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Conformation stability and organization of mefloquine molecules in different environments

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Abstract—The crystal structures of mefloquine base, $[C_{17}H_{16}F_6N_2O]$, and two salts of mefloquine: hydrochloride $[(C_{17}H_{17}F_6N_2O)^+]_3[Cl^-]_3 \cdot 3H_2O$ and hydrochloride tetrachlorocobaltate $[(C_{17}H_{17}F_6N_2O)^+]_3Cl^-[CoCl_4]^{2-} \cdot C_2H_6O \cdot H_2O$, were determined by X-ray diffraction measurements. A comparison of the crystal structures of mefloquine in three different crystalline environments shows that their conformations are stable regardless mefloquine being a base or a salt. In addition, the conformation of mefloquine is similar to that of crystalline *Cinchona* alkaloids. The CF_3 substituents in the quinoline moiety affect packing of molecules.
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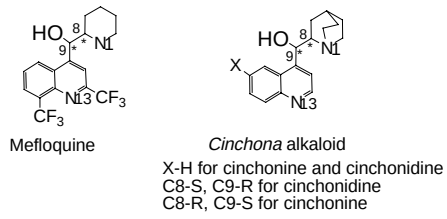
Mefloquine was effective antimalarial agent, when first introduced in 1971, and because of its long half life was a good prophylactic. In the end of 20th century a widespread resistance of *Plasmodium* sp. developed and this, together with undesirable side effects, such as neurotoxicity, have resulted in a decline in its use.[1]

This quinoline methanol derivative is related structurally to *Cinchona* alkaloids (Scheme 1). The formula of mefloquine could be derived from that of cinchonidine by exchanging the quinuclidine fragment for piperidine and introducing two trifluoromethyl substituents at positions: C14 and C21 of the quinoline part of the molecule.

Recently, it has been reported that mefloquine enantiomers, C8-R, C9-S and C8-S, C9-R, differ in the antimalarial activity. It was shown by in vitro studies that (+)-mefloquine had higher activity than (−)-mefloquine and both enantiomers are more active than the racemate.[2] These results agree well with the experiments in vitro.[3]

The generally accepted mode of action of 4-quinolinecarbinolamine antimalarial drugs is their hypothetic interaction with heme which is product of the host hemoglobin digestion by malaria parasites—*Plasmodium* sp.



Scheme 1. Structural formulae of mefloquine and *Cinchona* alkaloid.

The parasites detoxify heme, poisonous to them in its free form, by its biocrystallization leading to hemozoin, the so-called malaria pigment. In hemozoin microcrystals the heme molecules link to each other via their propionate carboxylic groups which form intermolecular bonds of the types: –Fe–O–C–O–Fe– and O–C–O–H···O–C–O.[4] The inhibition of hemozoin biocrystallization by antimalarials such as quinine and its synthetic derivative, mefloquine, may occur in two ways:[5]

- by formation of a complex with free heme after its release from digested hemoglobin, or to its derivative such as, e.g., μ-oxodimer,
- by 'capping' the growing hemozoin crystal by interaction with heme molecules on its surface.

In both cases the shape and the charge distribution of the drug molecules is essential for their efficacy. Since the structures of abovementioned heme-drug complexes or hemozoin-drug aggregates were not determined, many modelling studies can be found in the literature.[6,7]

Keywords: Mefloquine; Crystal structure; Conformation; Hydrogen bond.
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We have undertaken crystal structure studies of mefloquine in different environments in order to compare its behaviour, in point of view of conformation and intermolecular interactions, with that of *Cinchona* alkaloids. Our aim is also to confront these features with the molecular aspects of mefloquine neurotoxicity discussed recently by Dow et al.[1] Within our systematic studies, we have determined structures of mefloquine base[8] and two different salts of mefloquine.

The crystals of mefloquine base (Mefl) were kindly provided by Dr. W. Hofheinz from Pharma Preclinical Research, Infectious Diseases, F. Hoffmann-La Roche Ltd, Basel, Switzerland.

The crystalline samples of the salts were obtained as follows. Two saturated solutions of mefloquinium chloride and $CoCl_2 \cdot 6H_2O$ in 96% ethanol were prepared in room temperature. Then they were mixed (in volume ratio 1:2) and vigorously stirred. After two weeks blue crystals of the salt—trimefloquinium tetrachlorocobaltate(II) monochloride monohydrate (MeflCo) were obtained. The salt co-crystallized with a molecule of ethanol. A by-product of this crystallization was another salt, i.e., mefloquinium hydrochloride monohydrate (MeflCl).

Crystal data and details concerning the X-ray diffraction experiment, solution of the phase problem, and refinement of the single crystal structures are given in Table 1. The investigated mefloquine is a racemic mixture of the two isomers with the *S*, *R* and *R*, *S* configurations at C8 and C9 atoms, so Flack parameters for three centrosymmetric space groups are not given in Table 1.

Crystallographic data for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers (CCDC 282799 for Mefl, 282798 for MeflCl and 282800 for MeflCo). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK fax: +44(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

The asymmetric units of Mefl, MeflCl and MeflCo are depicted in Figures 1a–c, respectively.

The asymmetric unit of Mefl consists of two mefloquine molecules, linked by hydrogen bond O12–H12···N1 with formation of dimer. The asymmetric unit of MeflCl consists of three cations of mefloquine, three chloride anions and three disordered water molecules. In the asymmetric unit of MeflCo there are three ions of mefloquine, one chloride and one tetrachlorocobaltate(II) anions. These ions co-crystallized with one water molecule and one ethanol molecule. Each mefloquinium molecule in both salts is a cation with the positive charge at the protonated nitrogen atom, N1, of the piperidine moiety.

The conformation of the piperidine ring is defined by endocyclic torsion angles. The asymmetry parameters, introduced by Duax and Norton[13] and puckering parameters according to methods described by Cremer and Pople[14] confirm almost ideal chair conformation for piperidine rings.

The quinoline fragments of each mefloquine molecule are approximately planar, with carbon C14 atoms showing the maximum deviations from planarity of the quinoline rings, in the range from 0.02 to 0.03 Å. This situation is

Table 1. Experimental data for X-ray structure analysis

Structure code	Mefl	MeflCl	MeflCo
Empirical formula	$2[C_{17}H_{16}F_6N_2O]$	$[C_{17}H_{17}F_6N_2O]_3{}^+Cl_3{}^- \cdot 3H_2O$	$[C_{17}H_{17}F_6N_2O]_3{}^+Cl^-[CoCl_4]^{2-} \cdot C_2H_6O \cdot H_2O$
M (g/mol)	756.64	1316.39	1438.24
Diffractometer	CAD-4 Enraf-Nonius[9]	Nonius KappaCCD[10]	Nonius KappaCCD[10]
Crystal system, space group	Monoclinic, P2/n	Monoclinic, C2/c	Triclinic, $P\bar{1}$
Unit cell dimensions	$a = 17.946(3)$	$a = 28.633(1)$	$a = 10.105(2)$
a, b, c (Å) α, β, γ (°)	$b = 8.851(2)$	$b = 10.882(1)$	$b = 16.118(3)$
	$c = 22.210(4)$	$c = 41.176(1)$	$c = 20.013(4)$
	$\alpha = 90$	$\alpha = 90$	$\alpha = 81.29(1)$
	$\beta = 95.88(3)$	$\beta = 110.37(1)$	$\beta = 83.41(1)$
	$\gamma = 90$	$\gamma = 90$	$\gamma = 86.20(1)$
V (Å^3)	3509.3(2)	12027.0(5)	3196.8(1)
Z, d_x (g/cm^3)	4, 1.432	8, 1.454	2, 1.494
μ (mm^{-1})	0.134	0.261	0.579
$F(000)$	1552	5408	1466
Crystal size (mm)	$0.50 \times 0.30 \times 0.20$	$0.20 \times 0.15 \times 0.10$	$0.27 \times 0.20 \times 0.10$
θ range (°)	1.39 to 22.99	2.95 to 21.93	2.35 to 27.15
Limiting indices	$19 \leqslant h \leqslant 19$, $0 \leqslant k \leqslant 9$, $0 \leqslant l \leqslant 24$	$-30 \leqslant h \leqslant 30$, $-11 \leqslant k \leqslant 11$, $-43 \leqslant l \leqslant 43$	$-12 \leqslant h \leqslant 12$, $-20 \leqslant k \leqslant 20$, $-25 \leqslant l \leqslant 25$
Reflections collected/unique	4143/4143	25424/7285	37995/14034
Phase problem solution	SHELX97[11]	SIR92[12]	SIR 92[12]
Refinement method	Anisotropic refinement for non-hydrogen atoms on F^2		
Goodness of fit on F^2	1.047	1.024	1.016
R indices [$I > 2\sigma(I)$]	$R1 = 0.0588$, $wR2 = 0.1299$	$R1 = 0.0432$, $wR2 = 0.0998$	$R1 = 0.0592$, $wR2 = 0.1266$
R indices (all data)	$R1 = 0.0891$, $wR2 = 0.1427$	$R1 = 0.0773$, $wR2 = 0.1145$	$R1 = 0.1016$, $wR2 = 0.1479$

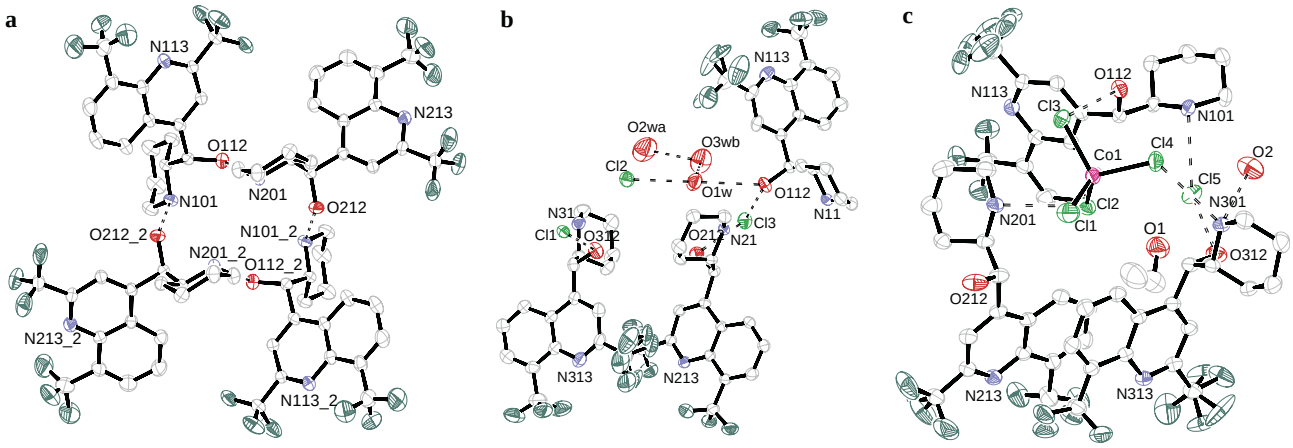

Figure 1. Projection of: (a) tetramer of MefI, (b) triplet of MefICl and (c) triplet of MefICo. In (b and c) also accompanying anions and solvent molecules are shown. Hydrogen bonds are indicated as dashed lines.

a result of binding trifluoromethyl groups to C14 atoms. These groups do not influence C21 atoms, probably because of rigidity of this fragment of quinoline. The values of angles C14–N13–C22 are comparable to those of *Cinchona* alkaloids which do not contain trifluoromethyl groups and are comprised in the range of 116.3(3)°–117.2(4)°. The trifluoromethyl groups do not affect the values of angles at carbon atoms, to which they are linked.

The values of torsion angles, which determine the overall conformation of mefloquine molecules, are listed in Table 2. The absolute magnitudes of these angles are similar to the relevant torsion angles, characteristic for *Cinchona* alkaloids. Since the structures of MefI, MefICl and MefICo are racemic mixtures, their molecules differ from each other in signs of torsion angles. However, from each pair of enantiomers one molecule can be selected, whose absolute configuration at C8 and C9 atoms is compatible with the configuration of cinchonine or cinchonidine. In the asymmetric unit of MefI, one molecule of mefloquine has the signs of the torsion angles, τ_1 = O12–C9–C16–C15 and τ_2 = N1–C8–C9–O12, compatible with those of cinchonine and the other with those of cinchonidine. The asymmetric unit of MefICl was chosen in such a way that one molecule has the signs of the torsion angles, τ_1 and τ_2, the same as those for cinchonine, while the other two have signs corresponding to cinchonidine. In the case of MefICo structure each mefloquine molecule in asymmetric unit has configuration of cinchonine. The conformations of molecules in the investigated structures are similar to those described by Karle and Karle[15] for mefloquinium chloride hydrate and for mefloquinium methylsulfonate hydrate.[16] On the basis of three torsion angles: C17–C16–C9–C8, C16–C9–C8–N1 and H12–O12–C9–C8 each molecule from three structures was qualified to conformation class according to Agranat method.[17] Interestingly, the conformations of all of mefloquine molecules were classified to *anti-open-γ* class, which was observed for most of the *erythro Cinchona* alkaloids in the crystalline state. The exception is only one molecule belonging to MefICl, with one torsion angle noticeably different and having *anti-open*-β conformation. The AM1 calculations performed by Menezes and co-authors[7] showed that the low-energy conformation of the mefloquine base corresponds to the torsion angles N1–C8–C9–O12 = −56.6° and C16–C9–C8–N1 = 179.5°. These values are in good agreement with those found in the crystalline state. Thus, a hypothesis can be put forward that similar conformation may also occur in solutions.

Table 2. Selected torsion angles describing mefloquine molecules in structures of MefI, MefICl and MefICo

Torsion angle (°)	MefI	MefICl	MefICo
τ_1 = O12–C9–C16–C15	13.9(5)	25.5(5)	13.6(4)
	−22.2(5)	−9.7(5)	23.1(5)
		−14.1(5)	16.9(5)
τ_2 = N1–C8–C9–O12	66.6(4)	72.8(4)	59.5(3)
	−61.3(4)	−63.8(4)	75.2(4)
		−55.2(4)	69.6(3)
C17–C16–C9–C8	71.8(5)	81.6(4)	75.8(4)
	−79.6(5)	−69.3(4)	79.1(4)
		−73.8(5)	76.8(4)
C16–C9–C8–N1	−169.8(3)	−164.5(3)	−179.1(3)
	175.8(3)	174.4(3)	−165.6(3)
		−177.9(3)	−168.9(3)
H12–O12–C9–C8	−155.21	139	−159
	142.13	122	−68
		148	−146

The main role in packing of mefloquine molecules in the three structures studied is played by hydrogen bonds and for MefICl and MefICo structures also—by hydrophobic contacts.

In MefI structure, molecules of mefloquine base linked to each other by hydrogen bonding system form 'tetramers' (fourfold rings), which consist of two dimers related by the twofold crystallographic axis, as shown in Figure 1a. In the unit cell of this structure there are two such tetramers.

In the salts, MefICl and MefICo, mefloquine cations are arranged in triplets, where hydrophilic sides of the cations are oriented towards solvent molecules or towards anions: chlorides and tetrachlorocobaltates (Figs. 1b and c).

In MeflCl structure, chloride anions, as proton acceptors, compose links between three mefloquine cations. In this structure, there are characteristic layers of molecules parallel to planes (1 0 0), separated from each other by 'empty' spaces, in which channels are found parallel to *y* axis. In these channels disordered water aggregates are located around inversion centres. The layers are built from two sheets. Every sheet exposes hydrophilic parts towards spaces with channels, and hydrophobic parts with trifluoromethyl groups towards the other sheet exposing the same groups. This arrangement resembles phospholipid membrane.

In MeflCo structure, the triplets of molecules are formed due to intermolecular hydrogen bonding, in which tetrachlorocobaltate anions are engaged. Each tetrahedral anion links to three mefloquine cations forming hydrophilic core around inversion centre of the unit cell. Hydrophobic fragments of the cations are directed outside the core. Trifluoromethyl groups form sort of puckered walls of the core, which coincide with the walls of unit cell: (0 1 0) and (0 0 1). In this structure there are also van der Waals interactions between fluorine atoms of one molecule and carbon atoms C5 of piperidine fragment and C17 of quinoline fragment of the another molecule.

In all the crystal structures hitherto determined mefloquine molecules, in form of triplets, tetramers or helical aggregates, expose their lipophilic surface towards the environments. This suggests that similar tendency of these molecules is retained in solution, which in turn may help them to pass the blood–brain barrier, thus inducing neurotoxicity.

In conclusion, the results of the crystal structure analysis of mefloquine base (Mefl) and two salts: hydrochloride (MeflCl) and hydrochloride tetrachlorocobaltate (MeflCo) show that, in agreement with Karle and Karle,[18] the conformation of mefloquine molecules is relatively stable. It does not markedly depend on the absolute configuration of mefloquine and its environment in the crystal. Despite of difference in the constitution, mefloquine and *erythro Cinchona* alkaloids have very similar conformation, which may explain their similar antimalarial activity.[19]

The CF_3 substituents in the quinoline fragment, due to their lipophilicity, seem to affect the mutual arrangement of the molecules in the crystalline state. This is revealed by the accumulation of the mefloquine cations around solvent molecules and around Cl^- and $[CoCl_4]^{2-}$ anions. The positively charged protonated nitrogen atoms and hydroxyl groups are directed towards the solvent molecules or anions, while the quinoline fragments containing CF_3 groups tend to interact with each other. In the case of the mefloquine base structure, though it does not co-crystallize with any solvent, the molecules also orient in a way similar to that observed in MeflCl and MeflCo. In the crystals of *Cinchona* alkaloids this tendency is not so obvious, which may be caused by lower hydrophobicity and/or by the quinuclidine fragment more bulky than piperidine.

The behaviour of mefloquine molecules in the crystalline state seems to confirm their affinity to hydrophobic environment, which may be relevant to the neurotoxicity of this drug. Also, the mutual orientation of the molecular fragments of mefloquine observed in all three structures corresponds well to that predicted for the 'neurotoxicity pharmacophore' by Dow et al.[1]

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