

## Unraveling the mechanism of 4-aminoquinoline antimalarial action

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### Introduction

Invasion of host erythrocytes by *Plasmodia* during a malarial infection allows deconstruction of haemoglobin within acidic vacuolar compartments, providing a vital nutrient source for parasite replication. Successive release of four, redox active, toxic porphyrins per molecule of haemoglobin prompts a biomineralization process producing an inert, redox inactive malarial pigment (haemozoin)<sup>1</sup>. Host defense mechanisms tightly regulate concentrations of toxic free haemin (heme) using a high affinity ( $K_d < 1$  pM) plasma protein scavenger, hemopexin. Current evidence suggests that certain 4-aminoquinoline compounds, especially amodiaquine and chloroquine, inhibit biomineralization allowing translocation of complexed haemin to loci sensitive to oxidative stress<sup>2, 3</sup>. Knowledge of both binding geometry and stoichiometry within putative antimalarial drug-receptor complexes may allow: i) a deeper understanding of the mechanism of *mono*- and *bis*-4-aminoquinoline antimalarial action, and ii) facilitate design of compounds superior to chloroquine. Previous investigations have allowed definition of a functional antimalarial receptor *in vivo* that has been used to design a highly active, orally bioavailable aryl bridged bisquinoline antimalarial called metaquine<sup>4</sup>. Since related cycloalkyl bridged compounds e.g. Ro 47-7737 demonstrate phototoxicity in animal models<sup>5</sup>, the potential for incurring phototoxicity within the metaquine class of compound requires investigation before further development.

Depiction of parallel  $\pi$ - $\pi$  interaction between the quinoline and the porphyrin is persistently favoured in antimalarial modeling studies<sup>6</sup> despite overwhelming evidence from geometrical investigations that confirm such face to face  $\pi$ - $\pi$  alignments, where the ring plane area overlaps, is a rare phenomenon<sup>7</sup>. The most common geometry is an offset, or slipped interaction (i.e. parallel displaced) revealing important contribution from  $\pi$ - $\sigma$  contacts<sup>7</sup>. Three possible binding geometries involving monomeric haem and 4-aminoquinolines merit further investigation namely  $\pi$ - $\pi$  alignments, parallel displaced and an exogenous axial ligand binding mode involving a Fe-N bond. However, the presence of a (+ $\pi$ , + $\sigma$ ) substituent e.g. chlorine, essential for high antimalarial activity, prevents binding to flat porphyrins.

This study consists of a combination of structural, computational and spectroscopic approaches to explore the putative mechanism(s) of action of 4-aminoquinoline antimalarials by defining their geometrical interaction(s) between drug and receptor.

### Methods

Modelling calculations were performed using Molecular Mechanics (Cerius2). A 440nm pulsed dye laser was used to investigate singlet oxygen generating capacity of putative drug candidates. An argon-ion laser with 457.9 and 514.5 nm output was employed for resonance Raman (RR) experiments<sup>8</sup>. Metaquine was synthesised according to reported methods<sup>4</sup>.

1:1 complexes were generated by mechanical grinding in the solid state between haemin chloride and corresponding antimalarial salt.

### Results and Discussion

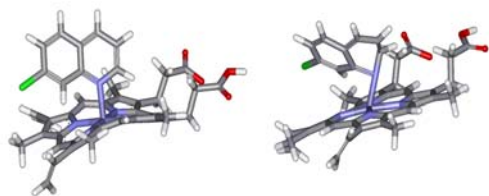
Conversion of an established binding assay<sup>9</sup> to a high throughput mode, confirms that a 1:1 haem: drug binding interaction persists in aqueous HEPES buffered DMSO (23° C pH 7.4: Log K chloroquine: 5.5; Amodiaquine 5.4; Metaquine 6.1 [pH 5.6: log K = 5.7, 23° C, MES).

Resonance Raman (RR) spectra between various drugs, including 4-aminoquinolines and their putative receptor, haemin, were recorded (using 457.9 and 514.5 nm outputs of the argon-ion laser) by probing into the absorption bands of the quinoline ring that interacts with the haem receptor. RR spectra of the hydroxyl and drug coordinated haemin (in SDS and wet DMSO) display the typical spectroscopic marker bands of a pentacoordinate high-spin ferric iron derivative<sup>10</sup>. RR spectra of metaquine and quinoline were obtained. Interaction of metaquine and haem in DMSO provided RR spectra were similar in terms of the peak frequencies of different marker bands. Only small shifts (within 2  $\text{cm}^{-1}$ ) are observed for the iron core size marker band ( $\nu_4$ ) as well as for other bands in the 1400-1600  $\text{cm}^{-1}$  region. In conditions favouring  $\mu$ -oxo dimer formation, a moderate (2-3  $\text{cm}^{-1}$ ) frequency shift was observed for the vinyl-stretching band ( $\nu_{\text{C}=\text{C}}$ ) at 1624  $\text{cm}^{-1}$  for the alkaline haemin with respect to 1629  $\text{cm}^{-1}$  for metaquine and amodiaquine-complexed haemin (See close contact G in Figure 2). In pure DMSO, molecular ions for such complexes were not detected in our high resolution (accurate) electrospray mass spectrometry (HRESMS) studies suggestive of weak complex formation.

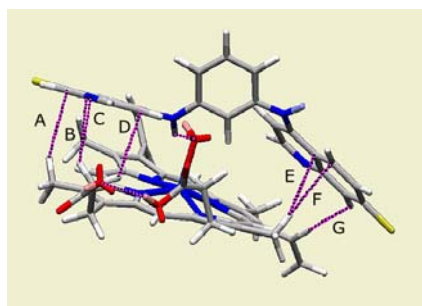
RR experiments reveal, in the absence of water, such interactions between the 4-aminoquinolines and haemin (or haemin chloride) are extremely weak. HRESMS confirm that haemin chloride forms a 1:1 complex with various 4-aminoquinolines (including chloroquine and amodiaquine) and bisquinolines (e.g. Ro 47-7737 and metaquine) in protic solvents such as methanol, but not in DMSO<sup>11</sup>. RR experiments with haemin (buffered SDS pH 4.7 - pH 14) and various antimalarials showed little evidence of covalent Fe-N bond formation (expected: 215-222  $\text{cm}^{-1}$ )<sup>10</sup>, suggesting that axial coordination of haemin by 4-aminoquinolines does not displace an axially coordinated hydroxyl group, a result consistent with modeling studies. Artemisinin, which also acts at haeme, generated an unstable radical cation complex which was detected in HRESMS and RR experiments in which solutions were flowed past the light source, a result that requires further TR<sup>3</sup> investigations.

Docking calculations between haemin and chloroquine (or metaquine) generate complexes of lowest energy favoring an out of plane displacement along the lowest frequency coordinate of the porphyrin<sup>12</sup> that predominate over parallel  $\pi$ - $\pi$  motifs. Furthermore, a 'normal coordinate analysis'<sup>13</sup>

suggests that such deformations are a generalized phenomenon in porphyrins and haem proteins. Modeling investigations (Figures 1 and 2) reveal a linear correlation between the total energy (of the monomeric complexes) and the sum of the Van der Waals and electrostatic energy terms (c.f. <sup>6</sup>) and note)



**Figure 1.** Highest energy conformation of 7-chloroquinoline bound to 7-chloroquinoline (restrained torsion angle =  $-40^\circ$ ) and the lowest energy conformation (restrained torsion angle =  $-140^\circ$ ). Drug complexation induced similar changes to ligand induced heme ruffling <sup>14</sup>.



**Figure 2.** Lowest energy docking interaction showing close contacts. Incorporation of suitable bioisosteres at E, F and G could be used to modulate antimalarial activity.

In contrast, experiments in the solid state suggest that reactions between 4-aminoquinoline and hemin chloride form stable 1:1 complexes, which persist in methanol, whose strong molecular ions in HRESMS experiments suggest axial ligation.

The structure of the reciprocal dimer subunit present in haemozoin, characterized using synchrotron studies <sup>11</sup>, has been detected in our HRESMS studies. Importantly, the currently favored ether bridged  $\mu$ -oxo dimer was not found either in Raman investigations (at pH 4.7) nor in corresponding HRESMS experiments and may be a desalting artifact or a complex formed at basic pH during preparation of solutions <sup>12</sup>. Binding to the reciprocal dimer involves weak  $\pi$ - $\pi$  stacking interactions ( $\sim 2 \text{ kJ mol}^{-1}$ ) <sup>7</sup> and displays correspondingly weak mass ions and, as this study shows, is unlikely to represent the currently favored haemin: 4-aminoquinoline binding geometry. Recent NMR investigations involving chloroquine-ferriprotoporphyrin IX complex in the solid state also support our conclusion that orthogonal binding to haemin is possible through the formation of a Fe-N bond. If indeed this is the case, than axial binding by chloroquine and related compounds is only possible with non-planar haemin <sup>13</sup>, because unfavorable contacts between the essential 7-halogen would prevent this type of complex formation. Monomeric haemin demonstrates antimalarial <sup>2</sup>) and immunological effects whereas haemozoin is pharmacologically inert (Dascombe *et al.* unpublished) providing additional support for the success of this *Plasmodial* detoxification mechanism. Experiments involving quantum mechanical calculations and crystallography are in progress to identify and quantify the binding interaction between various

4-aminoquinolines and porphyrins as well as investigating the spectroscopic properties of such adducts in the solid phase.

### Photochemical Investigations

In this study we also evaluated the capacity for singlet oxygen ( $^1\text{O}_2$ ) generation from known antimalarials such as quinine, amodiaquine, chloroquine and mefloquine in SDS micelles. As expected, quinine readily generated  $^1\text{O}_2$ , with amodiaquine and chloroquine less so. Gratifyingly, preliminary investigations confirm that mefloquine does not generate  $^1\text{O}_2$ . The recent introduction of a combined dosage form of artemisinin with a bisquinoline (Artekin) suggests that bisquinolines may be useful for treating multi-drug resistant malarials.

### Conclusion

A combination of *in silico*, spectroscopic and spectrometric experiments suggest that docking motifs other than cofacial  $\pi$ - $\pi$  interaction between the quinoline and the porphyrin could explain antimalarial drug action. Modeling investigations reveal close contacts between the porphyrin ring and selected atoms within the compounds such as mefloquine, which upon modification could be used to modulate antimalarial activity and further clarify the mechanism of antimalarial activity.

### References

1. T J Egan, J. Inorg. Biochem. **91** 19, (2002)
2. D Monti, B Vodopivec, N Basilio, P Olliaro D Taramelli. Biochemistry **38** 8858, (1999)
3. S E Francis, D J Sullivan Jr., D E Goldberg, Ann. Rev. Microbiol. **51** 97, (1997)
4. F M D Ismail, M J Dascombe, P Carr, S A M Merette, P Rouault, J. Pharm. Pharmacol. **50** 483 (1998)
5. J M Karle, A K Bhattacharjee, J L Vennerstrom, J. Chem Crystallography **32**, (2002)
6. P M O'Neill, D J Willock, S R Hawley, P G Bray, R C Storr, S A Ward, B K Park J. Med. Chem. **40**, 437, (1997). Inexplicably, these investigators have modeled a known isomeric form of haemin, PHIII [see: W A Klasbeck, A Ghosh, R K Pandey, K M. Smith, D F Bocian. J. Am Chem. Soc. **117**, 10959, (1995)], complexed with various 4-aminoquinolines. Consequently, inferences drawn from O'Neill *et al.*'s study are debatable.
7. C Janiak, J. Chem. Soc. Dalton Trans. 3885, (2000)
8. S M Tavender, J H Tinkler, A W Parker, R Goyal, L Mulroy, T G Truscott. CLF Annual Report, TR-RAL-94-042, 181, (1994)
9. T J Egan, W W Mavuso, D C Ross, H M Marques, J. Inorg. Biochem. **68** 137, (1997)
10. A Boffi, T K Das, S D Longa, Spagnuolo, D L Rousseau, Biophysical J. **77**, 1143 (1999)
11. T J Egan and K K Ncokazi. J Inorg Biochem, **98**, 144-152 (2003)
12. M J Dascombe, M G B Drew, F M D Ismail *et al.*, J. Med. Chem. Accepted for publication.
13. Molecular Simulations and Normal Coordinate analysis of Porphyrins and heme proteins, J. A. Shelnut, in 'The Porphyrin Handbook', Vol. 7. K. M. Kadish, K. M. Smith, R. Gullard (Eds.) (2000), Academic Press, London.167-223.
14. S A Roberts, A Weichsel, Y Qiu, J A Shelnut, F A Walker, W R Montfort, Biochemistry **40**, 11327, (2001)
15. de Dios AC, Tycko R, Ursos LMB, Roepe PD "NMR studies of chloroquine-ferriprotoporphyrin IX complex", J. Phys.Chem. **107** 5821, (2003)