Inorganica Chimica Acta 423 (2014) 52–59

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Novel levofloxacin zinc (II) complexes with N-donor heterocyclic ligands, as potential fluorescent probes for cell imaging: Synthesis, structural characterization and *in vitro* cytotoxicity

Anastasia Galani [a], Eleni K. Efthimiadou [b,*], Theodosis Theodosiou [c], George Kordas [d], Alexandra Karaliota [a]

[a] Department of Inorganic Chemistry, Faculty of Chemistry, National University of Athens, Panepistimioupoli Zographou, GR-15701 Athens, Greece
[b] Sol-Gel Laboratory, Institute for Advanced Materials, Physicochemical Processes, Nanotechnology & Microsystems, NCSR "Demokritos", 15310 Aghia Paraskevi Attikis, Greece
[c] Institute for Advanced Materials, Physical Chemistry Sector, Athens, Greece
[d] Institute of Advanced Materials, Physicochemical Processes, Nanotechnology and Microsystems, Department of Materials Science, Athens, Greece

ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 21 September 2014
Accepted 23 September 2014
Available online 12 October 2014

Keywords:
Zinc complexes
Heteroligands
MTT assay
Fluorescent microscopy

ABSTRACT

This paper deals with the synthesis, characterization and biological evaluation of mixed ligand zinc complexes with the third generation quinolones' representative, levofloxacin. Two novel zinc (II) complexes of fluoroquinolone drug levofloxacin (H-levo), containing 1,10-phenanthroline (phen) (complex **1**) and 2,2′-bipyridine (bipy) (complex **2**) were synthesized and evaluated as antimicrobial agents, antitumor antibiotics and fluorescent probes. The highlight of this work is the ability of the aforementioned complexes to penetrate the cell membrane inducing fluorescence. The complexes structural characterization was performed by means of elemental and thermogravimetric analysis, FT-IR, RAMAN, ^{1}H NMR, ^{13}C NMR, and UV–Vis. The complexation of zinc (II) metal ion with the deprotonated ligand levofloxacin and heteroligands reveals that levofloxacin coordinated to zinc through one pyridone and one carboxylato oxygen as well as with two nitrogen atoms from the heteroligands. Additionally, complexes have been tested for their antimicrobial activity, revealing an increased potency in comparison with the free H-levo ligand. The cytotoxic behavior of the synthesized mixed complexes in comparison with the free ligands was performed by MTT assay. It was found that the proliferation rate and viability of MCF-7 cells decreased after treatment with the above complexes. Zinc levofloxacin mixed complex with 1,10-phenanthroline (complex **1**) presents the highest effect in comparison with zinc levofloxacin complex with 2,2′-bipyridine (complex **2**). Furthermore, it was investigated the ability of mixed zinc complexes to penetrate the cell membrane inducing fluorescence. The results show that both complexes penetrate the cell membrane acting both, as fluorescent probes and as new cytotoxic drugs.

1. Introduction

Levofloxacin (H-levo), the active isomer of ofloxacin, is a third generation quinolone antimicrobial agent with a broad spectrum antibacterial activity similar to that of earlier quinolones, however, it has enhanced activity against Gram-positive and atypical organisms [1]. Levofloxacin is indicative for many infections such as those of the sinuses, lungs, ears, skin, bones and many others caused by susceptible bacteria, urinary infections, prostatitis, misstates and infectious diarrhoea caused by *Escherichia coli*, *Campylobacter jejuni* and *Shigella* bacteria [2]. The antibacterial activity of fluoroquinolones such as levofloxacin is depended on their bicyclic heteroaromatic pharmacophore as well as to the nature of the peripheral substituents. More specifically, fluoroquinolones provide additional affinity for bacterial enzymes; enhanced cell penetration, whereas they alter the relative pharmacokinetics. In addition, fluoroquinolone metal complexes have been thoroughly studied not only for their antibacterial activity against diverse micro organisms but also for their interaction with DNA, exemplifying in that way the importance of the metal ions in the mechanism of actions of these drugs [3].

Today, little articles have been reported on the coordination properties of levofloxacin with the zinc metal ion. Huber et al. refer a single zinc complex of levofloxacin and its antimicrobial properties [4] whereas Tarushi et al. presents a full study of an also single zinc complex with levofloxacin with distorted octahedral geometry and its biological properties [2]. This prompted us to

* Corresponding author at: Institute of Material Science, NCSR "Demokritos", 15310 Aghia Paraskevi Attikis, Greece. Tel.: +30 210 6503633; fax: +30 210 6547690.
E-mail addresses: elefth@chem.demokritos.gr (E.K. Efthimiadou), alkaraliota@yahoo.gr, akaraliota@chem.uoa.gr (A. Karaliota).

http://dx.doi.org/10.1016/j.ica.2014.09.034
0020-1693/

investigate both, chemistry and biological activity of mixed ligand zinc complexes of levofloxacin with the heteroligands 1,10-phenanthroline and 2,2′-bipyridine.

1,10-Phenanthroline is an attractive ligand due to its ability to act as strong binder for double-stranded DNA and facilitate the hydrogen atom abstraction from the sugar unit. For instance, metal complexes of 1,10-phenanthroline possess interesting anti-cancer properties [3,5].

It is also known that 1,10-phenanthroline complexes as well as its derivatives are of great importance as they exhibit numerous biological activities such as antitumor and antibacterial, interacting with double helix DNA. It is worth mentioning that their intercalating properties along with their function as artificial nucleases have also been reported [3].

Furthermore, many complexes with the 2,2′-bipyridine derivatives are potential antitumor agents. Recently, Gao and co-workers synthesized two binuclear cobalt (II) complexes of 2,2′-bipyridine derivatives, which exhibit cytotoxic activity against extracted HC-DNA and apoptotic effect on HeLa cells [6]. Efthimiadou et al. presents a full study of mixed ligand copper complexes of quinolones with 1,10-phenanthroline and 2,2′-bipyridine heteroligands which exert significant antibacterial and antitumor activity comparable to the free quinolone ligand [5,7,8–12].

Zinc is a trace element which is present in all biological organisms as its role is crucial in numerous cell processes. It is found in many tissues and tissue fluids whereas it is important for the activity of over 300 enzymes which is involved in metabolic pathways. In literature, zinc is referred to be essential in growth and development whereas in severe zinc deficiency, many disturbances occur such as chronic liver disease, diabetes, sickle cell disease, malabsorption syndrome, dermatitis, diarrhoea, delayed sexual and bone maturation, neurobehavioral changes, adverse pregnancy outcomes and so on [13].

Herein, we report the synthesis of two novel mixed ligand zinc complexes of levofloxacin and the heterocyclic ligands 1,10-phenantroline and 2,2′-bibyridine. The complexes structural characterization was studied through spectroscopic and analytical techniques such as: elemental analyses, thermo gravimetric, FT-IR, RAMAN, ^{1}H NMR, ^{13}C NMR, UV–Vis spectroscopy [14]. The evaluation of the complexes' antimicrobial activity against three different microorganisms *E. coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* was carried out through MIC determination and their anticancer activities against breast cancer cells was investigated by MTT assay. Calf-thymus DNA-binding properties of the complexes have been investigated using absorption titration techniques determining the binding constant [15]. According to our results, it is observed that the complexes interact with DNA possibly *via* intercalation mode. Furthermore, the fluorescent behavior and the complexes ability to penetrate the cancer cell membrane have been investigated.

2. Experimental

2.1. Reagents and materials

H-levo, $ZnCl_2$, CH_3ONa, 1,10-phenanthroline, 2,2′-bipyridine, and solvents used for complexes synthesis were of analytical grade. Zinc chloride and levofloxacin obtained from Sigma were used without further purification. Methanol obtained from Merck was used as received. Deuterated solvents for use in NMR (DMSO) were purchased from Merck.

2.2. Methods and instrumentation

IR spectra were recorded using a KBr pellet on a Perkin Elmer 880 IR spectrophotometer. RAMAN spectra were obtained with a Renishaw, inVia RAMAN Microscope. UV–Vis spectra were recorded using a Cary 3E spectrophotometer. The TGA was determined, using a Mettler Star SW9.30 differential thermal analysis apparatus operating at a heating rate of 10 °C per minute in the range 25–800 °C in N_2. DNA stock solutions were prepared by dilution of CT-DNA to buffer (containing 150 mM NaCl and 15 mM tris-sodium citrate at pH 7.0) followed by exhaustive stirring at 4 °C for three days and kept there for no longer than a week. The nucleotide concentrations were determined by their absorption at 260 nm using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$. H and N elemental analysis was performed on a Perkin–Elmer 240B elemental analyzer. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 500 MHz instrument and were processed by X-Win MR 2.6 (Bruker Analytic GmbH). Fluorescence Microscopy was determined by Leica Microsystems.

2.3. Synthesis of zinc complexes

2.3.1. Synthesis of the complex [Zn(levo)(phen)Cl]·MeOH complex (**1**)

A solution of $ZnCl_2$ (38.2 mg, 0.28 mmol) in methanol (10 ml) was added to a solution of 1,10-phenanthroline (phen) (55.0 mg, 0.28 mmol) in methanol (5 ml), followed by the addition of a previously prepared solution of levofloxacin (H-levo) (100.0 mg, 0.28 mmol) in methanol (5 ml). The pH of the levofloxacin solution was adjusted to ~8 using diluted methanolic CH_3ONa solution (0.1 M). The resulting solution was stirred for half an hour while heating, followed by concentrating it to half its volume. A fine amorphous product of yellow color was obtained, filtered off and washed with cold methanol and dried in vacuum desiccator over silica gel.Yield:65%. *Anal.* Calc. for $ZnC_{31}H_{31}FN_5O_5Cl$ (MW: 673.393) (**1**): C, 55.29; H, 4.64; N, 10.40. Found: C, 55.21; H, 4.41; N, 10.30%. IR (cm^{-1}, KBr disk) $\nu(C{=}O)_p$, 1574 (vs), $\nu(C{=}O_2)_{asym}$, 1618 (vs), $\nu(C{=}O_2)_{sym}$, 1462 (vs), $\nu(C{-}N)$, 728 (s). RAMAN (cm^{-1}): 316 m (Zn—Cl), UV–Vis in DMSO, λ_{max} (nm) 361, 333, 300, 265. As Nujol mull: 370, 305, 265, 222.

2.3.2. Synthesis of the complex [Zn(levo)(biby)Cl]·2MeOH complex (**2**)

A methanolic solution (10 ml) of $ZnCl_2$ (8.2 mg, 0.28 mmol) was added to a solution of 2,2′-bipyridine (bipy) (43.7 mg, 0.28 mmol) in methanol (5 ml), followed by the addition of a previously prepared solution of levofloxacin (levo) (100.0 mg, 0.28 mmol) in methanol (5 ml) deprotonated by using 0.1 M methanolic solution of CH_3ONa (pH 8). The resulting solution was stirred for half an hour while heating, followed by concentrating it to half of its volume. A fine amorphous product of yellow color was obtained, filtered off and washed with cold methanol and dried in vacuum desiccator over silica gel.Yield:61%. *Anal.* Calc. for $ZnC_{30}H_{35}FN_5O_6Cl$ (MW: 681.404) (**2**): C, 52.88; H, 5.17; N, 10.28. Found: C, 52.23; H, 4.50; N, 10.35%. IR (cm^{-1}, KBr disk) $\nu(C{=}O)_p$, 1585 (vs), $\nu(C{=}O_2)_{asym}$, 1617 (vs), $\nu(C{=}O_2)_{sym}$, 1468 (vs), $\nu(C{-}N)$, 774 (s). RAMAN (cm^{-1}): 320 m (Zn—Cl), UV–Vis in DMSO, λ_{max} (nm) 363, 335, 297, 253. As Nujol mull: 357, 306, 255, 222.

2.4. Biological experiments

2.4.1. Interactions with CT-DNA

The interaction of complexes **1**, **2** with calf thymus (CT)-DNA was examined using UV spectroscopy. According to literature DNA can furnish three distinctive binding sites for quinolone metal complexes: groove binding, binding to phosphate groups and intercalation. This behavior has a great impact in the biological role of quinolone in the living organism systems.

The concentration of calf-thymus (CT)-DNA was measured by using its standard extinction coefficient at 260 nm ($6600\ M^{-1}$-cm^{-1}). The purity of DNA was checked by measuring its absorbance's at 260 nm (A_{260}) and at 280 nm (A_{280}). The ratio of A_{260} to A_{280} was found to be 1.8, indicative of protein free DNA. The