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Synthesis, characterization, antibacterial activity, and interaction with DNA of the vanadyl-enrofloxacin complex

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Abstract—The neutral mononuclear vanadyl complex with the quinolone antibacterial drug enrofloxacin has been prepared and characterized with physicochemical and spectroscopic techniques and molecular mechanics calculations. The interaction of the complex with calf-thymus DNA has also been investigated and the antimicrobial activity has been evaluated against three different microorganisms.

Quinolones, a term commonly used for the quinolone-carboxylic acids or 4-quinolones, are a group of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton.[1] Diverse modifications of the skeleton based on structure–activity relationships (SARs) were made in order to isolate structurally related highly potent broad-spectrum antibacterial agents.[2] In this context, the introduction of a fluorine atom at position 6 and a piperazine ring at position 7 has led to a great enhancement of the activity spectrum. Fluoroquinolones are extremely useful for the treatment of urinary tract infections, soft tissue infections, respiratory infections, typhoid fever, sexually transmitted diseases, bone-joint infections, prostatitis, community-acquired pneumonia, acute bronchitis, and sinusitis.[2,3] The activity of quinolones as antibacterial drugs is mainly due to the effective inhibition of DNA replication.[1]

Enrofloxacin, Herx (Fig. 1), is a typical second-generation antimicrobial drug with a broad spectrum of activity against a wide range of Gram-negative and Gram-positive bacteria, including those resistant to β-lactam antibiotics and sulfonamides.[4,5] Enrofloxacin is the first fluoroquinolone developed for veterinary application and is potentially available for the treatment of some urinary tract, respiratory tract, and skin infectious diseases in pets and livestock.[5–8] It is also used for the treatment of uncomplicated and complicated urinary tract infections, pyelonephritis, sexually transmitted diseases, prostatitis, skin and tissue infections, and urethral and cervical gonococcal infections.[4,9,10]

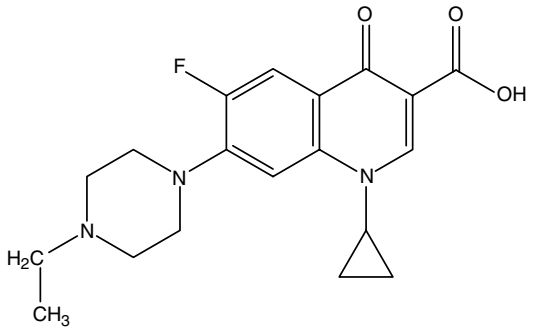

Figure 1. Enrofloxacin (Herx = 1-cyclopropyl-7-(4-ethyl-piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid).

Vanadium is a trace bioelement with interesting biological properties.[11,12] Vanadium is essential in chlorophyll synthesis[13] and it is present at the active site of several enzymes.[14,15] Several functions of insulin have been shown to be mimicked by simple vanadium salts as well as by oxovanadium(IV) species coordinated to organic ligands.[16]

Keywords: Enrofloxacin; Quinolones; Vanadyl complex; Biological activity; Interaction with calf-thymus DNA; MIC.
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The study of the interaction of the quinolone enrofloxacin with diverse metal ions has been initiated[17] in an attempt to examine the mode of binding and possible synergetic effects. In this paper, we report the synthesis and the characterization of the mononuclear VO^{2+} complex with the quinolone Herx, (aqua)bis(enrofloxacinato)oxovanadium(IV), **1**. Molecular modeling techniques have been employed to assess the lowest energy model structure of $VO(erx)_2(H_2O)$. The interaction of the complex with calf-thymus (CT) DNA has been also investigated with UV and circular dichroism (CD) spectroscopy and the antimicrobial efficiency of the complex has been evaluated by determining the minimum inhibitory concentration (MIC) against three different microorganisms.

A methanolic solution (10 mL) of enrofloxacin (0.6 mmol, 216 mg), deprotonated with KOH (0.6 mmol, 34 mg), was added dropwise to a methanolic solution (10 mL) of $VOSO_4{\cdot}5H_2O$ (0.3 mmol, 76 mg). The resulting reaction mixture was refluxed for 2.5 h. The solution was filtered and left for slow evaporation. After a few days, a light green microcrystalline product was deposited, collected with filtration, washed with methanol, and dried. Yield: 170 mg, 70%. Anal. Calcd for $VO(erx)_2(H_2O)$ ($C_{38}H_{44}N_6O_8F_2V$) (M_W = 801.73): C, 56.93; H, 5.53; N 10.48; found: C, 56.85; H, 5.42; N, 10.70. The complex is soluble in DMSO, DMF, and H_2O, and is non-electrolyte.

In the IR spectrum of complex (**1**) the band at 1733 cm^{-1} in the spectrum of Herx attributed to the absorption of the $\nu(C{=}O)_{carb}$ has disappeared. Instead, two very strong characteristic bands are present at 1602 cm^{-1} and 1395 cm^{-1} that could be assigned as ν(O–C–O) asymmetric and symmetric stretching vibrations, respectively, whereas $\nu(C{=}O)_p$ is slightly shifted from 1622 to 1629 cm^{-1} upon bonding (Table 1). The difference $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$, a useful characteristic for determining the coordination mode of carboxylate ligands, is 207 cm^{-1} indicating a monodentate coordination mode of the carboxylato group.[18] These changes of the IR spectra suggest that the enrofloxacinato ligand is coordinated to the metal via the O_{pyr} ($O_{pyridone}$) and one O_{carb} ($O_{carboxylate}$) oxygen atoms.[17]

The band at 3407 cm^{-1} in the IR spectrum of the complex can be attributed to the ν(O–H) vibration of the coordinated water molecule.[18] The existence and the number of the $\nu(V{=}O)$ absorptions is a useful diagnostic tool for the characterization of the complex. The appearance of the V=O stretching frequency in complex **1** at 927 cm^{-1} implies that a monoanionic ligand lies in *trans*-position to the O_v ($O_{vanadyl}$).[19] This acceptance is indicative of the arrangement of one O_{carb} or O_{pyr} atom in the axial position of the octahedron around V atom and excludes the O_w (O_{water}) atom, which consequently lies in *cis*-position to the O_v.

Table 1. Characteristic absorptions (in cm^{-1}) of IR spectra

	$\nu(C{=}O)_p$	$\nu(CO_2)_{asym}$	$\nu(CO_2)_{sym}$	Δ[a]
Herx	1622	1733[b]		
$Cu(erx)_2(H_2O)$[17]	1630	1611	1381	230
$VO(erx)_2(H_2O)$	1629	1602	1395	207

[a] $\Delta = \nu(CO_2)_{asym} - \nu(CO_2)_{sym}$.
[b] As ν(COOH).

The UV–vis spectra of the complex have been recorded as nujol mull and in DMSO solution. Practically, the spectra in DMSO solution are identical with those in nujol with the exception of a few nm shifts. The UV spectra of the complex are practically identical with that of the enrofloxacinato ligand but slightly shifted, indicative of coordination through the pyridone and one carboxylate oxygen atom.[17]

In the visible spectrum of $VO(erx)_2(H_2O)$, three low-intensity bands at 787 (band I), 603 (band II), and 500 (band III) nm attributed to d–d transitions are observed. Band I at λ = 787 nm (ε = 15 M^{-1} cm^{-1}) can be assigned to a $b_2(d_{xy}) \rightarrow e^*_\pi(d_{xz}, d_{yz})$ transition of the $V^{IV}O^{2+}$ and band II at λ = 603 nm (ε = 35 M^{-1} cm^{-1}) to a $b_2(d_{xy}) \rightarrow b_1^*(d_{x^2-y^2})$ transition.[20] Band III at λ = 500 nm (ε = 45 M^{-1} cm^{-1}) is also observed and can be attributed to a $d_{xy} \rightarrow d_{z^2}$ transition although it usually lies under the much stronger ligand-to-metal charge-transfer transition at λ = 431 nm (ε = 155 M^{-1} cm^{-1}) and can be difficultly distinguished.[21] These bands are typical for distorted octahedral VO^{2+} complexes.[19,22]

Although diverse crystallization techniques were employed, we did not manage to obtain a crystal of the complex suitable for the structure determination with X-ray crystallography. In order to present a model structure for the complex, eight diastereoisomers of $VO(erx)_2(H_2O)$ complex have been constructed (Scheme S1). In all these models the water molecule (O_w) occupies a *cis*-position with respect to the V=O (O_v), in accordance with the experimental results and just as in similar vanadyl complexes.[19–24] The resulted models of all $VO(erx)_2(H_2O)$ isomers exhibit comparable average total energy with the two *transOc* enantiomers (as are arbitrarily designated in Scheme S1) being more stable by ~1 kcal mol^{-1} (Table S1). After application of the distance restraints to M–L bonds (Table S2) and their geometry correction by minimizing the energy of the complex, the structure of the predicted most stable isomer is obtained (Fig. 2). However, we can neither exclude the formation of any of the other isomers, nor unambiguously distinguish between the favorable enantiomers.

The efficiencies of the ligand and the complex have been tested[25] against two Gram(−), *E. coli* and *P. aeruginosa*, and one Gram(+), *S. aureus*, microorganisms. Both the ligand and the complex (Table 2) have inhibitory action against all microorganisms tested and the coordination of Herx (MIC = 1–8 μg mL^{-1}) with vanadyl results in a diverse biological activity (MIC = 4–8 μg mL^{-1}). The complex $VO(erx)_2(H_2O)$ exhibits equal activity to that of Herx against *S. aureus* (MIC = 8 μg mL^{-1}) but it is less active (MIC = 4 μg mL^{-1}) than Herx (MIC = 1 μg mL^{-1}) against the two Gram(−) microorganisms. The antimicrobial activity of vanadyl sulfate has

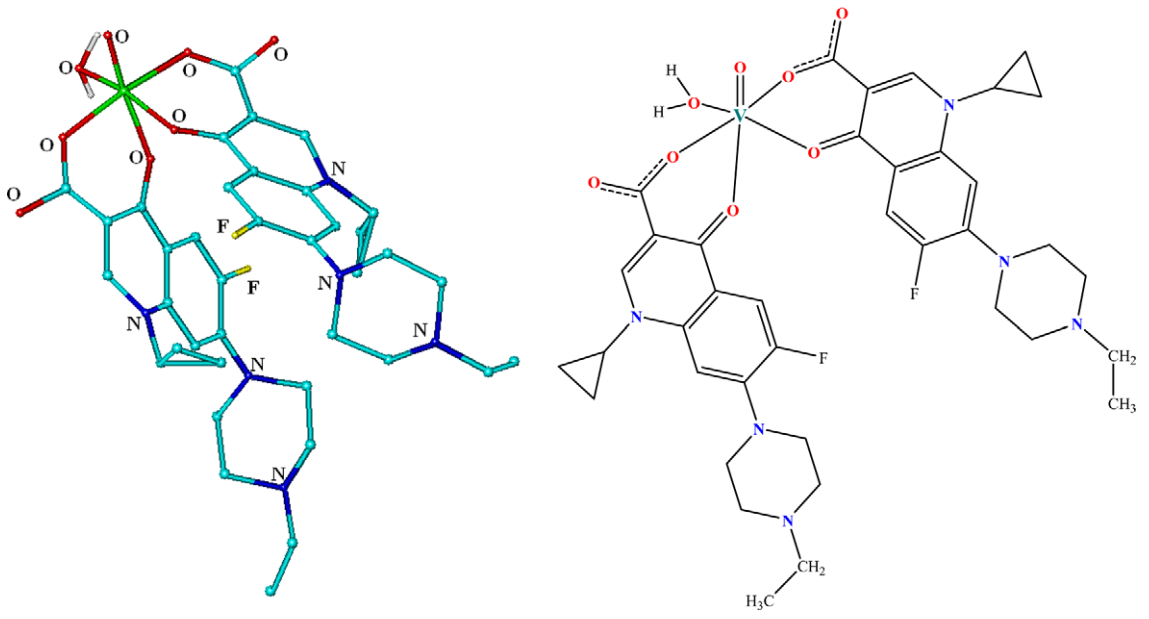

Figure 2. Lowest energy model structure for $VO(erx)_2(H_2O)$ complex.

Table 2. MIC in μg mL^{-1}

	E. coli	*P. aeruginosa*	*S. aureus*
Herx	1	1	8
$VO(erx)_2(H_2O)$	4	4	8
$Cu(erx)_2(H_2O)$[17]	0.125	0.125	4

also been investigated. It has been found that it does not exhibit antimicrobial activity at the concentration range used to assay the activity of the complex in this work.

When the antimicrobial activity of metal complexes is investigated, the following principal factors[26–28] should be considered: (i) the chelate effect of the ligands; (ii) the nature of the N-donor ligands; (iii) the total charge of the complex; (iv) the existence and the nature of the ion neutralizing the ionic complex; (v) the nuclearity of the metal center in the complex. For complex **1**, only the first of these factors is present, that is, the chelate effect of the ligand. This is probably one of the main reasons for the diverse antibacterial activity shown by the complex while the nature of the metal ion coordinated to enrofloxacinato ligand may have a significant role in this diversity. Indeed, the enrofloxacinato ligand shows better inhibition when coordinated to Cu(II) as $Cu(erx)_2(H_2O)$ (MIC = 0.125–4 μg mL^{-1})[17] than in $VO(erx)_2(H_2O)$.

DNA can provide three distinctive binding sites for quinolone–metal complexes; namely, groove binding, binding to phosphate group, and intercalation.[29] This behavior is of great importance with regard to the relevant biological role of quinolone antibiotics in the body.[30]

The absorption spectra of the interaction of complex **1** with CT DNA have been recorded for a constant DNA concentration (3.125×10^{-4} M) in different complex:CT DNA mixing ratios (*r*). The changes observed in the absorption spectra of the complex after mixing with CT DNA indicate that the interaction of the complex (**1**) with CT DNA takes place by a direct formation of a new complex with double-helical CT DNA.[31]

The CD spectra of the complex with double-stranded CT DNA for different *r* values can provide us with useful information concerning the complex–nucleotide interaction. The CD spectra of the interaction of the free ligand Herx with CT DNA do not show any transitions because there is not any asymmetry or chirality in the molecule.[32–37]

The CD spectra of CT DNA in the presence of complex **1** are shown in Figure 3. They consist of a positive band I at 274 nm and a strong negative one II at 246 nm. When *r* increases, the λ_{max} of band I is shifted from 274 nm to higher wavelengths followed by a decrease of the intensity. These changes indicate that complex **1** can be bound to CT DNA but we cannot safely suggest the exact mode of binding, although a B- → A-DNA transition attributed to complex interstrand cross-linking to the DNA pairs[36–38] cannot be ruled out.

The synthesis and characterization of the mononuclear complex of the second-generation quinolone antibacterial drug enrofloxacin with VO^{2+}, $VO(erx)_2(H_2O)$, has been realized with physicochemical and spectroscopic methods and molecular modeling calculations. Enrofloxacin is bound to the metal via the pyridone and one carboxylate oxygen atoms. Central vanadium(IV) atom is six-coordinate and the environment could be described as distorted octahedron. The antimicrobial activity of the complex has been tested on three different microorganisms. The complex shows a diverse biological activity in comparison to the free enrofloxacin. The investigation of the interaction of the complex with CT DNA has been

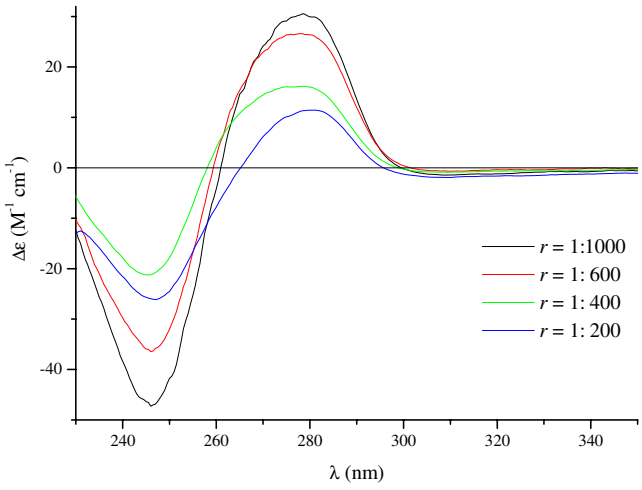

Figure 3. CD spectra of CT DNA in 5 mM buffer (containing 150 mM NaCl and 15 mM Tris–sodium-citrate at pH 7.0) in the presence of complex **1** in different *r* values. The spectra were recorded at 25 °C after samples had been incubated with CT DNA for 24 h at 37 °C.

performed with diverse spectroscopic techniques and has shown that complex **1** can be bound to CT DNA resulting probably to a B → A-DNA transition.

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Supplementary data

Experimental and materials data, antibacterial activity and MIC determination method, elemental analysis, IR and UV–vis spectral data of (**1**). Scheme S1 for the diastereoisomers of $VO(erx)_2(H_2O)$ employed in the simulated annealing calculations. Table S1 for the energy statistics from the simulated annealing results of $VO(erx)_2(H_2O)$ diastereoisomers. Table S2 for the metal–ligand distance restraints applied during the energy-minimization phase in order to adjust the geometry of $VO(erx)_2(H_2O)$ models. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.032.

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