

RESEARCH PAPER

Antimalarial drugs inhibit human 5-HT₃ and GABA_A but not GABA_C receptors

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Background and purpose: Antimalarial compounds have been previously shown to inhibit rodent nicotinic acetylcholine (nACh) and 5-HT₃ receptors. Here, we extend these studies to include human 5-HT_{3A}, 5-HT_{3AB}, GABA_A α1β2, GABA_A α1β2γ2 and GABA_C ρ1 receptors.

Experimental approach: We examined the effects of quinine, chloroquine and mefloquine on the electrophysiological properties of receptors expressed in *Xenopus* oocytes.

Key results: 5-HT_{3A} receptor responses were inhibited by mefloquine, quinine and chloroquine with *IC*₅₀ values of 0.66, 1.06 and 24.3 μM. At 5-HT_{3AB} receptors, the potencies of mefloquine (*IC*₅₀ = 2.7 μM) and quinine (15.8 μM), but not chloroquine (23.6 μM), were reduced. Mefloquine, quinine and chloroquine had higher *IC*₅₀ values at GABA_A α1β2 (98.7, 0.40 and 0.46 mM, respectively) and GABA_A α1β2γ2 receptors (0.38, 1.69 and 0.67 mM, respectively). No effect was observed at GABA_C ρ1 receptors. At all 5-HT₃ and GABA_A receptors, chloroquine displayed competitive behaviour and mefloquine was non-competitive. Quinine was competitive at 5-HT_{3A} and GABA_A receptors, but non-competitive at 5-HT_{3AB} receptors. Homology modelling in combination with automated docking suggested orientations of quinine and chloroquine at the GABA_A receptor binding site.

Conclusions and implications: The effects of mefloquine, quinine and chloroquine are distinct at GABA_A and GABA_C receptors, whereas their effects on 5-HT_{3AB} receptors are broadly similar to those at 5-HT_{3A} receptors. *IC*₅₀ values for chloroquine and mefloquine at 5-HT₃ receptors are close to therapeutic blood concentrations required for malarial treatment, suggesting that their therapeutic use could be extended to include the treatment of 5-HT₃ receptor-related disorders.

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Abbreviations: nACh, nicotinic acetylcholine; AChBP, acetylcholine binding protein

Introduction

For decades, quinine, chloroquine and mefloquine (Lariam) have provided an economical and effective approach for the treatment and prevention of malaria. It is thought that these compounds prevent the growth of parasites within infected erythrocytes, although the biochemistry of their therapeutic actions is not entirely known, and different compounds seem to have different modes of action (Bray *et al.*, 2005; Uhlemann *et al.*, 2005). These compounds have a good clinical record, with relatively few side effects reported. However, there is experimental evidence that they may affect the nervous system, as they have been shown to inhibit neurotransmission between nerves and at the neuromuscular junction (Sieb *et al.*, 1996; Ballestero *et al.*, 2005). In particular, there are detailed pharmacological

accounts of competitive and non-competitive behaviour at human nicotinic acetylcholine (nACh) α9α10 and mouse 5-HT₃ (type 3 serotonin) receptors (Ballestero *et al.*, 2005; Thompson and Lummis, 2007a). Given the strong structural and functional similarities between these receptors, it is possible that these effects will extend across species and to other members of this receptor family.

5-HT₃ and nACh receptors are members of a family of ligand-gated ion channels that are responsible for fast excitatory and inhibitory neurotransmission in the central and peripheral nervous systems. The group is known as the Cys-loop family and also includes GABA_A and glycine receptors. These receptors function as pentamers and have a subunit arrangement that can be either homomeric or heteromeric. Five 5-HT₃ receptor subunits (A–E) have been identified to date; the A subunit can form functional homomeric receptors, whereas subunits B–E only function as heteromeric receptors in combination with the A subunit (Davies *et al.*, 1999; Niesler *et al.*, 2003, 2007). In the human nervous system, 5-HT_{3A} subunits have been found

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throughout the adult brain, but are also widely distributed in internal organs and extraneuronal cells such as monocytes, T cells, synovial tissue and primary chondrocytes (Miyake *et al.*, 1995; Fiebich *et al.*, 2004). 5-HT_{3B} receptor subunits are not as widespread, but are still detectable throughout the adult brain, kidney and intestine (Davies *et al.*, 1999; Dubin *et al.*, 1999; Niesler *et al.*, 2003; Tzvetkov *et al.*, 2007). In contrast to the 5-HT₃ receptor, GABA_A receptor stoichiometry is more complicated and 16 different subunit types can potentially combine to form functional pentameric receptors (Barnard *et al.*, 1998; Akabas, 2004). The most common subunits are $\alpha 1$, $\beta 2$ and $\gamma 2$, all of which are abundantly expressed throughout the nervous system (Akabas, 2004). GABA_C receptors are classified as a subtype of the GABA_A receptors, and GABA_C $\rho 1$, $\rho 2$ and $\rho 3$ subunits can combine as homomeric or heteromeric complexes. They are largely restricted to retinal bipolar cells, but have been observed at low densities in various regions of the brain (Cutting *et al.*, 1991; Enz and Cutting, 1999).

The five subunits that constitute a functional receptor of the Cys-loop family are symmetrically arranged around a central ion-conducting pore and each subunit consists of three domains (Thompson and Lummis, 2007b). The N-terminal domain is located at the extracellular side of the membrane and is responsible for ligand binding. Structural details of the extracellular domain of nACh receptors have been revealed from cryo-electron microscopy and a more recent crystal structure of an nACh receptor monomer, but the most significant improvement in our understanding of this domain has come from high-resolution crystallography studies of the acetylcholine binding protein (AChBP) pentamer (Brejc *et al.*, 2001; Unwin, 2005; Dellisanti *et al.*, 2007). AChBP shares structural homology with the extracellular domain of Cys-loop receptors, and has confirmed previous biochemical studies that suggested that the ligand binding site is formed by six loops that converge at the interface of two adjacent subunits (Brejc *et al.*, 2001). Using AChBP crystal structures as templates for computer-generated homology models, a number of researchers have docked ligands into the extracellular domain of a range of Cys-loop receptors, enabling potential receptor–ligand interactions to be identified and experimentally tested (reviewed by Thompson and Lummis, 2006a, 2007b).

In a previous study, we provided a detailed account of the actions of antimalarial compounds at mouse 5-HT₃ receptors and performed automated docking of antimalarial compounds into homology models of this receptor (Thompson and Lummis, 2007a). In this paper, we extend these findings by reporting the effects of quinine, chloroquine and mefloquine on human 5-HT_{3A} and 5-HT_{3AB} receptors as well as providing the first direct evidence for their effects on human GABA_A receptors.

Methods

Cell culture and oocyte maintenance

Xenopus laevis oocyte-positive females were purchased from NASCO (Fort Atkinson, WI, USA) and maintained according to standard methods (Goldin, 1992). Harvested stage e–f

Xenopus oocytes were washed in four changes of ND96 (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 5 mM HEPES, pH 7.5), de-folliculated in 1.5 mg ml⁻¹ collagenase Type 1A for approximately 2 h, washed again in four changes of ND96 and stored in ND96 containing 2.5 mM sodium pyruvate, 50 mM gentamycin and 0.7 mM theophylline.

Receptor expression

Human 5-HT_{3A} (accession number P46098), 5-HT_{3B} (AF080582), GABA_A $\alpha 1$ (P14867), GABA_A $\beta 2$ (P47870), GABA_A $\gamma 2$ (P18507) and GABA_C $\rho 1$ (P24046) subunit cDNA was cloned into pGEMHE for oocyte expression (Liman *et al.*, 1992). cRNA was *in vitro* transcribed from linearized plasmid cDNA template using the mMessage mMachine T7 Transcription kit (Ambion, Austin, TX, USA). 5-HT_{3B}, GABA_A $\alpha 1$, GABA_A $\beta 2$, GABA_A $\gamma 2$ and GABA_C $\rho 1$ subunit cDNA was linearized with *NheI* and 5-HT_{3A} was linearized using *SphI*. Stage e and f oocytes were injected with 50 nl of 100–700 ng μ l⁻¹ cRNA (5–35 ng injected) and currents were recorded 1–4 days after injection. A ratio of 1:3 (A:B) was used for the expression of heteromeric 5-HT₃ receptors. GABA_A subunits were expressed in the ratio 1:1 ($\alpha 1$: $\beta 2$) or 1:1:10 ($\alpha 1$: $\beta 2$: $\gamma 2$).

Electrophysiology

Using two-electrode voltage clamp, *Xenopus* oocytes were clamped at –60 mV using an OC-725 amplifier (Warner Instruments, Hamden, CT, USA), Digidata 1322A and the Strathclyde Electrophysiology Software Package (Department of Physiology and Pharmacology, University of Strathclyde, UK; <http://www.strath.ac.uk/Departments/PhysPharm/>). Currents were filtered at a frequency of 1 kHz and sampled at 350 Hz. Micro-electrodes were fabricated from borosilicate glass (GC120TF-10, Harvard Apparatus, Edenbridge, Kent, UK) using a two-stage horizontal pull (P-87, Sutter Instrument Company, Novato, CA, USA) and filled with 3 M KCl. Pipette resistances ranged from 0.5 to 1.5 M Ω . Oocytes were perfused with saline at a rate of 15 ml min⁻¹. Drug application was via a simple gravity-fed system calibrated to run at the same rate. Extracellular saline contained (in mM) 96 NaCl, 2 KCl, 1 MgCl₂ and 5 HEPES (pH 7.4).

Analysis and curve fitting were performed using Prism V3.02 (GraphPad Software, San Diego, CA, USA, <http://www.graphpad.com>). Concentration–response data for each oocyte were normalized to the maximum current for that oocyte. For inhibition curves, antagonists were routinely co-applied in the presence of agonist or continuously applied for 20 s before and during the administration of 5-HT. For 5-HT₃ receptors, a 2 min wash was used between drug applications. For GABA_C receptors this was increased to 3 min, and for GABA_A receptors it was increased to 6 min. The mean and s.e.mean for a series of oocytes were plotted against agonist or antagonist concentration and iteratively fitted to the following equation:

$$y = I_{\min} + \frac{I_{\max} - I_{\min}}{1 + 10^{\log(EC_{50-x})/H}} \quad (1)$$

where I_{\min} is the baseline current, I_{\max} is the peak current evoked by agonist, EC_{50} is the concentration of agonist

needed to evoke a half-maximal response, x is the ligand concentration and n_H is the Hill slope. K_B was estimated from IC_{50} values using the Cheng–Prusoff equation with the modification by Leff and Dougall (1993):

$$K_B = \frac{IC_{50}}{\left(2 + \left(\frac{[A]}{[A_{50}]}\right)^{1/n_H}\right) - 1} \quad (2)$$

where K_B is the dissociation constant of the competing drug, IC_{50} is the concentration of antagonist required to produce half maximal response, $[A]$ is the agonist concentration, $[A_{50}]$ is the agonist EC_{50} and n_H is the Hill slope of the agonist.

Modelling and antagonist docking

The protein sequence of the extracellular domain of the GABA_A receptor $\alpha 1$, $\beta 2$ and $\gamma 2$ subunits (accession numbers P14867, P47870 and P18507, respectively) were co-aligned with the sequence of AChBP from *Lymnaea stagnalis* (P58154) using FUGUE, which takes into account secondary structures (Shi *et al.*, 2001). A three-dimensional homology model was generated using MODELLER 6v2 (Sali and Blundell, 1993) based on the crystal structure of AChBP at 2.7 Å resolution (PDB ID 1I9B) and the best fit identified as the model with the lowest energy and steric clashes (<http://www-cryst.bioc.cam.ac.uk/~ricardo/>).

The three-dimensional structures of quinine and chloroquine were extracted from the Cambridge Structural Database (reference codes KAMDAD and CLQUON01, respectively). Protonated forms of both molecules were constructed in Chem3D Ultra 7.0 (CambridgeSoft, Cambridge, UK) based on the crystal structures and energy-minimized using the MM2 force field.

Docking was performed using methods similar to those previously described (Thompson and Lummis, 2007a). Briefly, docking of the protonated ligands into the $\beta 2$ – $\alpha 1$ interface of the GABA_A receptor homology model was carried out using GOLD 3.0 (The Cambridge Crystallographic Data Centre, Cambridge, UK). The binding site centre was defined using the C_{e2} atom of Y157 or the C_c atom of Y205, both of which are known to be important binding residues. The binding site radius was defined as 7 Å for quinine and 10 Å for chloroquine. Ten genetic algorithm runs were performed on each docking exercise, giving a total of 20 solutions for each antagonist. The structures were analysed using the implemented GoldScore fitness function.

Materials

All cell culture reagents were obtained from Gibco (Invitrogen Ltd, Paisley, UK), except fetal calf serum, which was from Labtech International (Ringmer, UK). Quinine and chloroquine were from Sigma-Aldrich Co. Ltd (Poole, Dorset, UK). Mefloquine-HCl was kindly provided by CilagAG. All other reagents were of the highest obtainable grade. 5-HT_{3A} and 5-HT_{3B} receptor subunit cDNA was kindly donated by John Peters (University of Dundee), GABA_A $\alpha 1$, $\beta 2$ and $\gamma 2$ receptor subunit cDNA by Keith Wafford (Merck Sharp & Dohme) and GABA_C $\rho 1$ receptor subunit cDNA by David Weiss (University of Texas).

Results

Application of 5-HT or GABA to *Xenopus* oocytes expressing 5-HT_{3A}, 5-HT_{3AB}, GABA_A $\alpha 1\beta 2$ or GABA_A $\alpha 1\beta 2\gamma 2$ receptors produced concentration-dependent, rapidly activating, inward currents that desensitized over the time course of the application. GABA_C $\rho 1$ receptors also displayed a concentration-dependent fast activation, but did not desensitize during drug application. Plotting current amplitude against a series of 5-HT or GABA concentrations allowed curves to be fitted (Equation (1); Figure 1) and the p EC_{50} values and Hill slopes determined from these curves are given in Table 1.

Effects on the homomeric 5-HT_{3A} receptor

At 5-HT_{3A} receptors, a concentration-dependent inhibition of the 5-HT EC_{50} response was observed in the presence of quinine, chloroquine or mefloquine and was fully reversible (Figure 2 and Table 2). Application of antagonists alone did not elicit a response. The potency of the inhibition was unaltered by pre-application of quinine or chloroquine, but an increase in potency was seen for mefloquine with no further increase in the level of inhibition after 10 s pre-application (Figure 2a, inset). IC_{50} values for the three compounds had a rank order of potency of mefloquine > quinine > chloroquine. In previous studies, IC_{50} values for these compounds were used to calculate binding affinities (K_B) using the modified Cheng–Prusoff equation (Equation (2)), and the accuracy of this method was confirmed by comparing these values with binding affinities determined using competition binding, Schild analysis and an analysis of rate constants (Thompson and Lummis, 2007a). Here, binding affinities were similarly estimated from IC_{50} values, and yielded values of 1.76 and 40.3 μ M for quinine and chloroquine. A K_B value for mefloquine could not be estimated, as further investigations indicated that this compound was non-competitive (Leff and Dougall, 1993).

Concentration–response curves were compared in the presence and absence of antagonist. Increasing concentrations of quinine and chloroquine caused parallel rightward shifts in the concentration–response curves with no change in the maximal current, even when applied at concentrations far in excess (that is, 5–100 \times) of the IC_{50} values for these compounds (Figures 4a and b). In contrast, mefloquine caused an increase in the EC_{50} , a change in the Hill slope and a concomitant reduction in the maximal response at concentrations close to its IC_{50} value (Figure 4c).

Effects on the heteromeric 5-HT_{3AB} receptor

At 5-HT_{3AB} receptors, a concentration-dependent inhibition of the 5-HT EC_{50} response was observed in the presence of quinine, chloroquine and mefloquine. Similar to 5-HT_{3A} receptors, antagonists alone did not elicit a response and inhibition was fully reversible after washing (Figure 2b and Table 2). The potency of both quinine and mefloquine was increased by pre-application of these compounds, with no further increase after 10 s (Figure 2b, inset). Similar to 5-HT_{3A} receptors, the rank order of potency was mefloquine > quinine > chloroquine. Concentration–response curves in the

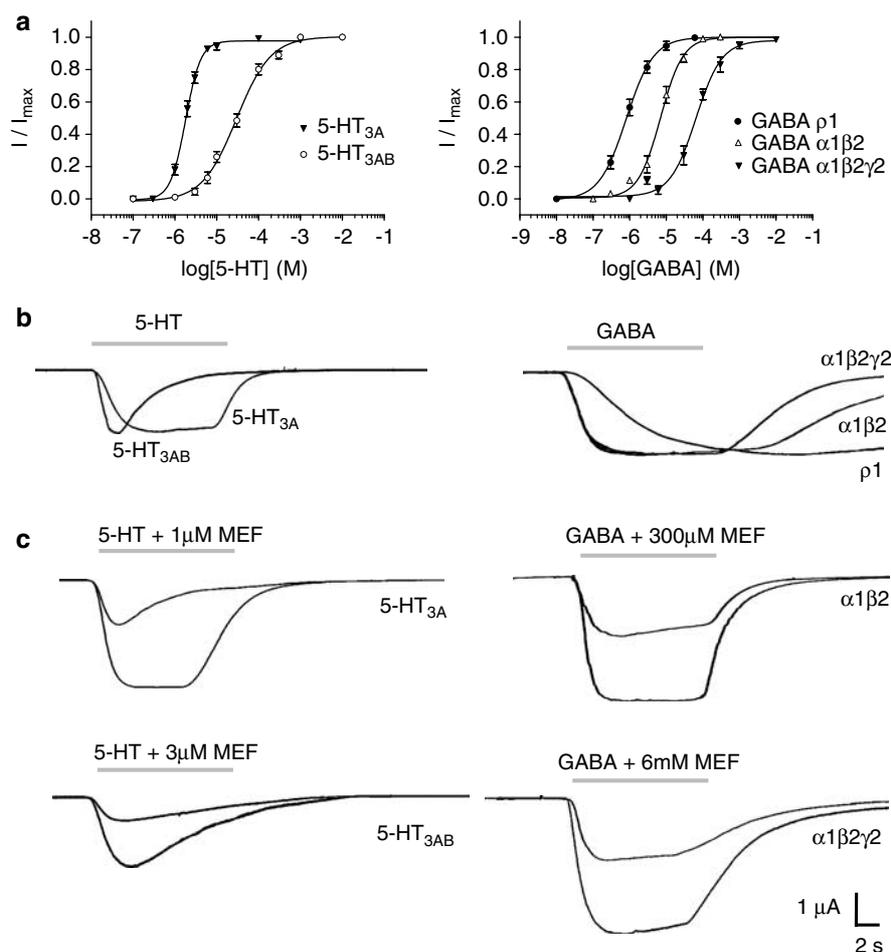


Figure 1 Concentration–response curves for 5-HT₃ and GABA receptors expressed in *Xenopus* oocytes (a). Parameters derived from these curves are shown in Table 1. Typical agonist EC₅₀ responses (b) and inhibition of these responses by mefloquine (c). Agonist application is indicated by a grey line above the current traces. Mefloquine (MEF) was pre-applied for 20 s and then co-applied at the concentrations shown.

Table 1 Parameters derived from concentration–response curves (shown in Figure 1) for 5-HT₃ receptors expressed in *Xenopus* oocytes

Receptor	pEC ₅₀ (μM)	EC ₅₀ (μM)	n _H	n
Mouse 5-HT _{3A} ^a	6.12 ± 0.02	0.75	2.21 ± 0.23	12
Human 5-HT _{3A}	5.76 ± 0.03	1.79	2.32 ± 0.28	6
Human 5-HT _{3AB}	4.53 ± 0.04	29.5	1.06 ± 0.09	6
GABA α1β2	5.15 ± 0.04	7.00	1.42 ± 0.18	4
GABA α1β2γ2	4.19 ± 0.05	64.8	1.23 ± 0.17	5
GABA ρ1	6.09 ± 0.05	0.81	1.17 ± 0.14	3

^aValues from Thompson and Lummis (2007a) were recorded at the same time as the current work and can be directly compared.

presence of antagonist were compared to those in the absence of antagonist and clearly showed that chloroquine caused a rightward shift in the concentration–response curve with no change in the maximal current, whereas quinine and mefloquine caused an increase in the EC₅₀ and a simultaneous reduction in the maximal response (Figure 4d). Ideally, additional curves would have been completed, but at higher concentrations the antimalarial compounds are insoluble. The K_B of chloroquine was calculated as 12.7 μM (Equation (2)). Estimates for quinine

and mefloquine could not be made, as further investigations indicated that these compounds were non-competitive (Leff and Dougall, 1993).

Effects on GABA receptors

Quinine, chloroquine and mefloquine inhibited the GABA_A α1β2 receptor and the GABA_A α1β2γ2 receptor EC₅₀ responses in a concentration-dependent manner (Figures 3a and b, and Table 3). In contrast, at a concentration of up to 1 mM, none of the three compounds inhibited the GABA_C ρ1 response (Figure 3c and Table 3). Application of antagonists alone did not elicit a response at any of the GABA receptors and inhibition at GABA_A α1β2 and α1β2γ2 receptors was fully reversible. The potency of all three antagonists was unaltered by pre-application. IC₅₀ values for all antagonists were higher than those observed at 5-HT₃ receptors, indicating that these compounds are less potent at GABA receptors. IC₅₀ values were higher at α1β2γ2 receptors than at α1β2 receptors. The rank order of potency for α1β2 receptors was mefloquine > quinine > chloroquine and at α1β2γ2 receptors it was mefloquine > chloroquine > quinine.

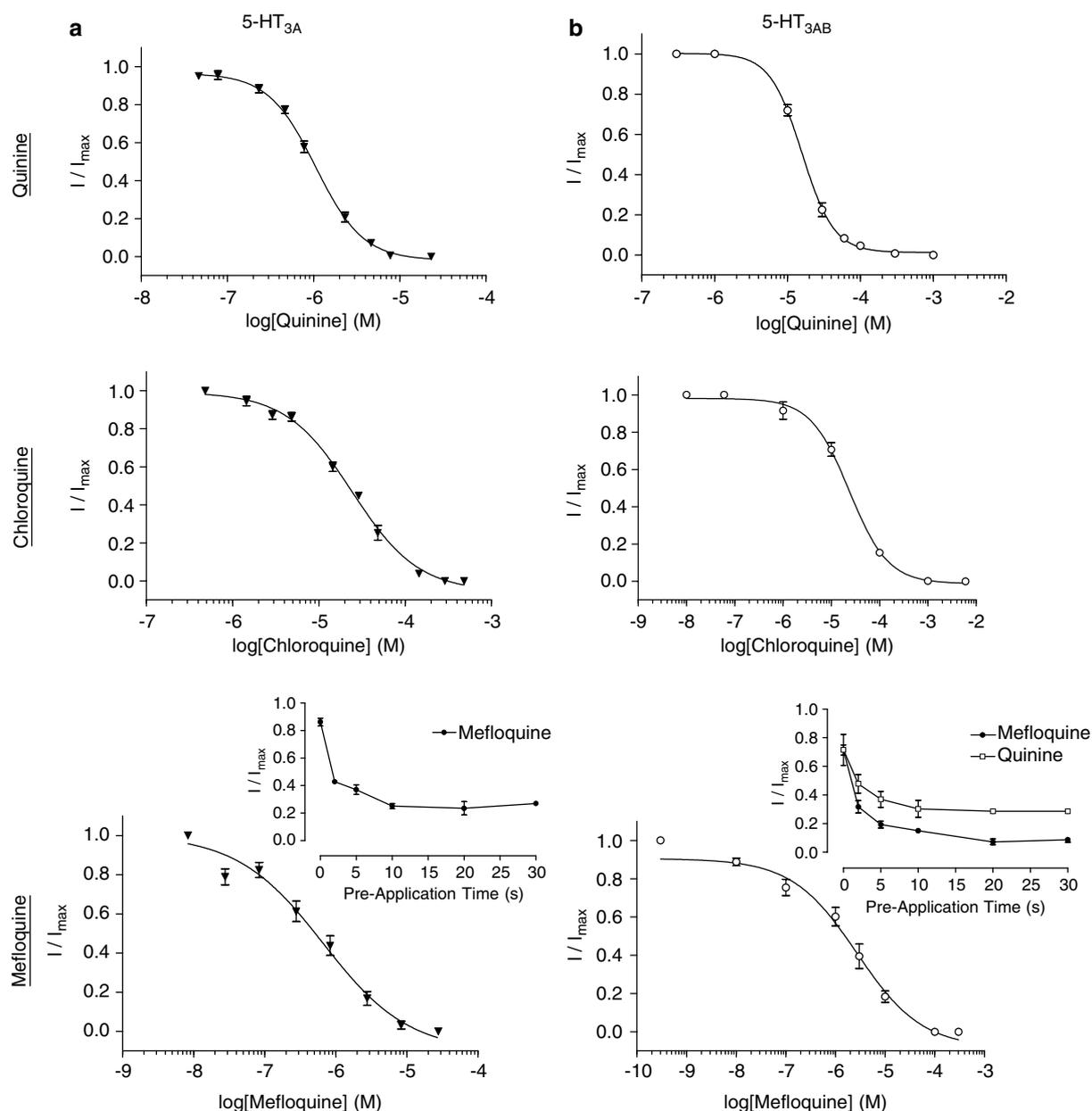


Figure 2 Concentration-dependent inhibition of homomeric (a) and heteromeric (b) 5-HT₃ responses. Inhibition was measured in the presence of EC_{50} concentrations of 5-HT. Values are shown as mean \pm s.e.mean, and parameters derived from these curves can be seen in Table 2. Inset: Inhibition of the EC_{50} response versus pre-application time: 5-HT_{3A} by 3 μ M mefloquine, 5-HT_{3AB} by 30 μ M quinine and 30 μ M mefloquine ($n=4$ for each curve).

A comparison of concentration–response curves in the presence or absence of antagonist showed that increasing concentrations of quinine and chloroquine resulted in increases in EC_{50} values with no change in maximal current at GABA_A $\alpha 1\beta 2$ receptors (Figures 4e and f), whereas mefloquine caused a significant decrease in the maximal current (Figure 4g). At the GABA_A $\alpha 1\beta 2\gamma 2$ receptor, problems of compound solubility prevented us from exploring higher mefloquine concentrations, but it was possible to see a shift in the EC_{50} and a reduction in the maximal current at 10 μ M (a concentration approximately 30-fold lower than its IC_{50}). At concentrations close to the IC_{50} values, the reduction in maximal response was absent from both the quinine and

chloroquine responses (Figure 4h). K_B estimates for quinine and chloroquine at the GABA_A $\alpha 1\beta 2$ receptor were 0.36 and 0.42 mM respectively, and at the GABA_A $\alpha 1\beta 2\gamma 2$ receptor they were 1.18 and 0.46 mM (Equation (2)). The K_B of mefloquine could not be determined, as it is a non-competitive antagonist (Leff and Dougall, 1993).

Docking studies

Docking quinine and chloroquine into the GABA_A receptor binding site ($\beta 2$ – $\alpha 1$ interface) yielded a series of docking solutions that were split into groups based on the orientation of the ligand and GOLD scores (Olsen *et al.*, 2004). Docking

of mefloquine was not performed, as this ligand did not display competitive behaviour.

Docking of chloroquine yielded ligand orientations that fell into two distinct groups designated a and b. In model a, the quinoline ring was close to F65 and the tertiary ammonium was orientated towards loop E (Figure 5a). The distance between the centroids of the quinoline ring and the aromatic ring of F65, and the distance between Y157 and the

secondary amine of chloroquine were both within 5 Å; these could indicate possible π - π and cation- π interactions, respectively. In model b (Figure 5b), the ligand orientation was reversed, with the tertiary ammonium located between the aromatic rings of Y157 and Y205 and the aromatic rings orientated towards loop E. The distance between the nitrogen atom of the ammonium group and the centroids of the two aromatic rings is ideal for a cation- π interaction. In this orientation, there was also a potential hydrogen bond

Table 2 Parameters derived from concentration-inhibition curves (shown in Figure 2) in the presence of EC₅₀ concentrations of 5-HT

Antagonist	pIC ₅₀ (μ M)	IC ₅₀ (μ M)	n _H	n
<i>Mouse 5-HT_{3A}</i>				
Quinine ^a	4.00 ± 0.02	101.0	2.66 ± 0.31	7
Chloroquine ^a	4.05 ± 0.04	89.9	2.00 ± 0.39	6
Mefloquine ^a	5.03 ± 0.02	9.36	2.08 ± 0.18	8
<i>Human 5-HT_{3A}</i>				
Quinine	5.98 ± 0.03	1.06	1.58 ± 0.13	14
Chloroquine	4.61 ± 0.03	24.3	1.13 ± 0.11	13
Mefloquine	6.18 ± 0.12	0.66	0.72 ± 0.15	7
<i>Human 5-HT_{3AB}</i>				
Quinine	4.80 ± 0.01	15.8	1.98 ± 0.12	3
Chloroquine	4.63 ± 0.06	23.6	1.08 ± 0.12	4
Mefloquine	5.57 ± 0.17	2.70	0.66 ± 0.16	6

^aValues taken from Thompson and Lummis (2007a) were performed at the same time as the current work and can be directly compared.

Table 3 Parameters derived from concentration-inhibition curves (shown in Figure 3) in the presence of EC₅₀ concentrations of GABA

Antagonist	pIC ₅₀ (μ M)	IC ₅₀ (μ M)	n _H	n
<i>GABAα1β2</i>				
Quinine	3.32 ± 0.04	400	1.32 ± 0.17	5
Chloroquine	2.33 ± 0.14	465	0.68 ± 0.16	5
Mefloquine	4.01 ± 0.17	98.7	0.48 ± 0.09	9
<i>GABAα1β2γ2</i>				
Quinine	2.77 ± 0.05	1694	1.00 ± 0.10	3
Chloroquine	3.17 ± 0.03	670	1.35 ± 0.11	3
Mefloquine	2.48 ± 0.11	328	0.85 ± 0.22	4
<i>GABAρ1</i>				
Quinine	NE	NE	NE	3
Chloroquine	NE	NE	NE	5
Mefloquine	NE	NE	NE	7

Abbreviation: NE, no effect.

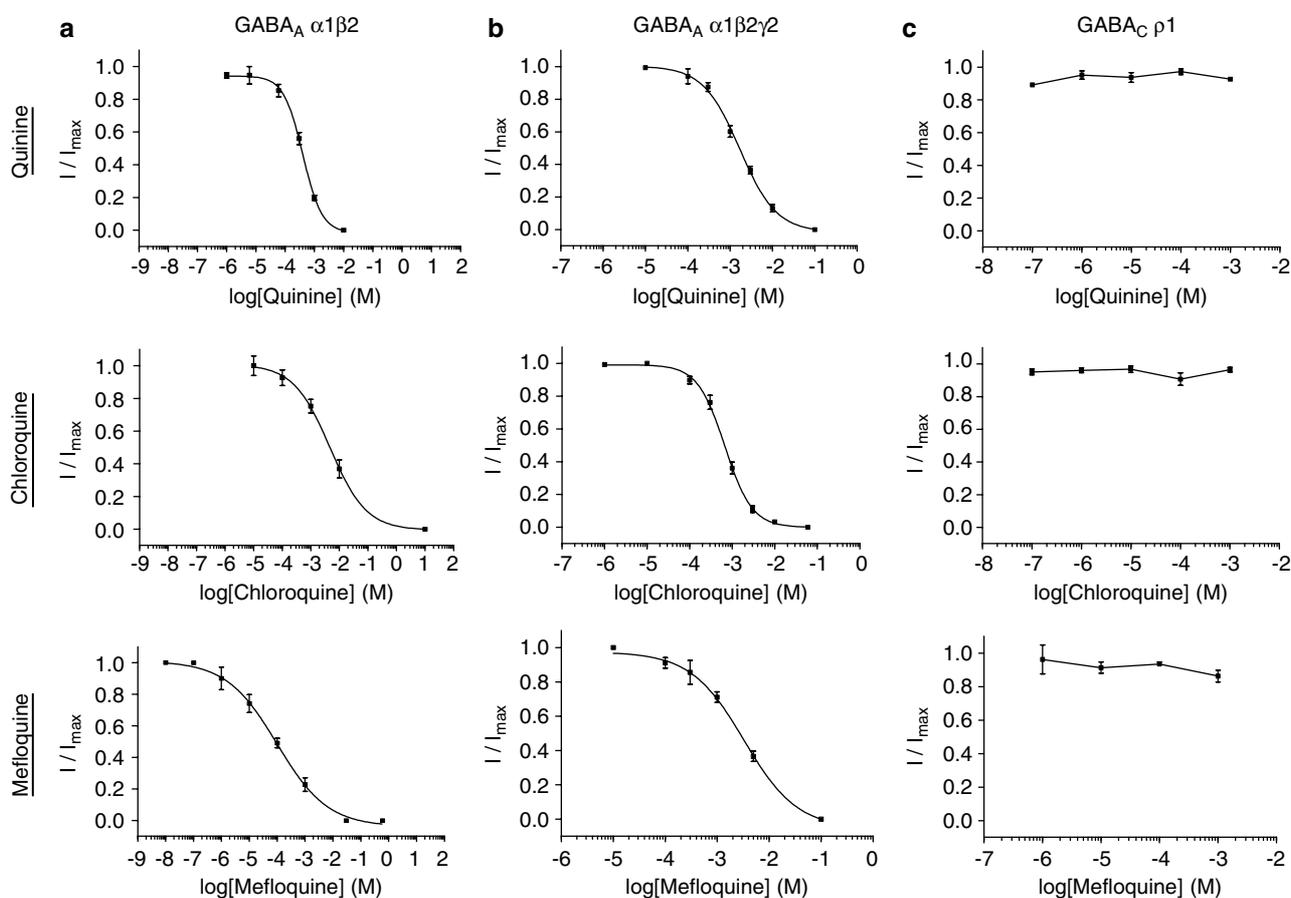


Figure 3 Concentration-dependent inhibition of GABA responses. Inhibition was measured in the presence of EC₅₀ concentrations of GABA for each receptor. Values are shown as mean ± s.e.mean, and parameters derived from these curves can be seen in Table 3.

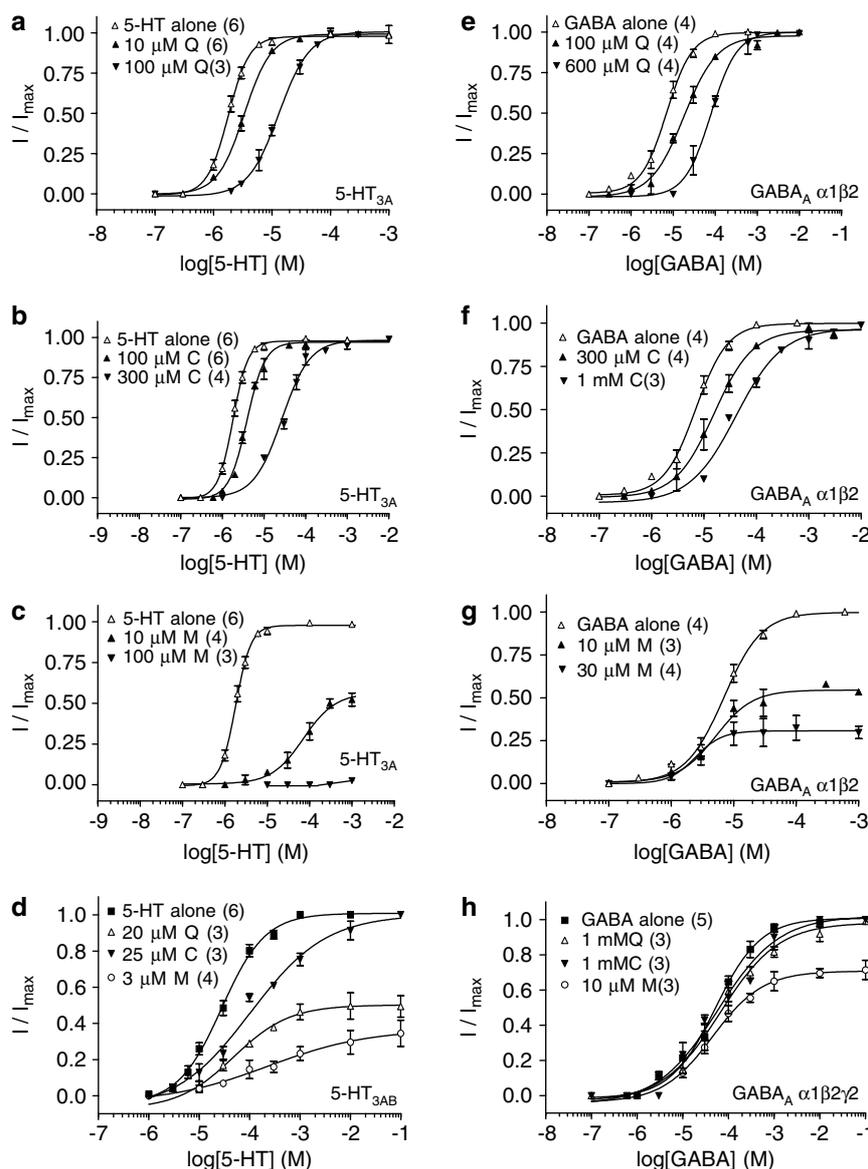


Figure 4 Inhibition by quinine, chloroquine and mefloquine at 5-HT_{3A} and GABA_A receptors. Concentration–response curves were performed in the presence or absence of antagonist. Antagonist concentrations are shown on each graph; Q = quinine, C = chloroquine, M = mefloquine. Values are shown as mean \pm s.e.mean. *n* values are shown in parentheses.

between the chlorine of the quinoline ring and the backbone amine of R120.

The results of docking quinine fell into three main groups that we categorized as c, d, e. In all three solutions, either the tertiary ammonium or the quinoline group was located between Y157 and Y205. In solution c, the quinoline ring was located 4–5 Å from the aromatic ring of Y205, indicating a possible π – π interaction, and the tertiary ammonium was orientated towards loop E (Figure 5c). In solution d, the tertiary ammonium was located between the aromatic rings of Y157 and Y205, and the distance between the centroid of Y157 and the tertiary amine of quinine was within 5 Å, suggesting a possible cation– π interaction (Figure 5d). In this orientation, the quinoline ring was positioned towards loop E, which would enable the formation of a hydrogen bond between the hydroxyl

group of this ring and the backbone amine of T130. Docking solutions c and d displayed similarities to the orientations of chloroquine in solutions a and b, but solution e was different (Figure 5e). In solution e, the tertiary ammonium was located between Y157 and Y205 and the nitrogen of this ammonium group was within 5 Å of Y157. The quinoline ring was orientated towards loop D, which would allow the formation of a hydrogen bond between the hydroxyl of the quinoline ring and S201 located within loop C.

Discussion

This study describes the effects of the antimalarial compounds quinine, chloroquine and mefloquine on human 5-HT₃, GABA_A and GABA_C receptors. At both 5-HT_{3A} and

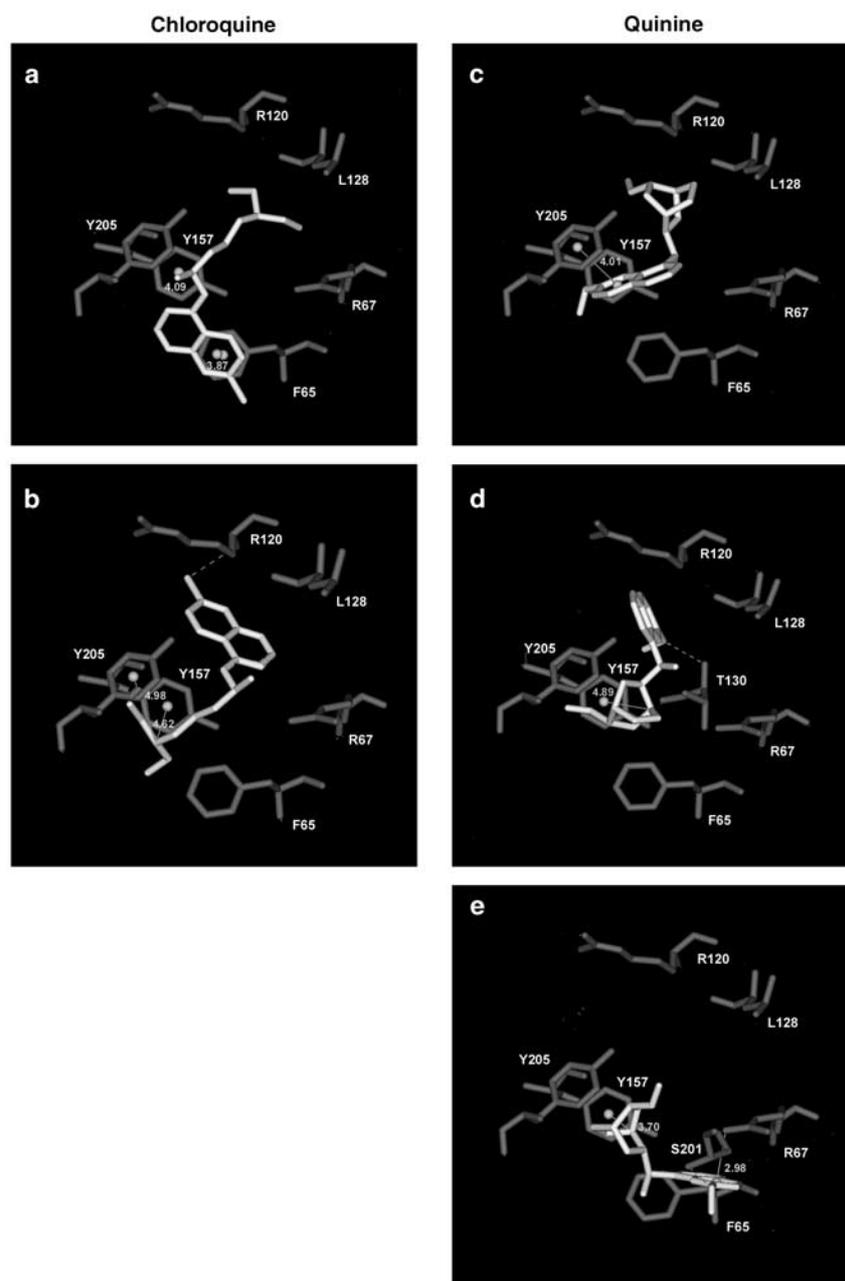


Figure 5 Chloroquine and quinine docked ligands in the GABA_A receptor $\beta 2/\alpha 1$ binding interface showing the orientation of the main residues that define these models. These residues are highlighted in Figure 6. Chloroquine (a, b) and quinine (c–e) are shown in white at the centre of each image.

5-HT_{3AB} receptors, all three compounds are relatively potent antagonists (IC_{50} = 0.66–24.3 μ M). GABA_A receptors are also inhibited by all three compounds, but at higher concentrations (IC_{50} = 98.7–1694 μ M). No inhibition was observed at GABA_C receptors.

At human 5-HT_{3A} receptors, and at both GABA_A $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2$ receptors, quinine and chloroquine displayed effects that were consistent with competitive antagonism, whereas mefloquine was non-competitive. These actions are equivalent to those reported at mouse 5-HT_{3A} receptors (Thompson and Lummis, 2007a). Interestingly, at heteromeric 5-HT₃ receptors, chloroquine and mefloquine displayed properties similar to those at homomeric receptors, but quinine had

effects that were consistent with non-competitive inhibition. Similarities in the actions of these compounds at 5-HT_{3A}, GABA_A and nACh receptors suggest the possibility of conserved sites of action for these compounds (Liu *et al.*, 1991; Sieb *et al.*, 1996; Ballesterro *et al.*, 2005; Thompson and Lummis, 2007a). For the compounds that showed competitive behaviour, our ligand docking provides further evidence for this. At the GABA_A receptor, we currently favour solution d (Figure 5) for the orientation of quinine and solution a for the orientation of chloroquine; these have the greatest potential to be energetically stable and are comparable to docking solutions for the same compounds in previous 5-HT_{3A} receptor studies (Thompson and Lummis,

2007a). Many of the residues involved in the ligand–receptor interaction are similar to those that have been experimentally identified in studies of other GABA_A and 5-HT₃ receptor ligands (Boileau *et al.*, 1999; Beene *et al.*, 2002; Lummis *et al.*, 2005; Thompson *et al.*, 2005; Harrison and Lummis, 2006; Thompson and Lummis, 2007b).

It was surprising that the three compounds could inhibit GABA_A but not GABA_C receptors, given the strong homology between the binding site residues in these two receptors and, in particular, the similarity of the residues that our model

predicts as being close to quinine and chloroquine (Figure 6). However, small changes in binding site residues can have a large impact on binding affinity, and it is possible that changes such as Phe65 (GABA_A) with Tyr (GABA_C) in loop D, and/or Thr130 for Ser in loop E (Figure 5), might significantly reduce ligand potency. For the GABA_A receptors, we have docked quinine and chloroquine into the $\beta 2/\alpha 1$ interface, as they compete with GABA. K_B values for the compounds were less than fourfold different in the $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2$ receptors, supporting this hypothesis.

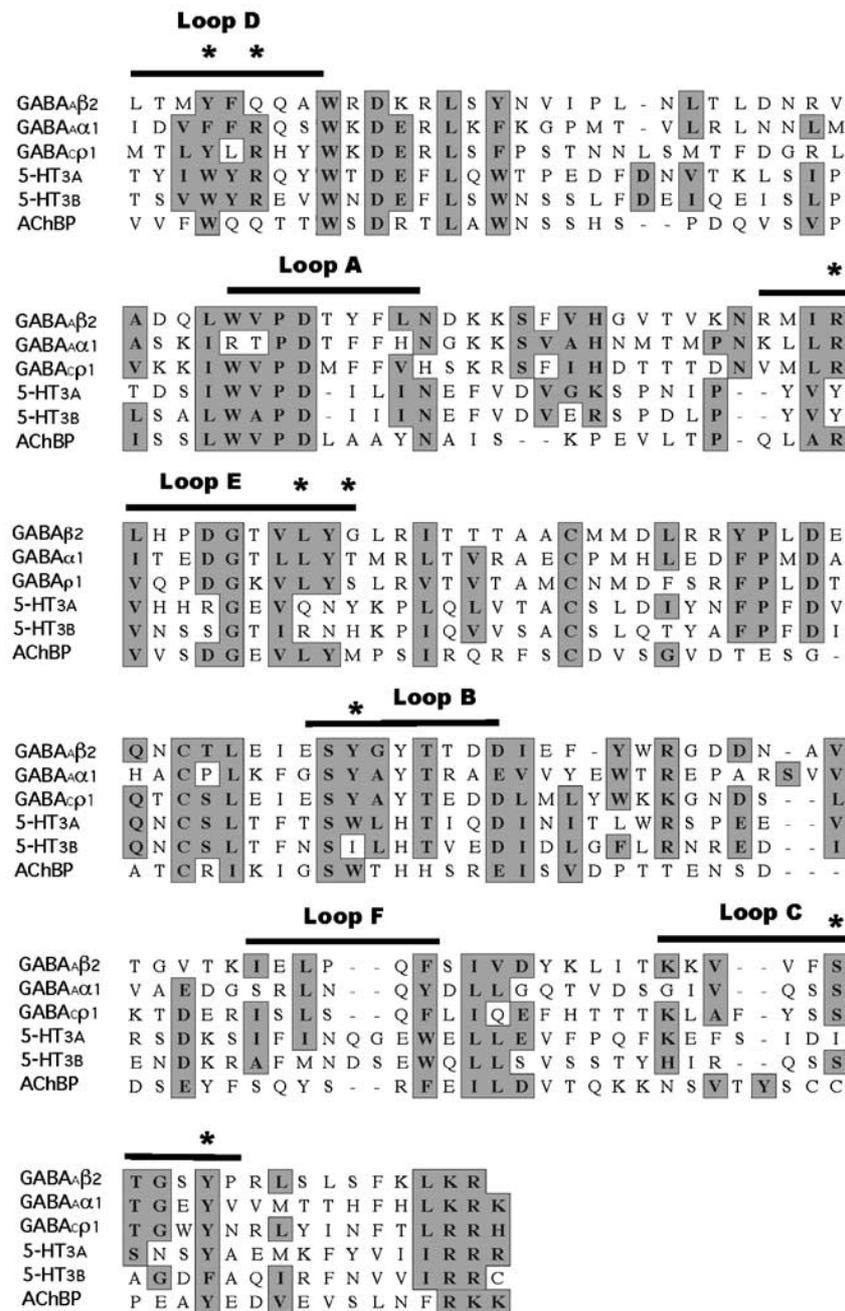


Figure 6 Sequence alignment of ligand-gated ion channel receptor subunits that have been examined for their susceptibility to antimalarial compounds. Residues with similar chemical properties are shown in grey and the approximate positions of the binding loops (A–F) are indicated with a bar. Residues that are shown in Figure 5 are marked with an asterisk (GABA_{β2}: Y157, S201, Y205; GABA_{α1}: F65, R67, L128, R120, T130). Accession numbers for the subunits are GABA_{β2} P47870, GABA_{α1} P14867, GABA_{ρ1} P24046, 5-HT_{3A} P46098, 5-HT_{3B} AF080582 and AChBP P58154.

In contrast to quinine and chloroquine, which showed competitive behaviour, mefloquine was non-competitive at both 5-HT₃ and GABA_A receptors. A similar action was observed for mouse 5-HT_{3A} receptors, where mefloquine displayed a small voltage dependence, suggesting that it may bind at a shallow position within the channel or channel vestibule (Thompson and Lummis, 2007a). Block by quinine was also found to be slightly voltage-dependent in the nACh $\alpha 9\alpha 10$ receptor, suggesting a similarly placed binding site, and it is possible that this site of action is conserved within the family (Ballesterio *et al.*, 2005). It is also possible that quinine and mefloquine may have mixed competitive/non-competitive activity at ligand-gated ion channels. At nACh $\alpha 9\alpha 10$ receptors, quinine competitively inhibited responses at concentrations up to 100 μM ($IC_{50} = 0.97 \mu\text{M}$), but was non-competitive at higher concentrations (Ballesterio *et al.*, 2005). Similarly, at mouse 5-HT_{3A} receptors, Schild analysis has shown that mefloquine is non-competitive, but displacement of a radiolabelled antagonist was recorded at higher concentrations, suggesting both competitive and non-competitive behaviours (Thompson and Lummis, 2007a). As members of the Cys-loop family share strong structural and pharmacological character, a similar mechanism is possible at the receptors described here (Rothlin *et al.*, 1999, 2003). Interestingly, some benzodiazepines (which bind at the $\gamma 2$ - $\alpha 1$ interface of the GABA_A receptor) can inhibit the growth of malarial parasite, indicating that some antimalarial compounds and benzodiazepines may also share binding sites (Dzierszynski *et al.*, 2002).

The blood concentration required to be 99% effective at reducing *Plasmodium falciparum* infection has been reported as 1.57 μM for quinine, 44.4 μM for chloroquine and 2.2 and 4.1 μM for mefloquine (Lobel *et al.*, 1993; Ramharter *et al.*, 2004). These blood concentrations are close to the IC_{50} values for chloroquine and mefloquine at both mouse and human 5-HT₃ receptors, but considerably lower than the IC_{50} values we found at GABA_A receptors. These whole blood values may not be representative of free blood concentrations, as these compounds can bind to plasma proteins; however, as they are able to freely pass the blood-brain barrier and have been found to accumulate in physiologically relevant concentrations in the brain and other tissues, we would expect that they would reach the CNS at concentrations where they could effect receptor responses (Adelusi and Salako, 1982; Baudry *et al.*, 1997; Dow *et al.*, 2003). Consequently, there may be 5-HT₃-mediated effects in patients taking these drugs, although reported side effects, such as nausea, are those that might be expected to be ameliorated by 5-HT₃ selective antagonists, which are used as antiemetics. However, the neuronal pathways that induce nausea and vomiting are varied, and 5-HT₃ antagonists are only effective against post-operative, chemotherapy-induced and radiation-induced nausea and vomiting, but do not inhibit the symptoms elicited by other agents, such as opiate administration, or motion (Bountra *et al.*, 1996; Lynch and Simpson, 2001). It must also be emphasized that for the majority of individuals, all of the drugs studied here rarely display severe adverse reactions when used at the recommended dose (Luzzi and Peto, 1993; Taylor and White, 2004). Indeed, because of their excellent safety record, it is

possible that these compounds could have measurable benefits for the treatment of 5-HT₃-related disorders, and could thereby circumvent the often slow development and high cost of new therapies, a range of which have already been proposed or are under development (Barann *et al.*, 1997, 2000; Piper *et al.*, 2002; Farber *et al.*, 2007; Thompson and Lummis, 2007b).

In summary, we have shown that the antimalarial compounds quinine, chloroquine and mefloquine antagonize both 5-HT₃ and GABA_A receptor responses. Inhibition is most potent at 5-HT₃ receptors, is only seen at higher concentrations at GABA_A receptors and is absent from GABA_C receptors. It is not yet clear if the 5-HT₃ receptors of patients taking these drugs are inhibited, but blood and tissue concentrations indicate that it is possible. These observations further extend the range of receptors known to be affected by antimalarial agents, and suggest that there may be potential benefits in assessing some of them for the treatment of 5-HT₃ receptor-related disorders.

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Conflict of interest

The authors state no conflict of interest.

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