

Monitoring the speciation of metal complexes with chloroquine ligand by NMR spectroscopy



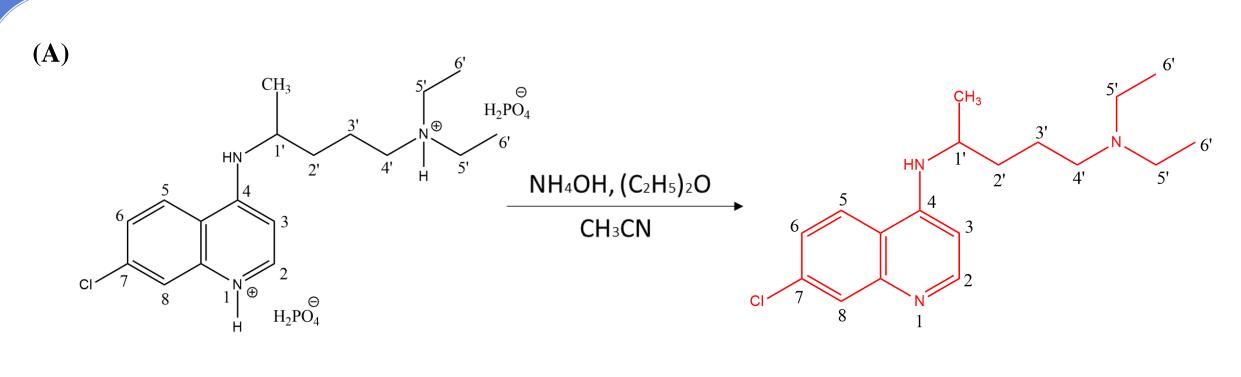
Daniele Vitone, 1 Alessandra Barbanente, 1 Fabio Arnesano. 1

¹ Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Via Orabona 4, 70125 Bari, Italy.

Introduction

Chloroquine is a 4-amino-quinoline and a first-choice drug for the treatment of malaria and autoimmune diseases with an important lysosomotropic character [1]. Endosome alkalinization is believed to be an antiviral mechanism [2]. Chloroquine is also considered a ionophore of zinc [3], able to inhibit the RNA-polymerase enzyme in vitro [4]. Based on the literature data [5], we decided to investigate the coordination chemistry of chloroquine mainly through Nuclear Magnetic Resonance (NMR) with the aim of inferring its mechanism of action in cell.

Purification of chloroquine from diphosphate salt confirmed by Nuclear Magnetic Resonance

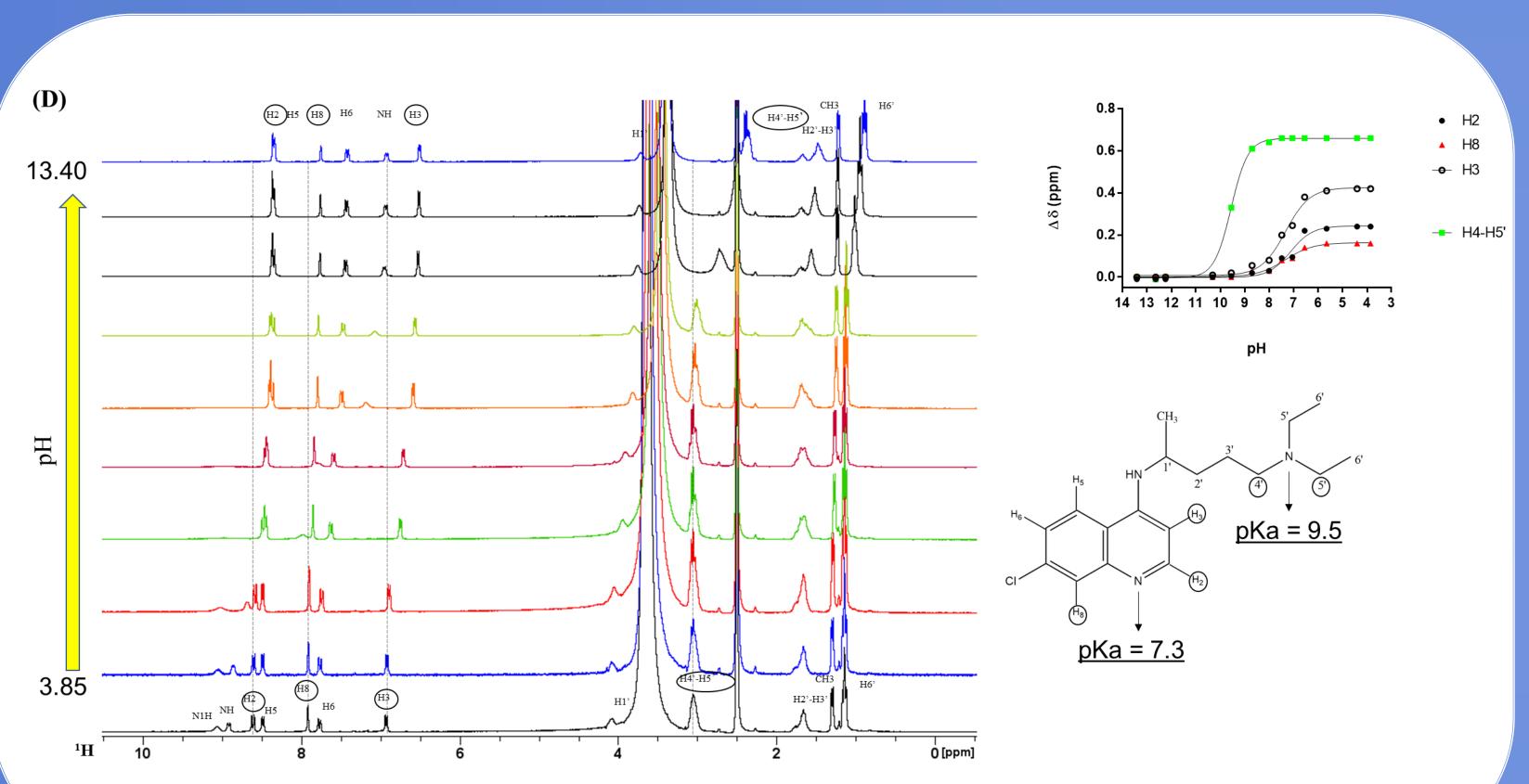


(A) Purification reaction of CQ (red) from the diphosphate salt CQDP (black)[6].



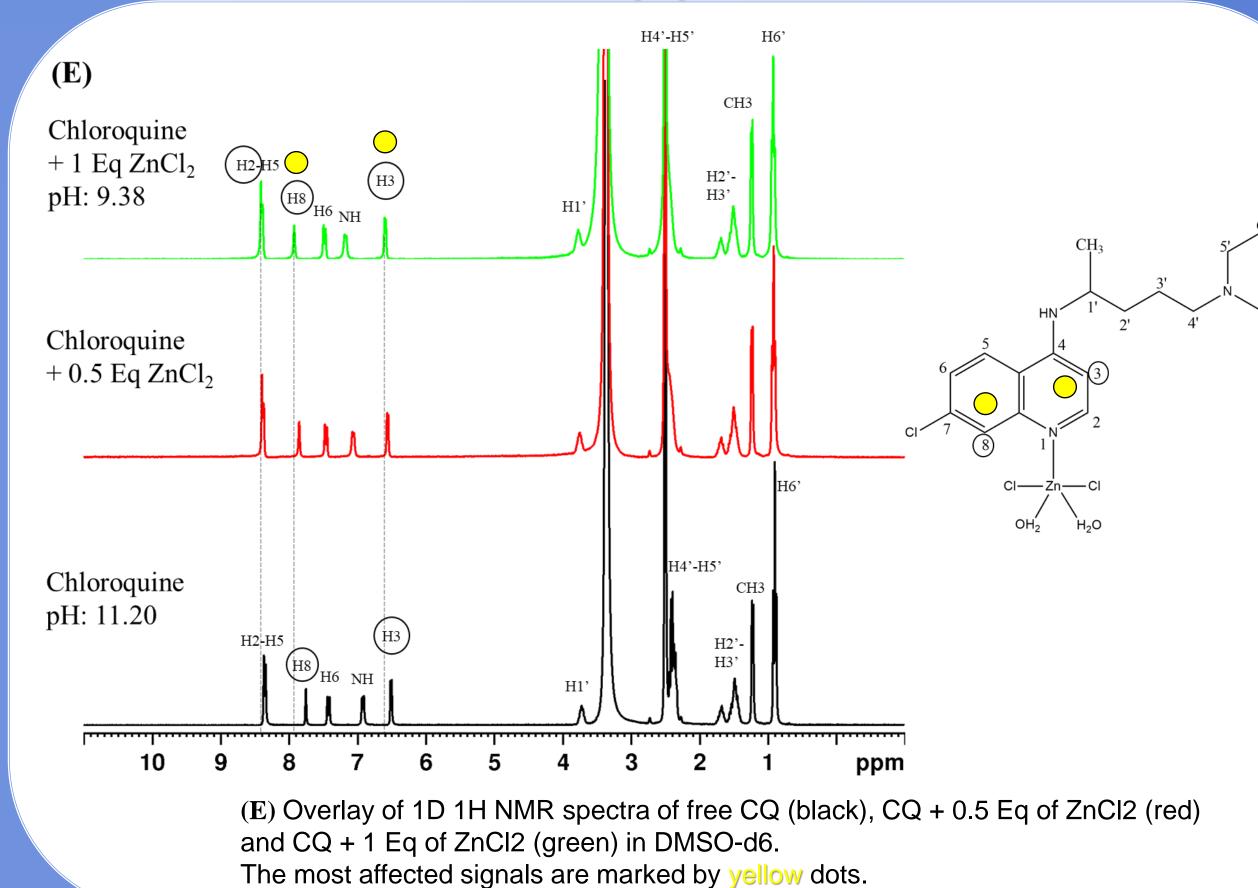
Overlay of 1D ¹H (**B**) and ³¹P (**C**) NMR spectra of CQ (red) and CQDP (black).

pKa determination of purified chloroquine

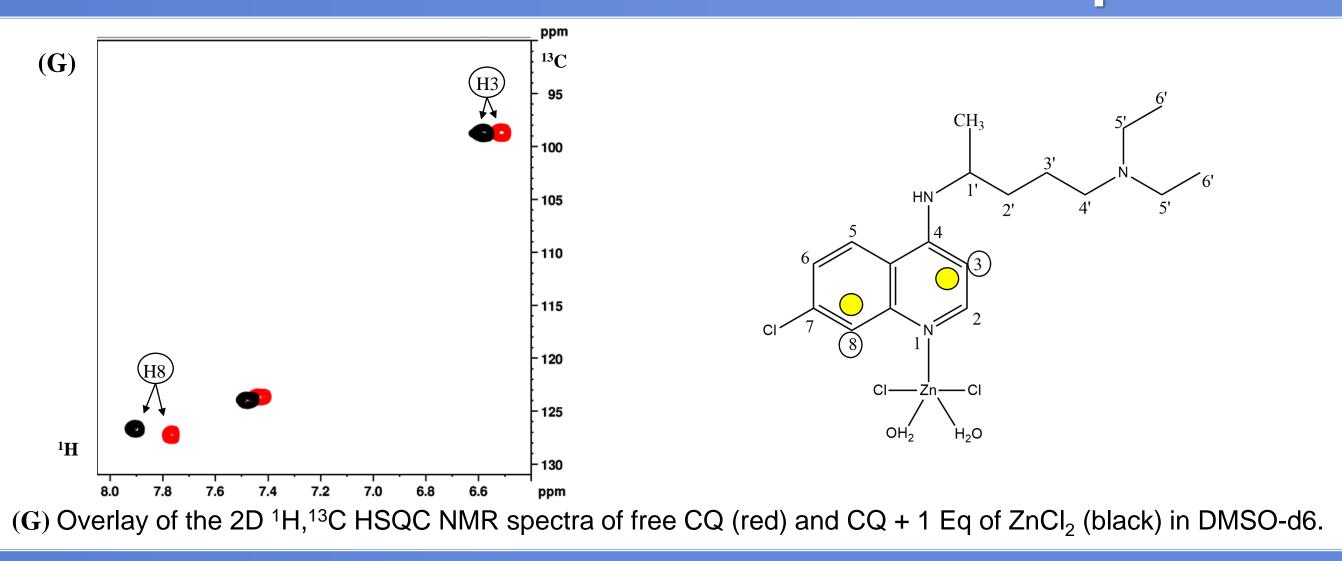


(D) pH-dependent titration of CQ in DMSO-d6.

NMR titration of Chloroquine with Zinc(II) Chloride



Characterization of Zinc-Chloroquine complex: 2D HSQC NMR and ESI-MS spectra

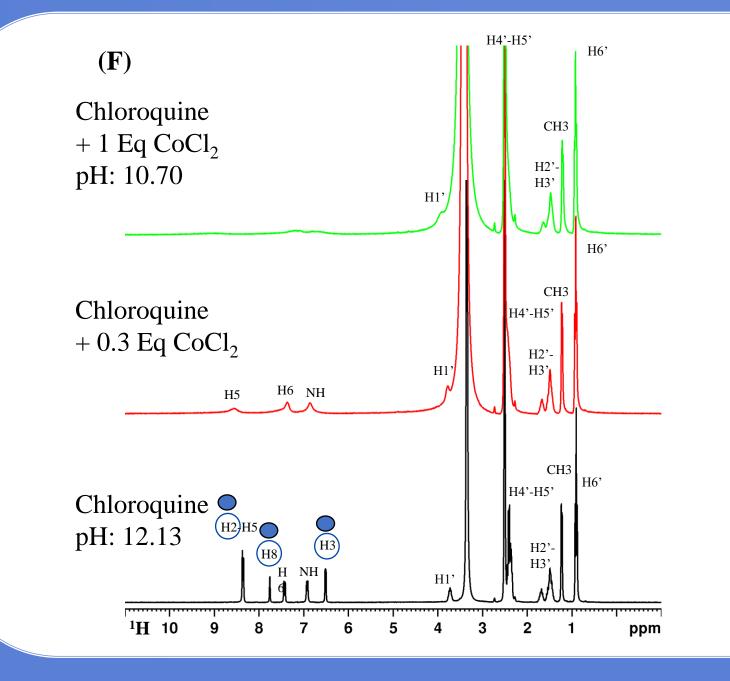


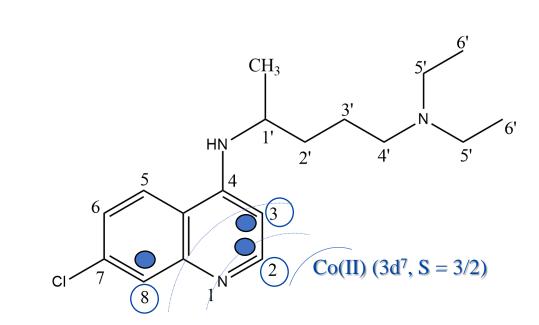
490.0328 495.0239

+ ESI-MS $C_{18}H_{30}CI_3N_3O_2Zn$

(H) Isotopic Pattern of $C_{18}H_{30}Cl_3N_3O_2Zn$ (MW: 489,069) in the Electrospray Mass Spectrum (+) of CQ + 1 Eq of ZnCl2.

NMR titration of Chloroquine with Cobalt(II) Chloride





(F) Overlay of 1D 1H NMR spectra of free CQ (black), CQ + 0.5 Eq of CoCl2 (red) and CQ + 1 Eq of CoCl2 (green) in DMSO-d6. Signals experiencing large paramagnetic broadening are marked by blue dots.

Conclusions

stoichiometry the pentacoordinate complex [Zn(CQ)(Cl)2(OH2)2] was obtained by ESI-MS. Heteronuclear NMR experiments were used to identify quinoline nitrogen as the coordination site of the Zn(II) ion and the paramagnetic Co(II) ion. Future studies will address the coordination of Zn(II) to CQ in lysosomes and the effect of pH modulation.

References

[1] Al Bari A., Pharmacol Res Perspect. (2017), doi: 10.1002/prp2.293 [2] Mercer J et al., Annu. Rev. Biochem. (2010), doi: 10.1146/annurev-biochem-060208-104626 [3] Xue J. et al., PLoS One. (2017), doi: 10.1371/journal.pone.0109180 [4] te Velthuis AJ et al., PLoS Pathog (2010), doi:10.1371/journal.ppat.1001176 [5] Navarro M. et al., J Inorg Biochem. (2005), doi: 10.1016/j.jinorgbio.2005.05.002

[6] Sánchez-Delgado R. A. et al., J. Med. Chem. (1996), doi: 10.1021/jm950729w