The speciation of zinc complexes with chloroquine ligand

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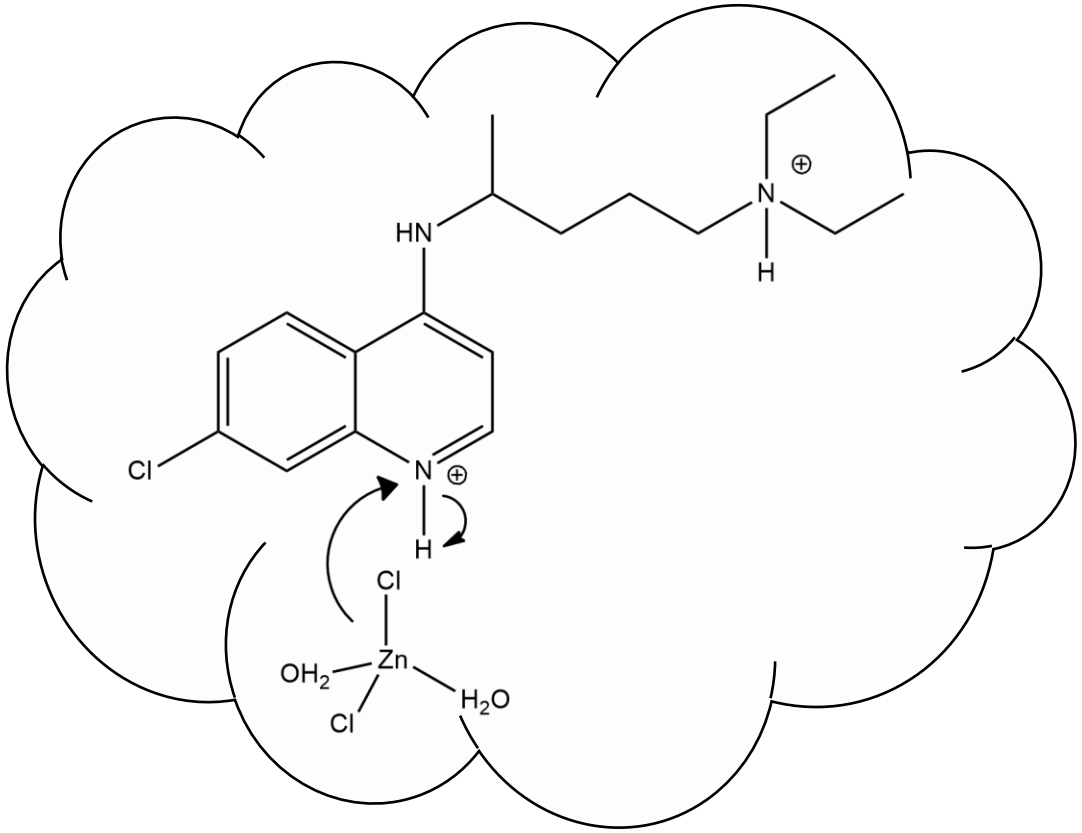
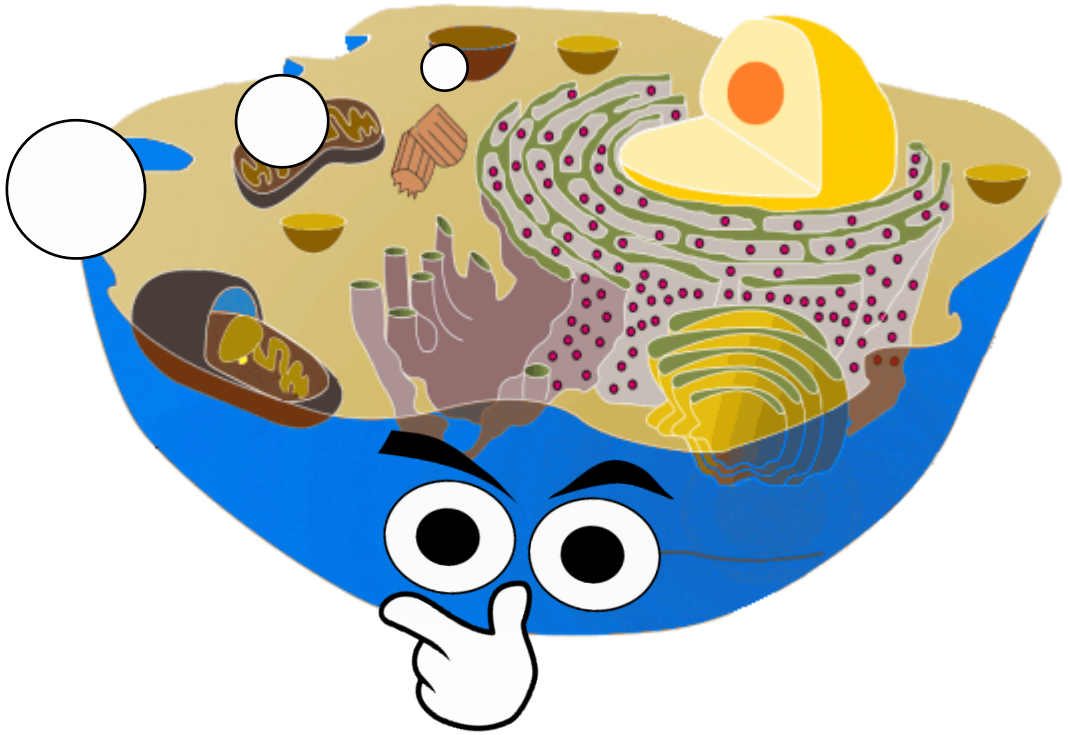


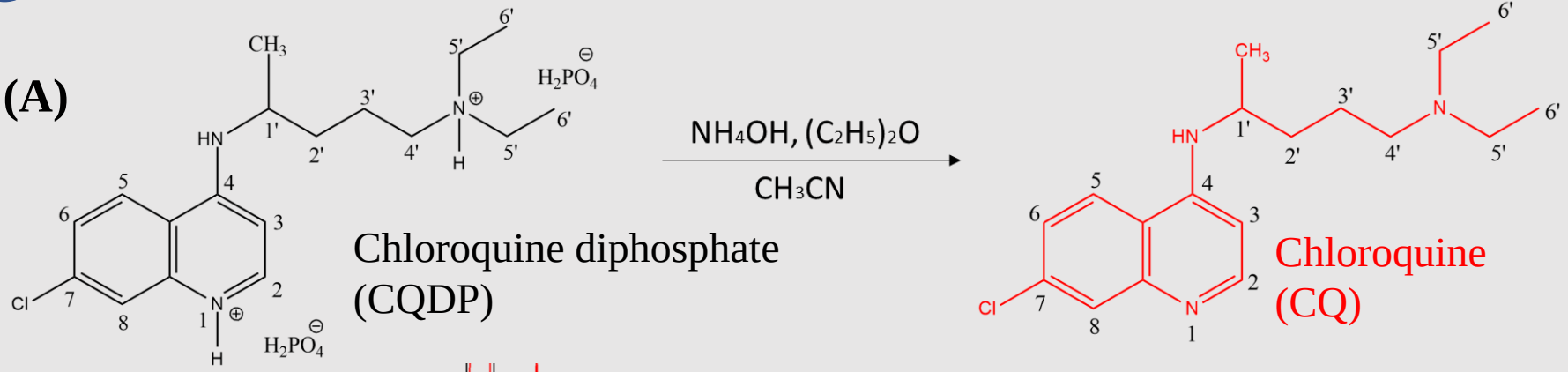


INTRODUCTION

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 with the aim of inferring its mechanism of action in cell.

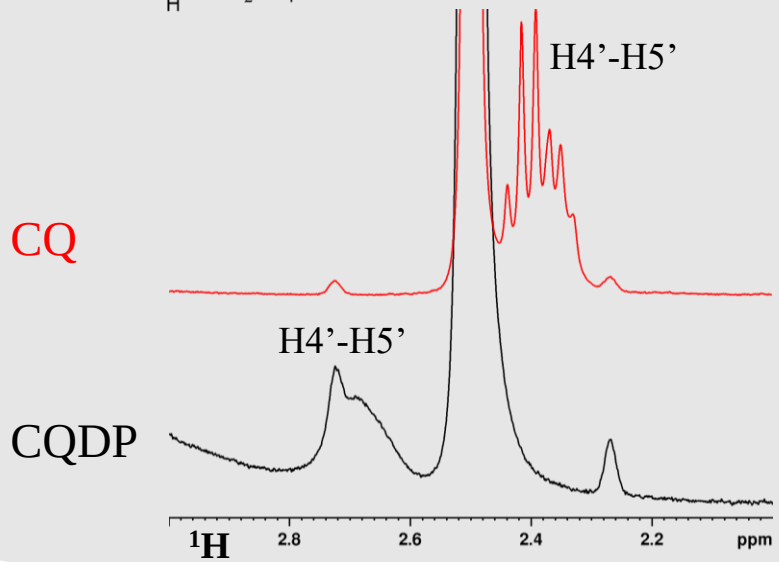
1 Purification of chloroquine from diphosphate salt confirmed by Nuclear Magnetic Resonance

(A)

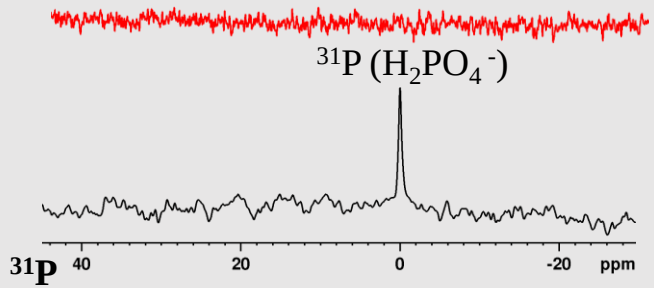


(A) Purification reaction of CQ (red) from the diphosphate salt CQDP (black)[6].

(B)



(C)



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

pH-dependent titration of purified Chloroquine: pKa determination

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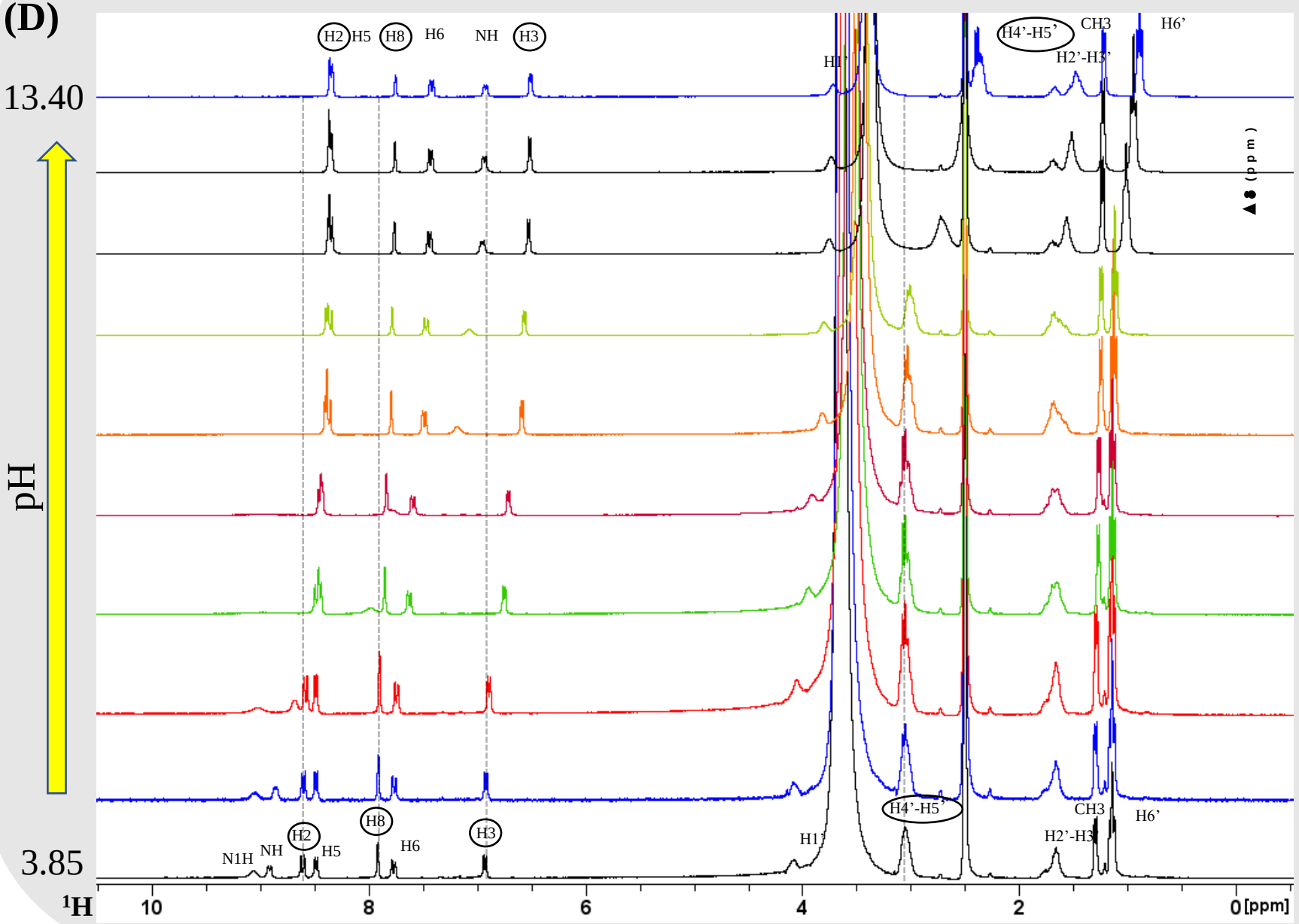
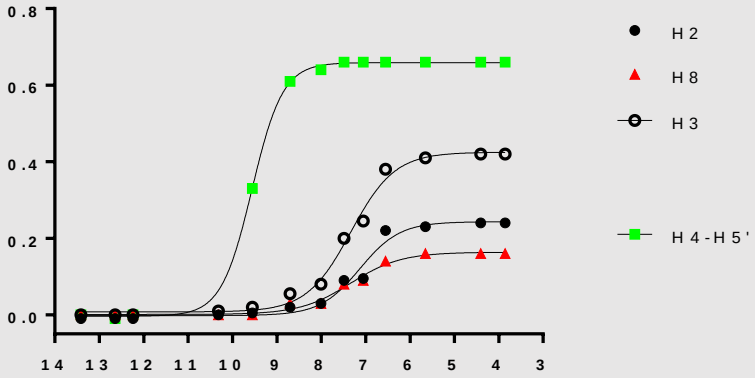
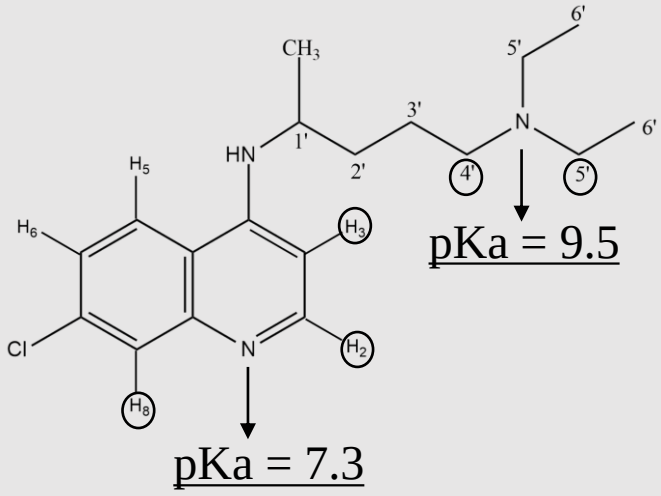
(D)



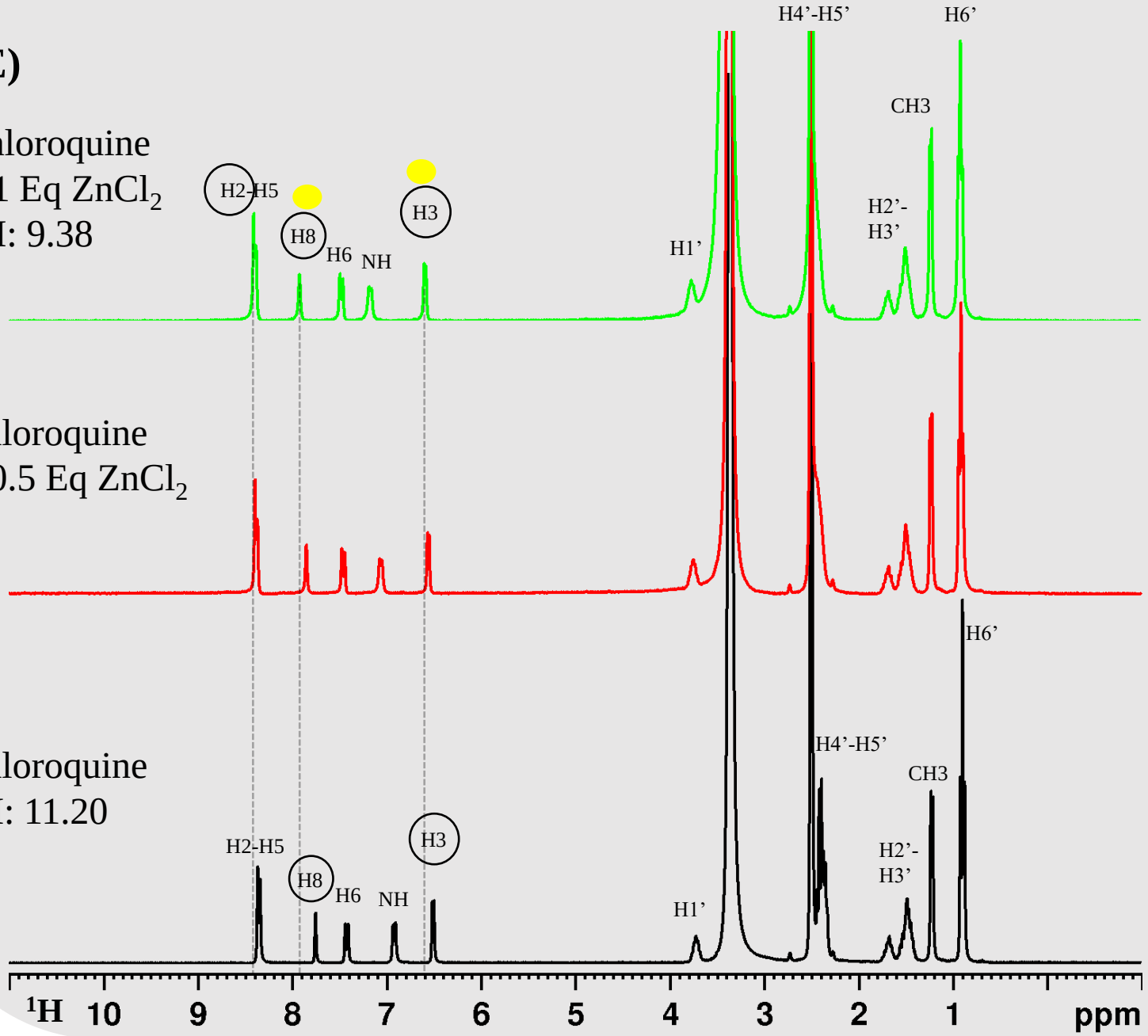


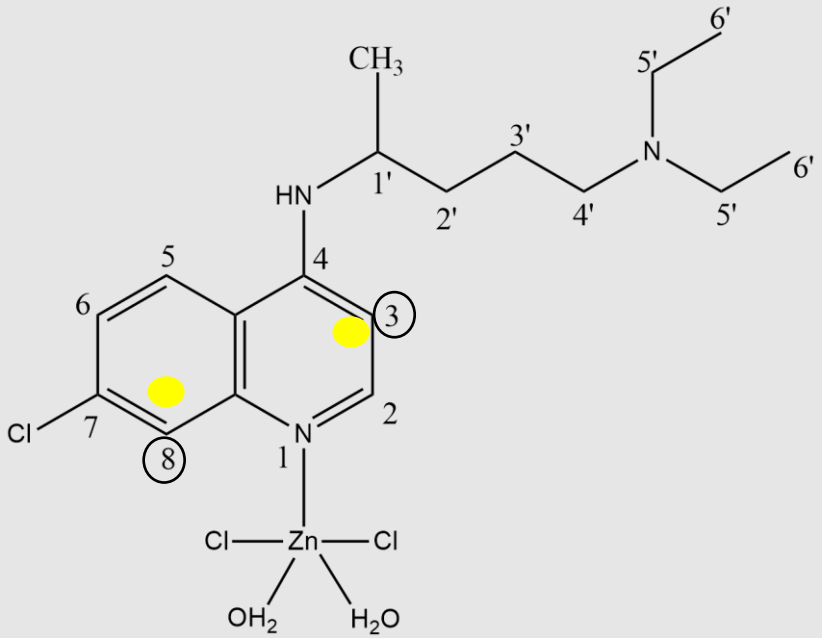


(D) pH-dependent titration of CQ in DMSO-d6.

3 Synthesis of Zinc-Chloroquine complex: NMR titration of Chloroquine with Zinc(II) Chloride

(E)

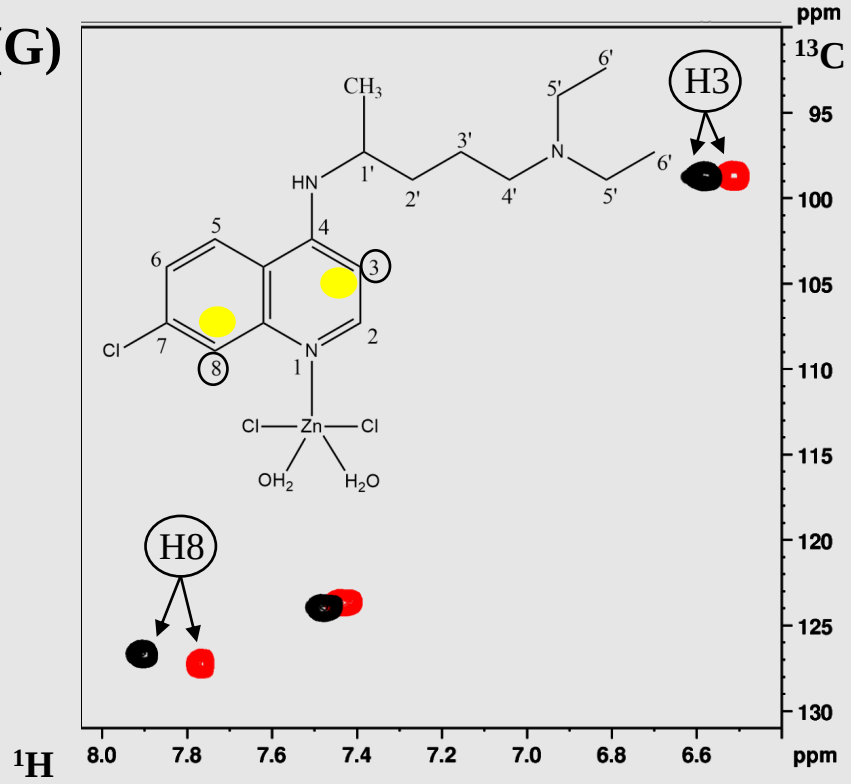




(E) Overlay of 1D ^1H NMR spectra of free CQ (black), CQ + 0.5 Eq of $ZnCl_2$ (red) and CQ + 1 Eq of $ZnCl_2$ (green) in DMSO-d6. The most affected signals are marked by yellow dots.

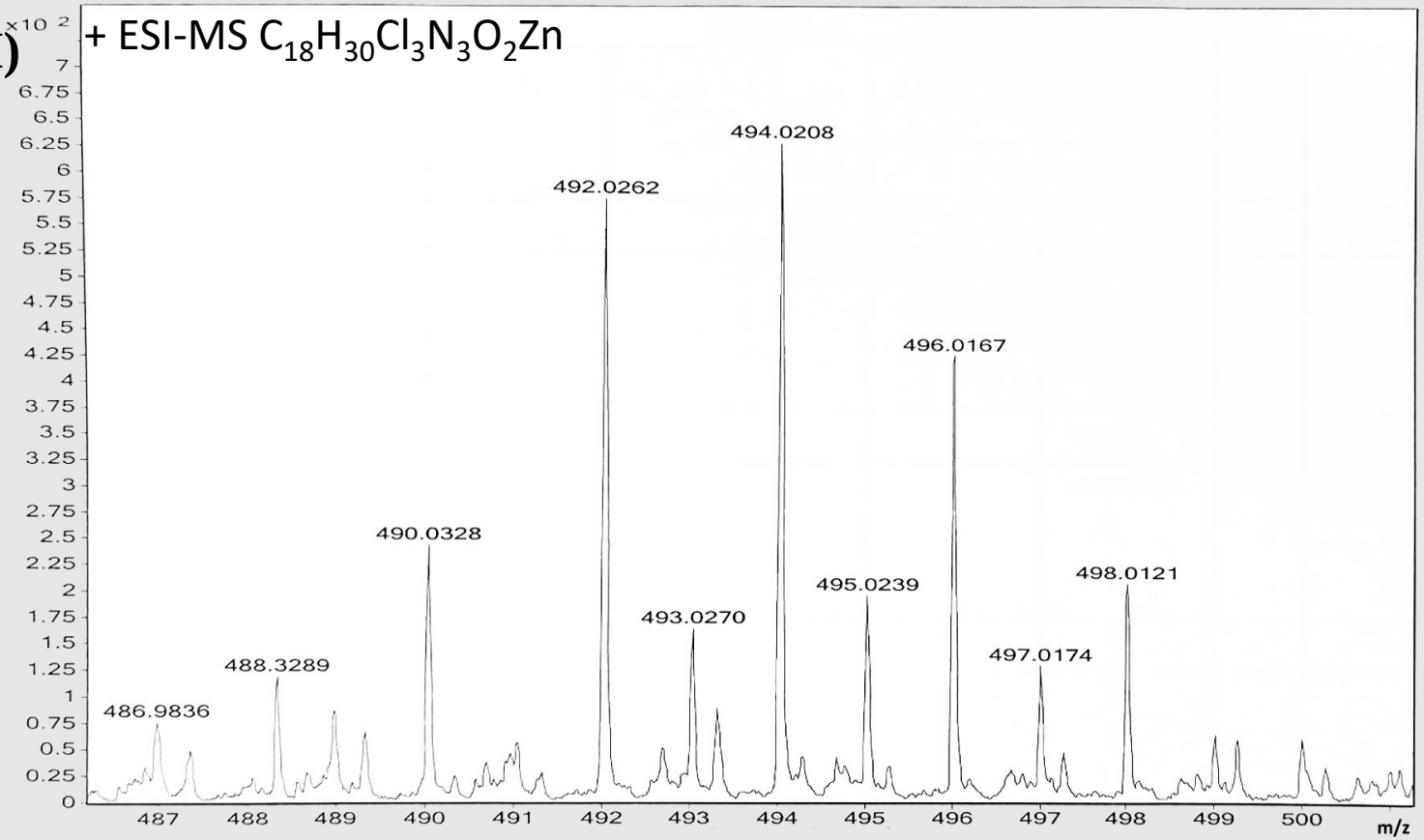
4 Characterization of Zinc-Chloroquine complex: 2D HSQC NMR and ESI-MS spectra

(G)



(G) Overlay of the 2D $^{1}H,^{13}C$ HSQC NMR spectra of free CQ (red) and CQ + 1 Eq of $ZnCl_2$ (black) in DMSO-d6.

(H)



(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 $ZnCl_2$.

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

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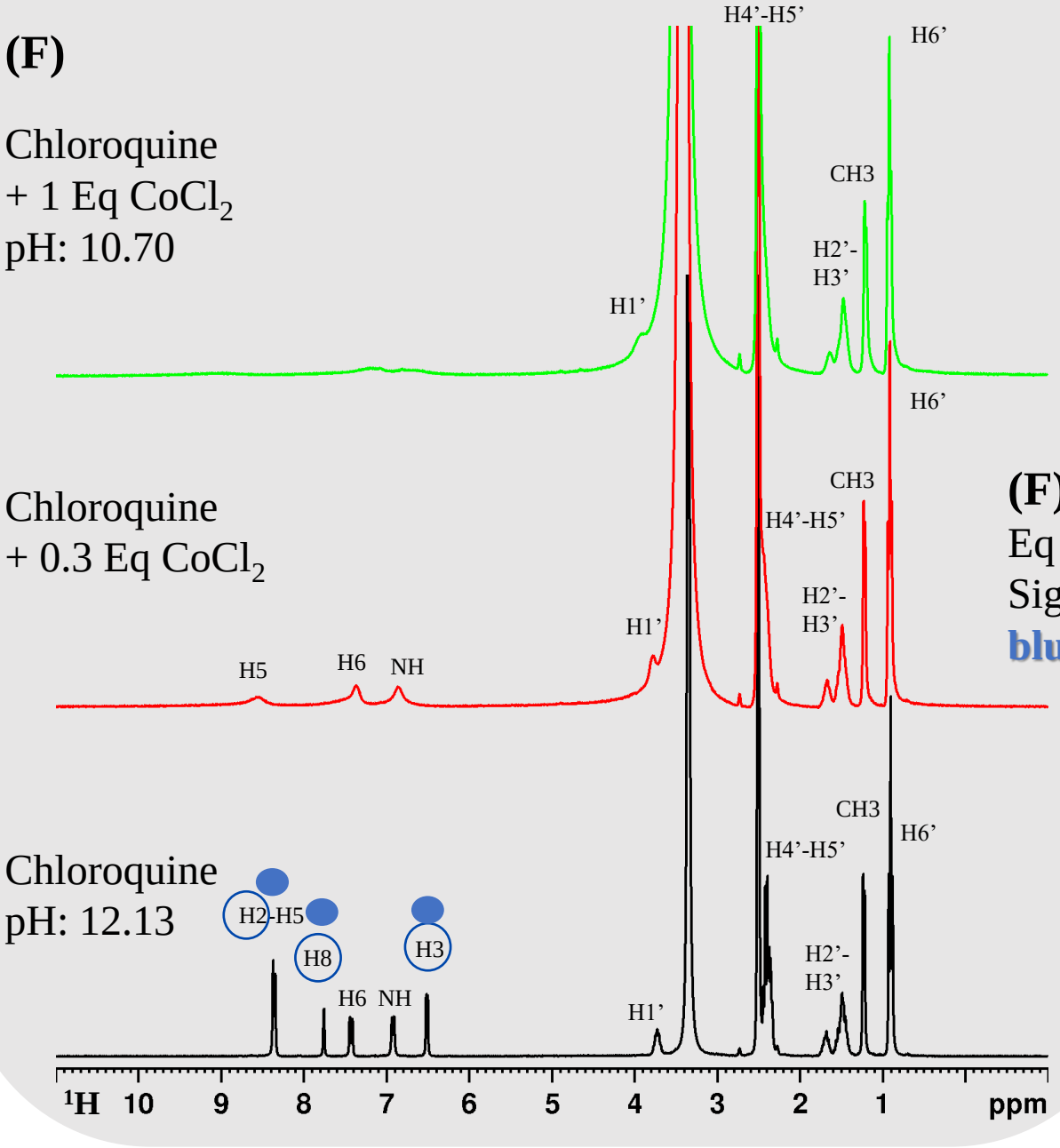
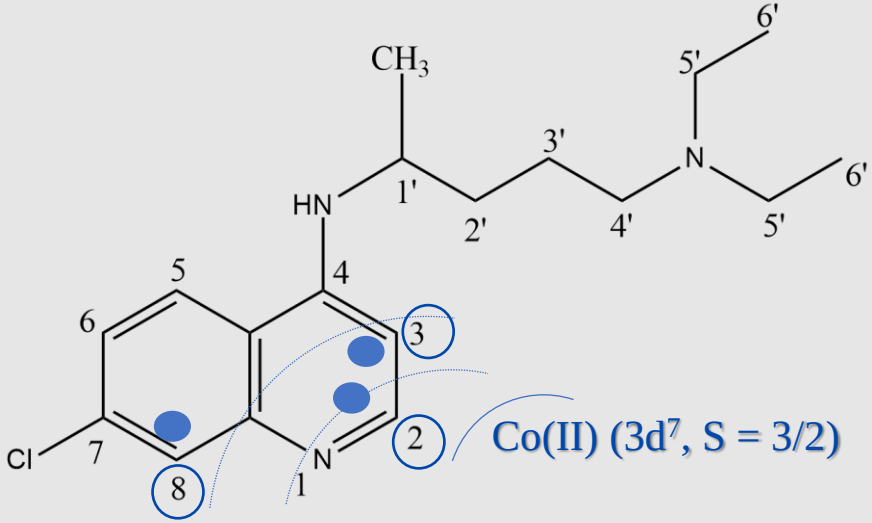
Identification of metal coordination site: NMR titration of Chloroquine with Cobalt(II) Chloride

(F)





(F) Overlay of 1D ^{1}H NMR spectra of free CQ (black), CQ + 0.5 Eq of $CoCl_2$ (red) and CQ + 1 Eq of $CoCl_2$ (green) in DMSO-d6. Signals experiencing large paramagnetic broadening are marked by **blue** dots.

CONCLUSIONS

The stoichiometry of the pentacoordinate complex $[Zn(CQ)(Cl)_2(OH_2)_2]$ 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.