

X-Ray crystal structures of Mg^{2+} and Ca^{2+} dimers of the antibacterial drug norfloxacin

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Received 21st August 2000, Accepted 9th October 2000

First published as an Advance Article on the web 23rd October 2000

The hydrothermal reactions of norfloxacin (H-Norf) with $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ yield two unprecedented dimers containing a direct coordinate bond between H-Norf and a metal $[\text{Mg}_2(\text{H}_2\text{O})_6(\text{H-Norf})_2]\text{Cl}_4 \cdot 4\text{H}_2\text{O}$ **1** and $[\text{Ca}_2(\text{Cl})(\text{H-Norf})_6]\text{Cl}_3 \cdot 10\text{H}_2\text{O}$ **2**.

Many organic compounds used in medicine do not have a purely organic mode of action; some are activated or bio-transformed by metal ions, others have a direct or indirect effect on metal ion metabolism.^{1,2} Norfloxacin (H-Norf, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid), a quinolone type compound, is a widely used antibacterial drug that targets the bacterial type II DNA topoisomerase (gyrase). Treatment with this drug leads to double-stranded DNA breaks and cell death.³ The cytotoxicity of the drug is achieved *via* binding strongly to the gyrase–DNA complex in the presence of Mg^{2+} .⁴ It is proposed that Mg^{2+} acts as a bridge between the phosphate groups of DNA and the carbonyl and carboxylate moieties of H-Norf,⁵ and binding of Mg^{2+} to H-Norf, which is zwitterionic at neutral pH, converts a repulsive negative charge to a positive attractive charge and promotes binding of the drug to DNA.⁶ It is also reported that quinolones interact with di- and tri-valent metal ions, and some of the metal complexes formed possess improved water solubility and antibacterial activity.⁷ Despite the important role that the divalent metal ions may play in this system, to date, to the best of our knowledge, no structural data for metal-coordinated H-Norf (Chart 1) appear to be available (however,

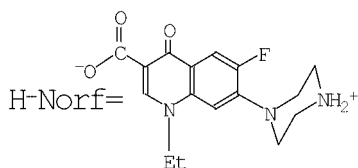


Chart 1

the metal (Cu^{2+} and Ag^{+}) complex crystal structures with related quinolones with a direct coordinate bond between quinolones and a metal ion were reported),⁸ although several binding modes of quinolones to Mg^{2+} have been proposed.^{9,10} To our surprise, the hydrothermal reactions of H-Norf with $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ yield two unprecedented dimers $[\text{Mg}_2(\text{H}_2\text{O})_6(\text{H-Norf})_2]\text{Cl}_4 \cdot 4\text{H}_2\text{O}$ **1** and $[\text{Ca}_2(\text{Cl})(\text{H-Norf})_6]\text{Cl}_3 \cdot 10\text{H}_2\text{O}$ **2**, respectively. Here we report their synthesis and solid state structures which provide a new insight into understanding the mode of action of quinolone type antibiotics and are very important not only for coordination chemists but also for biochemists.

The colorless block crystalline **1** and pale-yellow block crystalline **2** were obtained by the hydrothermal reactions of H-Norf with $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, respectively.[†] The IR

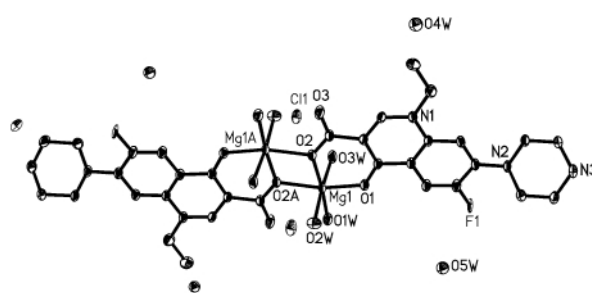


Fig. 1 An ORTEP¹³ diagram of $[\text{Mg}_2(\text{H}_2\text{O})_6(\text{H-Norf})_2]\text{Cl}_4 \cdot 4\text{H}_2\text{O}$ **1**. Selected bond lengths (Å) and angles (°): $\text{Mg}(1)–\text{O}(1)$ 1.997(2), $\text{Mg}(1)–\text{O}(2)$ 2.084(2), $\text{Mg}(1)–\text{O}(2A)$ 2.116(2), $\text{Mg}(1)–\text{O}(1W)$ 2.069(2), $\text{Mg}(1)–\text{O}(2W)$ 2.059(2), $\text{Mg}(1)–\text{O}(3W)$ 2.075(2); $\text{O}(1)–\text{Mg}(1)–\text{O}(2)$ 87.18(9), $\text{O}(1)–\text{Mg}(1)–\text{O}(2A)$ 166.91(10).

spectra of both **1** and **2** show two very strong peaks at 1620 and 1489 cm^{-1} for **1** and 1620 and 1490 for **2**, respectively, indicating that the carboxylic acid of H-Norf is deprotonated because of the absence of a medium peak at *ca.* 1700 cm^{-1} , compared to the free H-Norf. The N atom of the piperazine ring is protonated in both **1** and **2** and loses the coordination ability as confirmed by the crystal structure determination (see later).

Fig. 1 shows the crystal structure of a biologically-relevant complex formed between H-Norf and Mg^{2+} , $[\text{Mg}_2(\text{H}_2\text{O})_6(\text{H-Norf})_2]\text{Cl}_4 \cdot 4\text{H}_2\text{O}$ **1**.[‡] Complex **1** can be described as a 2:2 dimer in which the two Mg^{2+} ions are bridged by two oxygen atoms from carboxylate groups of the two drug molecules to give rise to a four-membered ring $[\text{Mg}(1)\text{O}(2)\text{Mg}(1A)\text{O}(2A)]$. Each Mg^{2+} is coordinated in an octahedral coordination environment, with the oxygen atom of the quinolone carbonyl and one of the two oxygen atoms in the carboxylate chelating to Mg^{2+} ions, resulting in the formation of a stable six-membered ring. The coordination mode of carboxylate in **1** can be considered as a monodentate bridging type.¹¹ This structural feature is unexpected and quite different from those proposed previously for Mg^{2+} –quinolone complexes.^{5b,10} It has been suggested that quinolone drugs form 2:2 dimer or even higher equimolar drug: Mg^{2+} complexes in solution,^{5b,10} however, this appears to be the first structural evidence for such complexes.

Unlike **1**, although **2**, shown in Fig. 2, is also a dimer, the bridging group is a chloride ion rather than a carboxylate oxygen atom. In addition, a higher molar ratio (3) of drug: Ca^{2+} is observed in the reaction system of H-Norf with Ca^{2+} ion. The coordination geometry around each Ca^{2+} ion can best be described as approximately pentagonal bipyramidal in which three H-Norfs act in a bidentate coordination mode through the oxygen atom of the quinolone carbonyl and one of the two oxygen atoms in the carboxylate moiety to chelate Ca^{2+} ions, resulting in the formation of a stable six-membered ring, and chloride ion completes the seven-coordination around the Ca^{2+} ion. The coordination mode of three bulky H-Norfs with Ca^{2+}

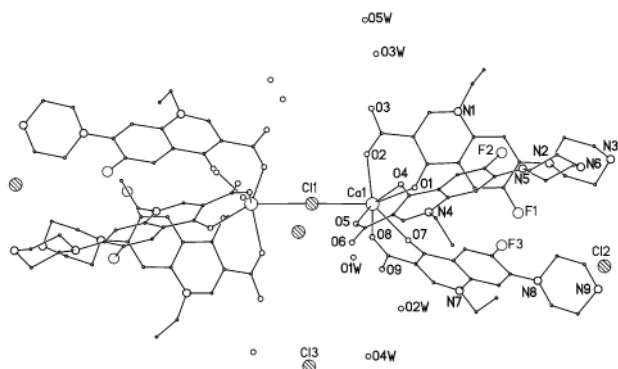


Fig. 2 A perspective view of $[\text{Ca}_2(\text{Cl})(\text{H-Norf})_6]\text{Cl}_3 \cdot 10\text{H}_2\text{O}$ **2**. Selected bond lengths (Å) and angles ($^\circ$): $\text{Ca}(1)\text{--Cl}(1)$ 2.8629(6), $\text{Ca}(1)\text{--O}(1)$ 2.413(2), $\text{Ca}(1)\text{--O}(2)$ 2.387(3), $\text{Ca}(1)\text{--O}(4)$ 2.384(2), $\text{Ca}(1)\text{--O}(5)$ 2.395(3), $\text{Ca}(1)\text{--O}(7)$ 2.410(2), $\text{Ca}(1)\text{--O}(8)$ 2.383(3); $\text{O}(1)\text{--Ca}(1)\text{--O}(2)$ 71.62(9), $\text{O}(4)\text{--Ca}(1)\text{--O}(5)$ 72.06(9), $\text{O}(7)\text{--Ca}(1)\text{--O}(8)$ 72.01(8), $\text{O}(8)\text{--Ca}(1)\text{--Cl}(1)$ 82.86(6), $\text{O}(2)\text{--Ca}(1)\text{--Cl}(1)$ 81.43(7), $\text{O}(5)\text{--Ca}(1)\text{--Cl}(1)$ 80.05(6).

can be considered as a three-fold package if the chloride ion is omitted. Thus, the shape of **2** looks like a molecular dumbbell. Compared to Mg^{2+} in **1**, the larger ionic radius of Ca^{2+} may allow the chloride ion to sit between two Ca^{2+} ions. Also, the higher coordination-number of Ca^{2+} is normal in main group metals. The bond distance of $\text{Ca}(1)\text{--Cl}(1)$ (2.8629(6)) is, as expected, slightly longer than those found in $\text{Ca}\text{--Cl}_{\text{bridging}}$ (2.711–2.750) and $\text{Ca}\text{--Cl}_{\text{monodentate}}$ (2.841–2.847 Å).¹²

In conclusion, this study provided the first direct evidence of the metal-bound antibacterial drug norfloxacin. The structural data show that H-Norf binding to divalent metal ions is mainly ion-radius-dependant. Such metal-driven structural alterations of H-Norf such as those seen in the Mg^{2+} and Ca^{2+} complexes may influence greatly drug properties such as cell membrane permeability. It remains to be seen whether the metal-modified H-Norf binds to DNA or DNA-gyrase complexes differently from the parent drug.

Acknowledgements

This work was supported by The Major State Basic Research Development Program (Grant No. G2000077500) and the National Natural Science Foundation of China.

Notes and references

† Compound **1**: Samples of 1 mmol of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and 1 mmol of H-Norf were thoroughly mixed in a mortar with a pestle, and placed in thick-walled Pyrex tubes (ca. 20 cm long). After addition of 0.5 ml of EtOH and 1.5 ml of H_2O (pH ca. 6.0), the tube was frozen with liquid N_2 , evacuated under vacuum and sealed with a torch. The tube was heated at 110 $^\circ\text{C}$ for one day to give colorless block crystals (only one phase, 0.277 g) in 55% yield based on H-Norf (Found: C, 38.12; H, 5.45; N, 8.03; Calc.: C, 38.08; H, 5.59; N, 8.33%). IR (KBr, cm^{-1}): 3251(vs, br), 1620(s), 1571(m), 1489(s), 1372(m), 1342(m), 1327(m),

1262(s), 1174(w), 1131(w), 1025(w), 927(w), 892(w), 819(m), 748(w) and 620(w).

Compound **2**: The procedures are identical to those of **1** and $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (molar ratio of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$:H-Norf is 1:3) (the mixture solution pH is also about 6.0). The pale-yellow block crystals (one phase, 0.753 g) were harvested, with a yield of 65% based on H-Norf (Found: C, 49.58; H, 5.49; N, 11.13; Calc.: C, 49.74; H, 5.57; N, 10.88%). IR (KBr, cm^{-1}): 3400(s), 2998(w), 1620(s), 1580(m), 1490(s), 1380(s), 1330(s), 1270(s), 1190(m), 1140(w), 1120(w), 1020(m), 930(m), 900(w), 820(m), 750(m), 700(w) and 630(w).

‡ Crystal data for **1**: $\text{C}_{16}\text{H}_{28}\text{Cl}_2\text{FMgN}_3\text{O}_8$, $M_r = 504.62$, triclinic, $P\bar{1}$ (No. 2), $a = 8.8109(5)$, $b = 10.8325(7)$, $c = 11.6965(7)$ Å, $\alpha = 85.8250(10)^\circ$, $\beta = 87.1270(10)^\circ$, $\gamma = 85.4620(10)^\circ$, $V = 1108.80(12)$ Å³, $Z = 2$, $\rho_{\text{calc}} = 1.511$ g cm⁻³, $\mu = 0.377$ mm⁻¹, $R1 = 0.0632$, $wR2 = 0.1653$ for 2751 observed reflections from 5207 independent reflections, GOF = 0.908.

Crystal data for **2**: $\text{C}_{96}\text{H}_{128}\text{Ca}_2\text{Cl}_4\text{F}_6\text{N}_{18}\text{O}_{28}$, $M_r = 2318.12$, monoclinic, $C2/c$, $a = 26.49430(10)$, $b = 15.35450(10)$, $c = 27.8604(2)$ Å, $\beta = 109.2440(10)^\circ$, $V = 10700.50(11)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.439$ g cm⁻³, $\mu = 0.301$ mm⁻¹, $R1 = 0.0771$, $wR2 = 0.1733$ for 6500 observed reflections from 12224 independent reflections, GOF = 0.947. CCDC reference number 186/2216. See <http://www.rsc.org/suppdata/dt/b0/b006806n/> for crystallographic files in .cif format.

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