Receptor–drug association studies in the inhibition of the hematin aggregation process of malaria

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Abstract Docking studies were performed to investigate the binding of several antimalarial compounds to the putative drug receptors involved in the hematin aggregation process. These studies reveal a binding profile that correlates with the complementarity of electrostatic potentials between the receptors and the active molecules. These results allow a possible explanation for the same molecular mechanism shown by 4-aminoquinolines, quinine, mefloquine, halofantrine and hydroxylated xanthones. The docking data presented in this work offer an interesting approach to the design of new molecules with potential antimalarial activity.

Key words: Ab initio quantum mechanics; Antimalarial compound; Docking; Hematin aggregation; Stereoelectronic property; Xanthone

1. Introduction

Malaria is a disease of major relevance in the world, with a prevalence of 300–500 million clinical cases and 2 million deaths each year [1]. The loss of efficacy of the available antimalarial compounds due to drug resistance increases the concerns about this health problem [1]. The continuing spread of drug-resistant malaria imposes the need for the development of new potential antimalarial molecules. This can be accomplished with the understanding of the molecular mechanisms that can lead to an antimalarial action. One of these mechanisms is the inhibition of the detoxification of the heme released from the hemoglobin digestion [2]. The malaria parasite has a limited capacity for amino acid synthesis, acquiring the essential nutrients from hemoglobin proteolysis [2]. This digestion of hemoglobin releases heme, which oxidizes to hematin and originates a toxic action against the parasite [3]. Plasmodial species detoxify by sequestering this by-product into a chemically inert crystal of hemozoin, overcoming the lack of the heme oxygenase, the defensive mechanisms used by vertebrates [3–5]. Hemozoin is an aggregate of several units of hematin linked by carboxylate–iron(III) and carboxylate–carboxylate coordinated bonds [6,7].

Several antimalarial compounds interfere in this metabolic pathway by associating with a derivative form of hematin, thus avoiding the aggregation of this porphyrin in the hemozoin formation process [8]. This provides a sufficient amount of free hematin to develop an antimalarial action [9]. Thus, the referred derivative form of hematin constitutes a pharmacological receptor of interest.

The most defended possibility is that the derivative form referred to corresponds to a μ-oxo-dimer of hematin [8,10,11], a complex of two molecules of hematin connected by an oxygen bridge between the two iron atoms [7].

The definition of the structure of the malaria pigment by high-resolution X-ray powder diffraction allowed the establishment of hemozoin as another possibility for a drug receptor [6,12]. The antimalarial drug action could arise from surface adsorption on the growing hemozoin crystallites, limiting new hematin uptake [6]. The consequently free hematin would develop its toxicity against the parasite.

The antimalarial 4-aminoquinolines mefloquine, halofantrine and quinine can inhibit parasite growth by preventing the formation of hemozoin [13–17]. Hydroxylated xanthones also have shown antimalarial activity, pointing to the same molecular mechanism [18–21].

Several studies have been performed on antimalarial compounds, relating their activity with their electrostatic potential [22–26]. We present a study relating different antimalarial molecules with the two putative receptors that are involved in their antiplasmodial action. Electrostatic interactions are one of the phenomena that rule the association between a molecule and its receptor. The electrostatic potential profiles determined for the active molecules offer a possibility of complementarity with the electrostatic potentials determined for the two putative receptors. To corroborate our findings we have performed docking studies between the referred active compounds and the putative receptors hematin μ-oxo-dimer and hemozoin. The results obtained agree with the molecular electrostatic potentials.

2. Materials and methods

Geometry optimizations and energy calculations for all the ligands in the neutral form were performed at an ab initio quantum mechanical level by using density functional theory (DFT) with the Becke3–Lee–Yang–Parr (B3LYP) functional, and the 3-21G basis set, followed by single point calculations with the 6-31G(d) basis set.

The three-dimensional structure of the μ-oxo-dimer of hematin had to be modeled, because it has not been resolved experimentally. A conformational search was first performed using the CHARMM force field [27,28] to find out the most likely position of the porphyrin subunits relative to each other, as well as the propionic and vinyl substituents. A geometry optimization was then carried out with the

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AM1(d) semi-empirical method [29,30] and the resulting neutral structure was further subjected to a single point calculation using DFT with the B3LYP functional and the 6-31G(d) basis set.

The three-dimensional structure of the hemozoin dimer was obtained from the Cambridge Crystallographic Database [6]. The structure was further subjected to a single point calculation using DFT with the B3LYP functional and the 6-31G(d) basis set.

Both hemozoin and the μ-oxo-dimer of hematin show a spin state of $^{5}/_{2}$, as determined by Mössbauer spectroscopy [31]. This was the value considered in the calculations performed on the hematin complexes.

All ab initio calculations were performed with the Gaussian 98 package [32].

Molecular electrostatic potential surfaces were drawn using the CUBEGEN utility present in Gaussian 98 [32], applied to the optimized geometries of all molecules. The visualization of all quantum calculations results was performed with Molekel 4.2 [33].

The docking procedure was performed with GOLD-Genetic Optimization for Ligand Docking, using both scoring functions (Goldscore and Chemscore) included in the software [34–39]. The Goldscore fitness function has four components: protein–ligand bond energy, protein–ligand van der Waals energy, ligand internal van der Waals energy, and ligand torsional strain energy. This fitness function has been optimized for the prediction of ligand binding positions. The Chemscore function was derived empirically from a set of 82 protein–ligand complexes for which measured binding affinities were available. This function estimates the total free energy that occurs in ligand binding as a summation of several physical contributions: hydrogen bonding, metal binding, lipophilic terms, rotatable bond freezing terms. The final Chemscore value is obtained by adding in a clash penalty and internal torsion terms. GOLD constitutes one of the most respected programs for docking studies [38,39]. The application of this method was performed with the active molecules and receptors in the protonation state that occurs with the pH conditions inside the parasite vacuole [6,40,41]. The work was performed in the standard default settings for the best possible predictive accuracy, with 10 docking runs for each ligand. Visualization of docking results was performed with DS ViewerLite from Accelrys.

3. Results and discussion

3.1. Molecular electrostatic potential calculations

Three-dimensional surfaces of molecular electrostatic potentials at the constant values of −10 kcal/mol and 10 kcal/mol were generated to determine the profile of the electrostatic potential of a compound when approaching another molecule. These surfaces allow the determination of points of possible interaction based on the concept of electrostatic interactions. The quantities of −10 kcal/mol and 10 kcal/mol correspond to limit values defining a long-range interaction. The definition of isosurfaces using other values does not show any significant differences.

The study of the electrostatic properties of the μ-oxo-dimer of hematin shows that the most negative potential is located on the central part of the complex, corresponding to the iron atoms and the tetrapyrrole system, with the most positive potential found in the surrounding area. The carboxyl oxygen atoms also present negative electrostatic potential (Fig. 1). This profile is represented by electrostatic potential isosurfaces at values of −10 kcal/mol, with a large surface centered on the dimer, corresponding to the system containing the iron, oxygen bridge and the pyrrolic nitrogen atom system. Smaller surfaces can be found over the carboxylic oxygens. The area around these surfaces corresponds to a positive electrostatic potential.

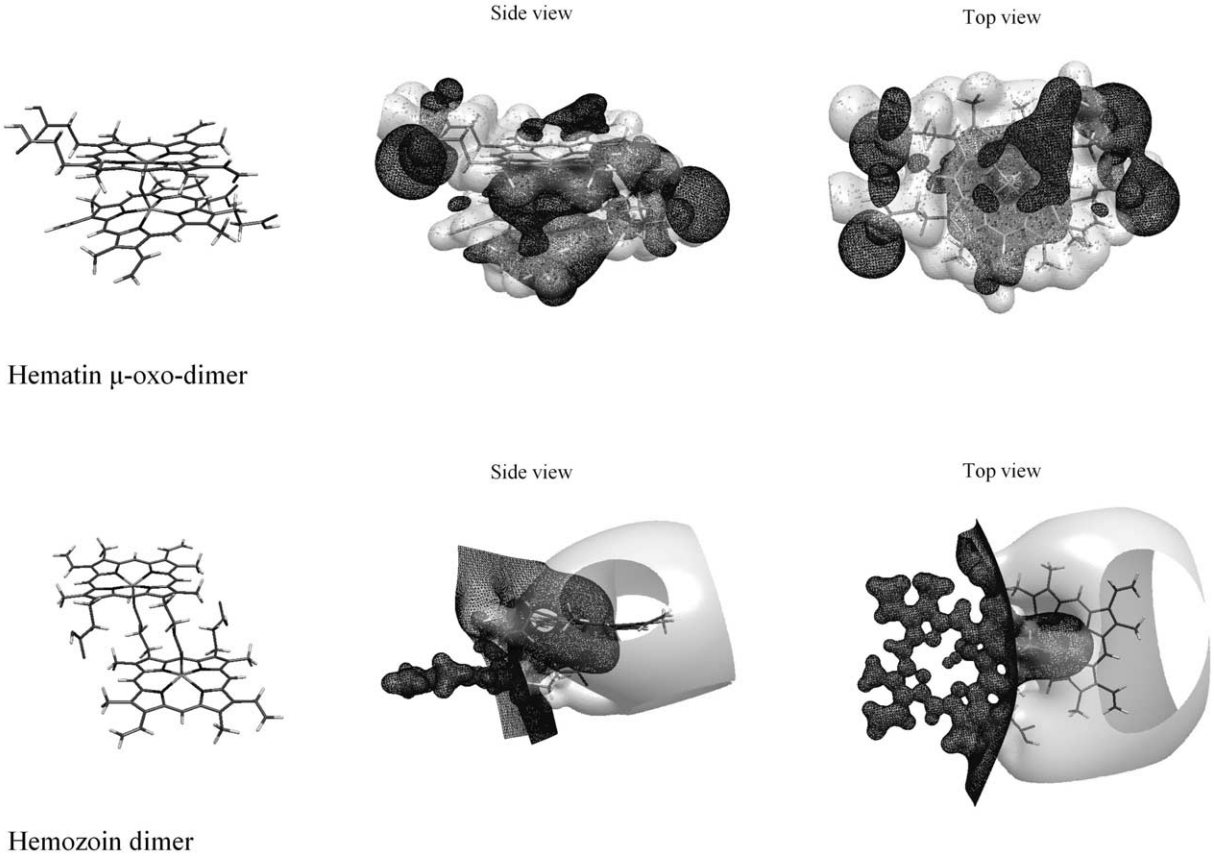

Fig. 1. Three-dimensional electrostatic potential isosurface at −10 kcal/mol (black chickenwire) and 10 kcal/mol (light gray transparent flat-shade) for hematin μ-oxo-dimer and hemozoin (side view and top view).

Observing the distribution of electrostatic potential in this receptor, we can define a possibility of attachment with an active molecule, based on the concept of complementary electrostatic profiles. The active compound should present a central point of positive potential and peripheral points of negative potential. The central point of positive potential would interact with the negative central zone of the hematin dimer, with the peripheral points of negative potential interacting with the peripheral positive area of this receptor. As a consequence, the iron(III) atoms would not be available to establish a coordination bond with a propionic chain from another hematin unit, inhibiting the formation of hemozoin.

The observation of the electrostatic potential isosurfaces at values of −10 kcal/mol of hemozoin denotes the presence of a negative value of potential at the points of carboxylate–iron (III) coordination, with a peripheral positive potential (Fig. 1).

The previous considerations about the possibility of association between the hematin μ-oxo-dimer putative receptor and an active compound are also applicable to hemozoin.

The antimalarial compounds can therefore act by stabilizing the receptor due to the electrostatic interactions determined by their complementary electrostatic profiles, avoiding a further uptake of hematin in the aggregation phenomenon.

The observation of the electrostatic profiles of the inhibitors of hematin aggregation is in accordance with the complementarity-based possibility of drug–receptor association (Fig. 2). These compounds present a null or positive potential placed over at least a part of the aromatic rings, complemented by the existence of peripheral bulks of negative potential [22–26].

3.2. Docking studies

The docking study was performed to predict how the potential antimalarial molecules presented here would bind to both putative receptors, by using a non-deterministic sampling method. The GOLD algorithm performs a stochastic search for preferred orientation and conformation of the ligand in relation to the receptor [38].

The calculations were performed on the receptors in the deprotonated form, which is the form that occurs in vivo [6]. The results presented here correspond to the best-scored solutions of docking for each structure. Both scoring functions present in GOLD were used. The results obtained with Goldscore and Chemscore for the studied compounds correlated with the complementarity of the electrostatic potentials.

The association of all 4-aminoquinolines with the hematin μ-oxo-dimer gave similar results for both scoring functions. The area around the amino group connected to the quinolinic ring, corresponding to a positive electrostatic potential, interacts with the central negative zone of the hematin dimer. The negative potential located over the aromatic area interrelates with the peripheral positive area of the μ-oxo-dimer receptor (Fig. 3a).

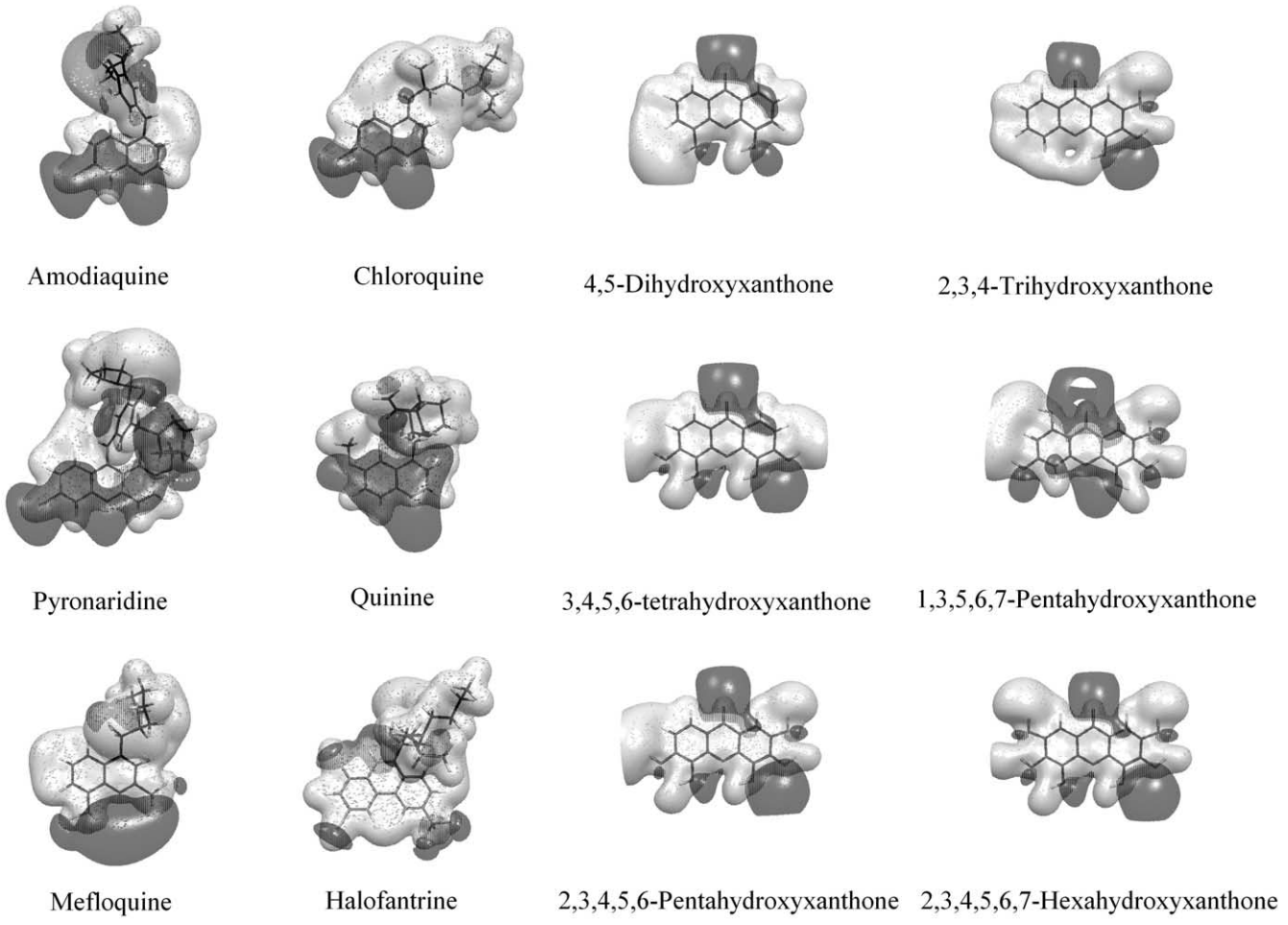

Fig. 2. Three-dimensional electrostatic potential isosurfaces at −10 kcal/mol (dark gray transparent flatshade) and 10 kcal/mol (light gray transparent flatshade) for compounds that present antimalarial activity as an inhibition of hematin aggregation.

Halofantrine presents two ways of associating with the hematin μ-oxo-dimer, both agreeing with the complementarity of electrostatic profiles. In the Chemscore result the negative central area of the hematin complex interacts with the positive aromatic rings of the drug (Fig. 3b). For the Goldscore function, the iron–tetrapyrrole system interacts with the hydroxyl group of halofantrine (Fig. 3c). Mefloquine and quinine also present an association between their hydroxyl group and the hematin dimer iron–tetrapyrrole system (Fig. 3d,e). The structural components corresponding to the peripheral bulks of

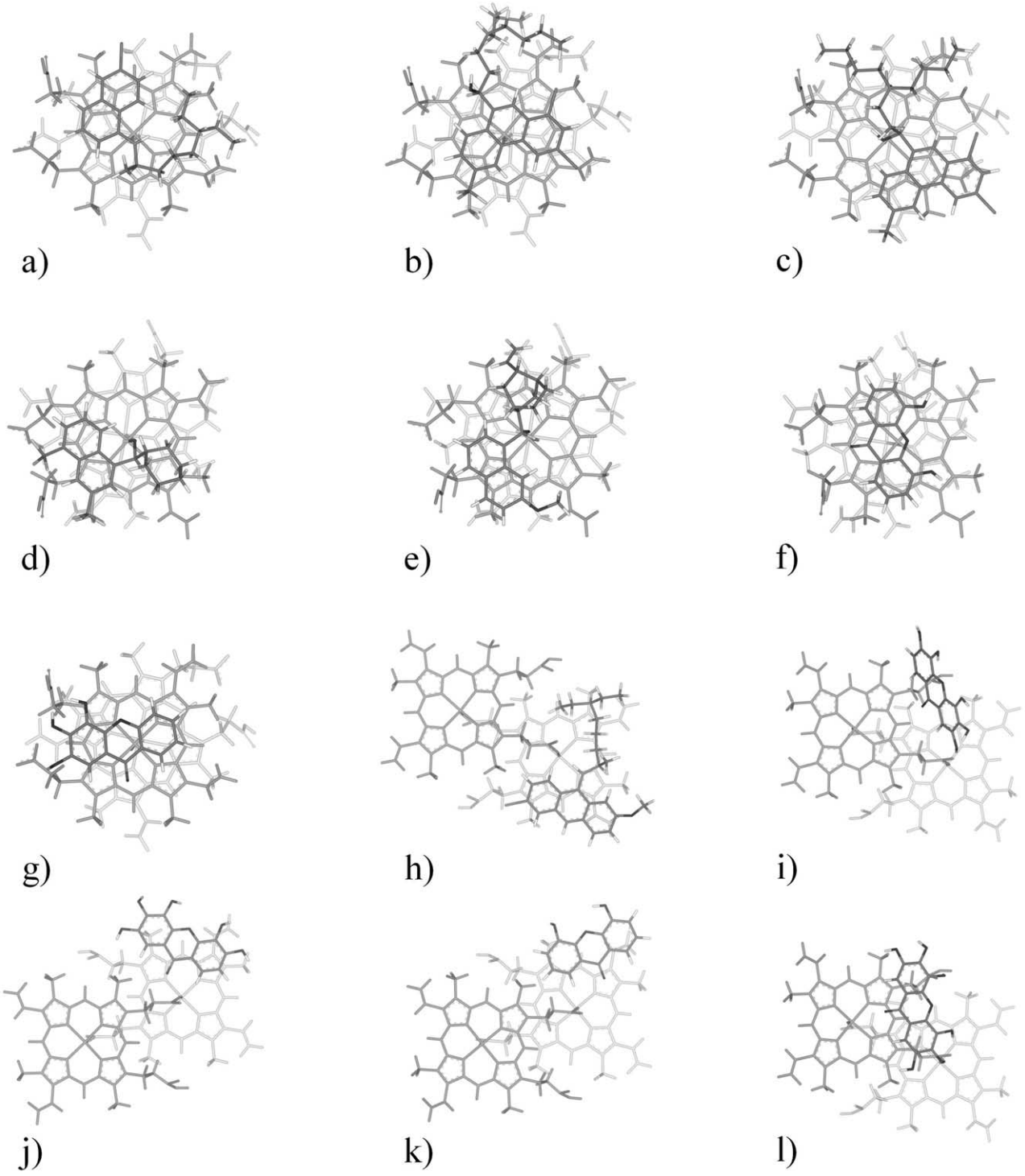

Fig. 3. Docking results (top view) for: (a) chloroquine over hematin μ-oxo-dimer, with Chemscore; (b) halofantrine over hematin μ-oxo-dimer, with Chemscore; (c) halofantrine over hematin μ-oxo-dimer, with Goldscore; (d) mefloquine over hematin μ-oxo-dimer, with Goldscore; (e) quinine over hematin μ-oxo-dimer, with Chemscore; (f) 4,5-dihydroxyxanthone over hematin μ-oxo-dimer, with Goldscore; (g) 2,3,4-trihydroxyxanthone over hematin μ-oxo-dimer, with Chemscore; (h) mepacrine over hemozoin, with Chemscore; (i) 4,5-dihydroxyxanthone over hemozoin, with Chemscore; (j) 2,3,4,5,6-pentadihydroxyxanthone over hemozoin, with Chemscore; (k) 2,3,4,5,6-pentahydroxyxanthone over hemozoin, with Goldscore; (l) 2,3,4,5,6,7-hexahydroxyxanthone over hemozoin, with Goldscore.

negative potential interact with the zone of positive potential of the hematin μ-oxo-dimer. These results show the influence of the hydroxyl group in the action of halofantrine, mefloquine and quinine.

The interaction profile of the hydroxylated xanthones is also based on electrostatic complementarity, defined between the central aromatic area of the xanthone derivatives, with positive potential, and the central area of the μ-oxo-dimer receptor, with negative potential (Fig. 3f). In addition, the Chemscore function defined the hydrogen bonding of the hydroxyl groups in positions 3 and 4 of the xanthone nucleus to one of the carboxyl groups of the hematin subunit (Fig. 3g). The observation of the stereoelectronic profile for the active xanthones shows that the hydroxyl in position 4 is always present, reinforcing the importance of this substituent.

In all results obtained on the hematin μ-oxo-dimer, the iron atom is protected from coordination with a propionic chain of another hematin, avoiding further aggregation to hemozoin crystallites.

The docking with hemozoin occurred laterally to the carboxylate–iron(III) coordination system. The association of 4-aminoquinolines mefloquine, halofantrine and quinine happened in the same way as mepacrine, presented in Fig. 3h. These docking solutions are also coherent with the concept of complementarity of electrostatic potentials. The interaction occurs between the negative area over the carboxylate–iron (III) system of hemozoin and the area of positive potential of the drug (corresponding to the zone around the connection of the side chain to the aromatic ring-conjugated system). These results do not show how the inhibition of new hematin uptake would occur.

The hydroxylated xanthones also presented different results for each scoring function, for hemozoin. In the Chemscore results, the xanthone derivatives associated with the carboxyl groups by hydrogen bonding as presented in Fig. 3i. The Goldscore function produced different results, but also with hydrogen bonding with a carboxyl group (Fig. 3j). With this scoring function, the hydroxylated xanthones are placed side by side with the carboxylate–iron(III) coordination system, except for 4,5-dihydroxyxanthone and 2,3,4,5,6,7-hexahydroxyxanthone, which gave similar results for both scoring functions. 4,5-Dihydroxyxanthone did not establish hydrogen bonds (Fig. 3k). 2,3,4,5,6,7-Hexahydroxyxanthone established hydrogen bonds with both carboxyl groups of one of the hematin subunits of hemozoin (Fig. 3l). This hydrogen bonding can interfere with the carboxylic groups' availability to coordinate with an iron atom of other hematin molecule.

The present communication suggests that the association between active molecules and their receptor can be ruled by the complementarity of electrostatic potentials. The results of the docking studies performed agreed with the opposite distribution of electrostatic potential between the receptors and the active compounds. Furthermore, the orientation results obtained with the docking process are in agreement with the nuclear magnetic resonance work performed with chloroquine and quinine [8,42]. The docking studies can be of great help in the design of new structures, which could be applicable in the inhibition of hemozoin crystallites formation. The design of new potential antimalarials based on this information is under way [43]. These new molecules are chlorinated xanthones and present a stereoelectronic profile comparable to the electrostatic properties of the compounds presented in this study.

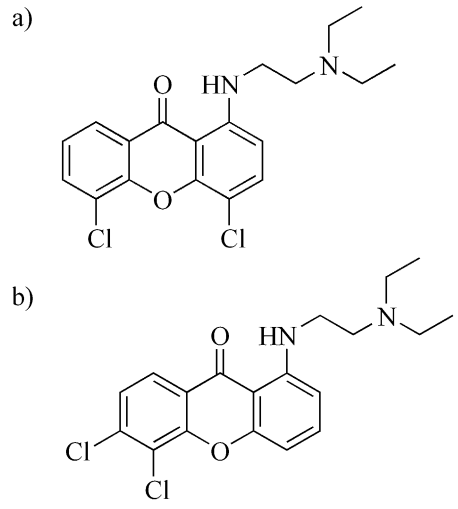

Fig. 4. Structures of new potential antimalarial compounds: (a) 4,5-dichloro-1-(2-diethylamino-ethylamino)-xanthone, (b) 5,6-dichloro-1-(2-diethylamino-ethylamino)-xanthone.

We have included such two examples in Fig. 4. We are hopeful that, under their action, the resultant free hematin will develop its toxic activity against the *Plasmodium* parasite, producing the antimalarial action.

4. Supporting information

Three-dimensional electrostatic potential isosurfaces and docking visual results for all compounds studied have been presented as the article's supporting information.

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