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Nickel–quinolones interaction
Part 5—Biological evaluation of nickel(II) complexes with first-, second- and third-generation quinolones

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ABSTRACT

The nickel(II) complexes with the quinolone antibacterial agents oxolinic acid, flumequine, enrofloxacin and sparfloxacin in the presence of the N,N′-donor heterocyclic ligand 2,2′-bipyridylamine have been synthesized and characterized. The quinolones act as bidentate ligands coordinated to Ni(II) ion through the pyridone oxygen and a carboxylato oxygen. The crystal structure of [(2,2′-bipyridylamine)bis(sparfloxacinato)nickel (II)] has been determined by X-ray crystallography. UV study of the interaction of the complexes with calf-thymus DNA (CT DNA) has shown that they bind to CT DNA with [(2,2′-bipyridylamine)bis(flumequinato) nickel(II)] exhibiting the highest binding constant to CT DNA. The cyclic voltammograms of the complexes have shown that in the presence of CT DNA the complexes can bind to CT DNA by the intercalative binding mode which has also been verified by DNA solution viscosity measurements. Competitive study with ethidium bromide (EB) has shown that the complexes can displace the DNA-bound EB indicating that they bind to DNA in strong competition with EB. The complexes exhibit good binding propensity to human or bovine serum albumin protein having relatively high binding constant values. The biological properties of the [Ni(quinolonato)$_2$(2,2′-bipyridylamine)] complexes have been evaluated in comparison to the previously reported Ni(II) quinolone complexes [Ni(quinolonato)$_2$(H$_2$O)$_2$], [Ni(quinolonato)$_2$(2,2′-bipyridine)] and [Ni (quinolonato)$_2$(1,10-phenanthroline)]. The quinolones and their Ni(II) complexes have been tested for their antioxidant and free radical scavenging activity. They have been also tested *in vitro* for their inhibitory activity against soybean lipoxygenase.

1. Introduction

Quinolones are synthetic antibacterial agents used for the treatment of a variety of infections, including urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, prostatitis, community-acquired pneumonia, acute bronchitis and sinusitis [1,2]. Nalidixic acid was the first quinolone introduced in 1963 as a therapeutant for urinary tract infections [3]. Since then many quinolones have been synthesized showing a broad spectrum of activity and they are classified in generations based on their activity [4]. Each generation presents an enhanced spectrum of activity in comparison to a previous one. First-generation quinolones (e.g. nalidixic acid, oxolinic acid, flumequine, cinoxacin) include those that are active against Gram-negative organisms (but not *Pseudomonas* species) and they are used for the treatment of uncomplicated urinary tract infections. Second-generation quinolones including norfloxacin, enrofloxacin, ciprofloxacin, ofloxacin show an activity spectrum covering Gram-negative organisms (including *Pseudomonas* species), some gram-positive organisms (including *Staphylococcus aureus* but not *Streptococcus pneumoniae*) and some atypical pathogens and they are used for the treatment of uncomplicated and complicated urinary tract infections and pyelonephritis, sexually transmitted diseases, prostatitis, skin and soft tissue infections. Sparfloxacin, moxifloxacin and gatifloxacin are third-generation quinolones with expanded activity against gram-positive organisms (including penicillin-sensitive and penicillin-resistant *S. pneumoniae*) and atypical pathogens and are used for acute exacerbations of chronic bronchitis and community-acquired pneumonia [3,4].

Oxolinic acid (=Hoxo, (Fig. 1(A)) is a first-generation quinolone antimicrobial drug [1,4] with a pharmaceutical role known for the last four decades [5], but only four metal (a Cu(II) [6], a Ni(II) [7] and two

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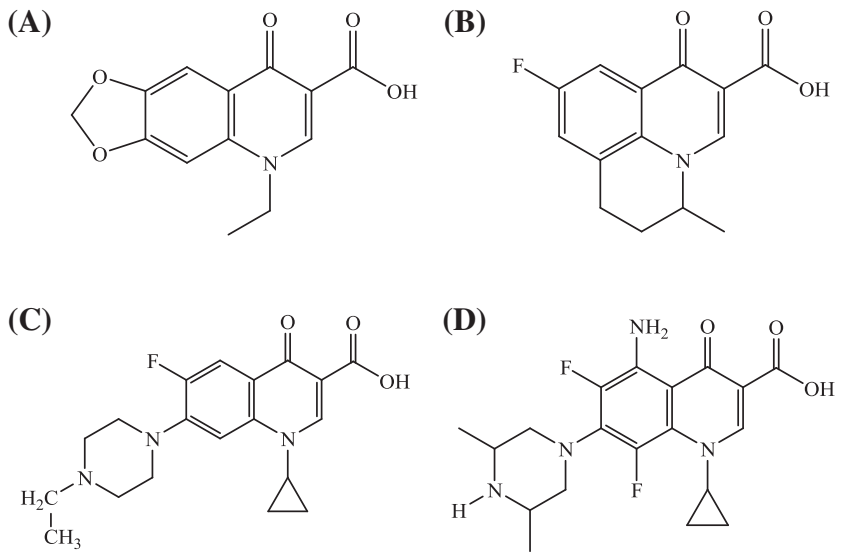

Fig. 1. The quinolone ligands (A) Oxolinic acid (=Hoxo), (B) Flumequine (=Hflmq), (C) Enrofloxacin (=Herx) and (D) Sparfloxacin, (=Hsf).

Zn(II) [8]) complexes structurally characterized, all reported by our lab.

Flumequine, Hflmq (Fig. 1(B)), is a synthetic first-generation quinolone mainly used in veterinary medicine for the treatment of animal diseases caused by a wide-range of Gram-negative bacteria for the last three decades [9–11]. Nevertheless, only three Ni(II) complexes of flumequine have been structurally characterized recently by our group [12].

Enrofloxacin (=Herx, Fig. 1(C)) is a typical second-generation quinolone antimicrobial drug and is usually used for the treatment of infectious diseases in pets and livestock [13]. A thorough survey of the literature has revealed that a Ni(II) [14], two Zn(II) [15,16] and two out of three structurally characterized Cu(II) enrofloxacin complexes [17,18] have been reported by our lab.

Sparfloxacin (=Hsf), (Fig. 1(D)) is the first marketed aminodifluoroquinolone with increased absorption and has good bioavailability and long half-life permitting once-daily dosing, which may contribute to improved adherence to therapy and cost-effectiveness [19,20]. In the literature, two Ni(II) [21,22] and one of two structurally characterized Cu^{2+}[23,24] sparfloxacin complexes have been reported by our group.

Nickel is an element of expanding biological interest [25]; not only it is present in the active centre of some enzymes but also diverse nickel complexes of biological activity have been reported in the literature. More specifically, nickel complexes have been reported to act as anticonvulsant [26], antiepileptic [27] agents or vitamins [28], while other Ni(II) complexes have shown antibacterial [29,30], antifungal [30,31], antimicrobial [32] and antiproliferative/anticancer [33–35] activities. One of the main target of metals in the cancer cells is the coordination with DNA, bound selectively to it through the oxygen of phosphates and/or to heterocyclic nitrogen atoms of DNA bases [36,37]. In this context, the interaction of Ni(II) complexes with DNA has been mainly dependent on the structure of the ligand exhibiting intercalative behavior [37,38] and/or DNA cleavage ability [39]. To the best of our knowledge, the crystal structures and biological properties of Ni(II) complexes with the quinolones oxolinic acid [7], flumequine [12], enrofloxacin [14] and sparfloxacin [21,22] have been found, all reported by our lab.

Taking into consideration the reported biological role and activity of nickel and its complexes, the significance of the quinolones in medicine and the fact that metal complexes with drugs may exhibit more pronounced biological properties in comparison to the free drugs, we have initiated the study of nickel(II) complexes with quinolone antimicrobial agents in the absence or presence of diverse N- (pyridine = py and 4-benzylpyridine = 4bzpy) and N,N′-donor (2,2′-bipyridine = bipy and 1,10-phenanthroline = phen) heterocyclic ligands [7,12,14,21,22]. In this context, we report the synthesis, the structural characterization, the electrochemical and the biological properties of the nickel(II) complexes with the quinolone antibacterial drugs sparfloxacin, oxolinic acid, flumequine and enrofloxacin in the presence of the N,N′-donor heterocyclic ligand 2,2′-bipyridylamine (= bipyam). The crystal structure of the complex $[Ni(sf)_2(bipyam)]\cdot 7H_2O$, $\mathbf{1}\cdot 7H_2O$ has been determined by X-ray crystallography. The study of the biological properties of the complexes has been focused on (i) the binding properties of the complexes with CT DNA investigated by UV spectroscopy, viscometry measurements and cyclic voltammetry (ii) competitive binding studies with ethidium bromide (EB) performed by fluorescence spectroscopy in order to investigate the existence of a potential intercalation of the complexes to CT DNA and (iii) the affinity for bovine (BSA) and human serum albumin (HSA), proteins involved in the transport of metal ions and metal–drug complexes through the blood stream, investigated by fluorescence spectroscopy. The results have been evaluated in relation to the generation of the quinolone ligand. Additionally, a comparison of the biological behavior between previously reported Ni(II)–quinolone complexes $[Ni(quinolonato)_2(H_2O)_2]$, $[Ni(quinolonato)_2(bipy)]$ and $[Ni(quinolonato)_2(phen)]$ [7,12,14,21,22] and the present compounds is being attempted.

It has been recently reported that drugs, plant extracts and metal complexes possessing antimicrobial activity have also presented antioxidant effect [40–44]. Therefore, the antioxidant capacity of the quinolones and all the nickel-quinolones complexes has been studied in an attempt to explore their potential use in medicine as analgesics and antiinflammatories, activities which are related to free radicals scavenging.

2. Experimental

2.1. Materials—instrumentation—physical measurements

Sparfloxacin, oxolinic acid, enrofloxacin, flumequine, CT DNA, BSA, HSA and EB were purchased from Sigma, NaCl, 1,1-diphenyl-2-picrylhydrazyl (DPPH), soybean lipoxygenase, linoleic acid sodium salt 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), caffeic acid and all solvents were purchased from