxacin motif: Spectroscopic properties, *in vitro* cytotoxicity and interactions with DNA and human serum albumin

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ABSTRACT

In this paper we present lomefloxacin's (HLm, 2nd generation fluoroquinolone antibiotic agent) organic and inorganic derivatives: aminomethyl(diphenyl)phosphine (PLm), its oxide as well as new copper(I) iodide or copper(I) thiocyanate complexes with PLm and 2,9-dimethyl-1,10-phenanthroline (dmp) or 2,2′-biquinoline (bq) as the auxiliary ligands. The synthesized compounds were fully characterised by NMR, UV–Vis and luminescence spectroscopies. Selected structures were analysed by theoretical DFT (density functional theory) methods. High stability of the complexes in aqueous solutions in the presence of atmosferic oxygen was proven. Cytotoxic activity of all compounds was tested towards three cancer cell lines (CT26 - mouse colon carcinoma, A549 - human lung adenocarcinoma, and MCF7 - human breast adenocarcinoma). All complexes are characterised by cytotoxic activity higher than the activity of the parent drug and its organic derivatives as well as cisplatin. Studied derivatives as well as parent drug do not intercalate to DNA, except Cu(I) complexes with bq ligand. All studied complexes caused single-stranded cleavage of the sugar–phosphate backbone of plasmid DNA. The addition of H_2O_2 caused distinct changes in the plasmid structure and led to single- and/or double-strain plasmid cleavage. Studied compounds interact with human serum albumin without affecting its secondary structure.

1. Introduction

Despite of a growing number of different approaches, the use of metal complexes in anticancer therapy or cancer imaging is still very promising and probably will bring applicable results in a near future [1]. Examples of the recently studied complexes cover a wide range of ligands and geometries. The most extensively investigated groups of the complexes are those with copper [2,3] platinum [4–6], palladium [6], ruthenium [7–9], and iridium ions [9–12].

For a few years we have been studying mixed copper(I) complexes with aminomethylphosphines and diimines as new potential antimicrobial and cytotoxic agents [13,14]. Recently we have started to study phosphine derivatives of fluoroquinolones (FQs) (Fig. 1). They are broad-spectrum antibiotics used for treatment of bacterial infections not only in human but also in veterinary medicine [15–18]. Since the WHO declared that increasing microbial resistance against antibiotics is one of the most imminent health dangers [19,20], FQs have been a frequent subject of structural modifications [21–32], including formation of the coordination compounds [33,34]. Syntheses of the complexes can improve their solubility, pharmacokinetics and bioavailability. Interestingly, apart from the strong antimicrobial activity, FQs have also proved to cause immunomodulation and antitumor effects [35–37]. As a consequence, the novel strategies in the design of metal-FQ complexes have also led to compounds significantly more active towards cancer cells than the parent drugs [33,38].

We have already characterised the derivatives and complexes of three FQs: ciprofloxacin (HCp), norfloxacin (HNr) and sparfloxacin (HSf) [29–31,39–41]. We have demonstrated the diversified biological activity of the derivatives. This has enabled us to get some insight into the structure-activity relationships. HSf turned out to be the most promising FQ studied by us. It has been characterised by a small cytotoxic effect towards selected cancer cell lines, but its attachment to the copper(I) complex *via* $-CH_2PPh_2$ moiety highly elevated the cytotoxicity [41]. This prompted us to study the physicochemical and biological properties of the derivatives of another FQ - lomefloxacin (HLm). It is a 2nd generation antibiotic, known for a broad spectrum of antimicrobial activity against a wide range of gram-negative and gram-positive bacteria [42]. The structure of a lomefloxacin molecule is similar to that of norfloxacin but it has an extra fluorine atom in position 16, and a methyl substituent on the piperazine ring (Fig. 1).

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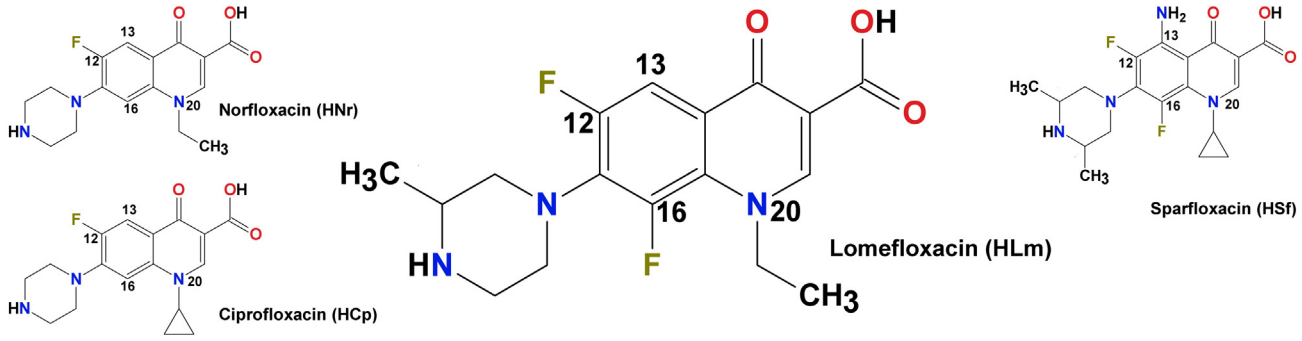

Fig. 1. Schemes of ciprofloxacin, norfloxacin, lomefloxacin and sparfloxacin structures with atomic enumeration.

2. Experimental

2.1. Materials

Reactions were carried out under a dinitrogen atmosphere using standard Schlenk techniques. $PPh_2(CH_2OH)_2Cl$ was synthesized according to a literature procedure [43]. Lomefloxacin, Ph_2PH, calf thymus DNA (CT DNA), plasmid DNA, human serum albumin (HSA) and other small chemicals and solvents were purchased from Sigma-Aldrich (Germany) and used without further purifications. All cell culture fluids were purchased from IMMUNIQ (Poland). All solvents were deaerated prior to use.

2.2. Methods

Elemental analyses were performed on a Vario EL3 CHN analyser for C, H, and N, and they were within 0.3% of the theoretical values. NMR spectra were recorded on a Bruker AMX 500 spectrometer (at 298 K) with traces of solvent as an internal reference for ^1H (CDCl3: 7.27 ppm) and ^{13}C spectra (CDCl3: 77.0 ppm) and 85% H_3PO4 in H_2O as an external standard for ^{31}P. The signals in the spectra are defined as: s = singlet (* – strongly broadened signal), d = doublet, dd – doublet of doublets, t = triplet and m = multiplet. Chemical shifts are reported in ppm and coupling constants are reported in Hz. Absorption spectra were recorded on a Beckman DU 7500 spectrophotometer in the 800–200 nm range. Photoluminescence measurements were recorded at 298 K on a Cary Eclipse Fluorescence Spectrophotometer. Spectra of circular dichroism were measured using Spectropolarimeter JASCO J-715 (CD and MCD).

2.3. Synthesis

2.3.1. Preparation of Ph_2PCH_2Lm (PLm)

$PPh_2(CH_2OH)_2Cl$ (0.9478 g, 3.35 mmol) was dissolved in 20 mL of methanol and after 10 min an excess of NEt_3 (triethylamine; 1.4 mL, 10.0 mmol) was added. The mixture was stirred for 40 min. Then, in the darkness, lomefloxacin (1.1770 g, 3.35 mmol) was added. After 10 min of stirring a white precipitate formed. The solid was filtered off, washed several times with water and methanol 50:50 (V:V) and dried under reduced pressure for several hours.

Yield: 91%, **Molar mass**: 549.55 g/mol. **Anal. Calcd** for $PC_{30}H_{30}F_2N_3O_3$: C, 65.57; H, 5.50; N, 7.65%. **Found:** C, 65.54; H, 5.54; N, 7.62%.

NMR ($CDCl_3$, 298 K): $^{31}P\{^1H\}$: −28.82 s ^1H: H^{Ph}: 7.60–7.03 (12*H*); H^1: 2.80 s* (2H); H^2, H^3, H^5, H^6: 3.45–2.95; H^7: 0.97 d (J = 5.72) (3H); H^{13}: 7.84 d (J = 11.4) (1H); H^{19}: 8.52 s (1H); H^{21}: 4.39 d (J = 3.64) (2H); H^{22}: 1.48 t (J = 3.67) (3H); H^{23}: 14.65 s* (1H); $^{13}C\{^1H\}$: $C^{Ph(i)}$: 138.38 d (J = 13.7); $C^{Ph(o)}$: 137.93 d (J = 12.3); $C^{Ph(m)}$: 132.89 d (J = 8.04); $C^{Ph(p)}$: 133.19 s; C^1: 51.14 s*; C^2: 54.49 d (J = 16.6); C^3: 56.01 d (J = 8.51); C^5,C^6: 57.00–58.50; C^7: 15.00 s; C^{11}: 134.53 dd (J = 13.6, J = 1.82); C^{12}: 145.64 dd (J = 248.2, J = 6.2); C^{13}: 107.94 s; C^{14}: 108.32 s; C^{15}: 127.18 d (J = 8.17); C^{16}: 154.97 dd (J = 252.0, J = 6.62); C^{17}: 176.22 s; C^{18}: 106.87 s; C^{19}: 149.92 s; C^{21}: 53.15 s; C^{22}: 16.31 s; C^{23}: 166.69 s.

2.3.2. Preparation of $Ph_2P(O)CH_2Lm$ (OPLm)

The oxide derivative of phosphine was prepared in the reaction of PLm (0.8316 g; 1.47 mmol) with equimolar amount of H_2O_2 (35% solution in water) in chloroform (20 mL). After 1 h of stirring at room temperature the solution was evaporated to dryness and light yellow solid was obtained. Recrystallization from methanol and acetonitrile (1:1; V:V) gave a white powder.

Yield: 84%, **Molar mass**: 565.55 g/mol **Anal. Calcd** for $PC_{30}H_{30}F_2N_3O_4$: C, 63.71; H, 5.35; N, 7.43%. **Found**: C, 63.70; H, 5.37; N, 7.41%.

NMR ($CDCl_3$, 298 K): $^{31}P\{^1H\}$: 28.15 s ^1H: H^{Ph}: 7.78–7.16 (12H); H^1: 2.66 d (J = 3.45) (2H); H^2,H^3,H^5,H^6: 3.35–2.70 (7H); H^7: 0.95 s* (3H); H^{13}: 7.78 d (J = 7.06) (1H); H^{19}: 8.51 s (1H); H^{21}: 4.38 d (J = 4.01) (2H); H^{22}: 1.46 s* (3H); H^{23}: 14.64 s* (1H); $^{13}C\{^1H\}$: $C^{Ph(i)}$: 131.87 d (J = 99.0); $C^{Ph(o)}$: 131.89 d (J = 4.54); $C^{Ph(m)}$: 131.03 d (J = 9.08); $C^{Ph(p)}$: 131.33 d (J = 6.08); C^1: 50.94 s*; C^2: 52.70 d (J = 6.63); C^3: 57.52 d (J = 8.16); C^5,C^6: 56.02–57.52; C^7:14.17 s; C^{11}: 134.35 d (J = 4.54); C^{12}: 145.70 dd (J = 258.80, J = 3.63); C^{13}: 107.09 s; C^{14}: 108.24 s; C^{15}: 127.06 d (J = 8.16); C^{16}: 154.97 dd (J = 259.7, J = 4.54); C^{17}: 176.16 s; C^{18}: 108.06 s; C^{19}: 149.89 s; C^{21}: 53.50 s;C^{22} = 16.26 s; C^{23}: 166.57 s.

2.3.3. Preparation of [CuI(dmp)PPh_2CH_2Lm], [CuNCS(dmp)PPh_2CH_2Lm], [CuI(bq)PPh_2CH_2Lm] and [CuNCS(bq)PPh_2CH_2Lm] - general method

Phosphine (PLm 0.300–0.350 g), diimine (dmp or bq) and copper (pseudo)halide (CuI or CuNCS) in equimolar ratios were dissolved in 20 ml of deaerated CH_3CN:$CHCl_3$ (4:1 V:V). After a short time of stirring, cloudy solutions formed, that became clear with time. They were stirred in the dark and the solid complexes precipitated out after 7 h. They are well soluble in DMSO, $CHCl_3$, CH_2Cl_2 moderately in and CH_3CN, slightly in methanol and ethanol, insoluble in water but soluble in water with 2% DMSO.

2.3.3.1. Characterization of [CuI(dmp)PPh_2CH_2Lm] (1-PLm). Dark yellow solid. **Yield**: 61%, **Molar mass** = 948.26 g/mol. **Anal. Calcd.** for $PCuIC_{44}H_{42}F_2N_5O_3$: C, 55.71; H, 4.46; N, 7.39%. **Found**: C, 55.70; H, 4.48; N, 7.38%.

NMR ($CDCl_3$, 298 K): $^{31}P\{^1H\}$: −21.22; ^1H: H^{Ph}: 7.45–7.18 (12H); H^1: 2.47 s* (2H); H^2,H^3,H^5,H^6: 3.71–2.72 (7H); H^7: 0.86 d (J = 5.05) (3H); H^{13}: 7.91 d (J = 11.78) (1H); H^{19}: 8.59 s (1H); H^{21}: 4.44 dd (J = 6.94, J = 3.16) (2H); H^{22}: 1.56 t (J = 5.89) (3H); H^{23}: 14.70 s* (1H); $_{dmp}H^{3,8}$: 7.53 d (J = 8.41) (2H); $_{dmp}H^{4,7}$: 7.99 d (J = 8.20) (2H); $_{dmp}H^{5,6}$: 7.77 s (2H); $_{dmp}H^{15,16}$: 2.90 s (6H); $^{13}C\{^1H\}$: $C^{Ph(i)}$: not observed; $C^{Ph(o)}$: 133.38 d (J = 9.46); $C^{Ph(m)}$: 128.39 d (J = 3.78); $C^{Ph(p)}$: 129.69 s; C^1: 50.88 s*; C^2: 54.55 d (J = 17.87); C^3: 55.53 d (J = 7.57); C^5,C^6: 55.85–57.04; C^7: 13.59 s; C^{13}: 108.12 s; C^{14}: 108.49 s; C^{15}: 127.09 s*; C^{16}: 153.24 dd (J = 222.7, J = 4.25); C^{17}: 176.33 s; C^{18}: