Effects of novel triple-stage antimalarial ionic liquids on lipid membrane models

Ricardo Ferraz [a,b,f], Marina Pinheiro [c,f], Ana Gomes [a,b], Cátia Teixeira [a,b], Cristina Prudêncio [b,d], Salette Reis [c], Paula Gomes [a,*]

[a] LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, 4069-007 Porto, Portugal
[b] ESS – Escola Superior de Saúde, Politécnico do Porto, Rua Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal
[c] LAQV-REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[d] i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal

A B S T R A C T

Primaquine-based ionic liquids, obtained by acid-base reaction between parent primaquine and cinnamic acids, were recently found as triple-stage antimalarial hits. These ionic compounds displayed significant activity against both liver- and blood-stage Plasmodium parasites, as well as against stage V *P. falciparum* parasites. Remarkably, blood-stage activity of the ionic liquids against both chloroquine-sensitive (3D7) and resistant (Dd2) P. *falciparum* strains was clearly superior to those of the respective covalent (amide) analogues and of parent primaquine. Having hypothesized that such behaviour might be ascribed to an enhanced ability of the ionic compounds to permeate into *Plasmodium*-infected erythrocytes, we have carried out a differential scanning calorimetry-based study of the interactions between the ionic liquids and membrane models. Results provide evidence, at the molecular level, that the primaquine-derived ionic liquids may contribute to an increased permeation of the parent drug into malaria-infected erythrocytes, which has relevant implications towards novel antimalarial approaches based on ionic liquids.

Keywords: Ionic liquids Liposomes Malaria Primaquine Red Blood Cells

Ionic Liquids (IL) derived from active pharmaceutical ingredients (API) may open new perspectives towards low-cost rescuing of classical drugs, given that most available API are found in the cationic or anionic form.[1–3] One example is that of antimalarial drugs, most of which are administered as salts (e.g., primaquine bisphosphate, chloroquine bisphosphate, mepacrine hydrochloride, proguanil hydrochloride, sodium artesunate, among others).[4,5] As such, antimalarial API can be combined with either an inert counterion or a counterion displaying additional biological properties, eventually producing novel drug-derived IL (API-IL) with therapeutic interest. In this connection, and following our long-term research focus in rescuing antimalarial drugs like primaquine (PQ, **1**),[6–12] or chloroquine (CQ, **2**),[13,14] we have recently developed PQ-derived ionic liquids (PQ-IL, **3a,b**).[15] These compounds were found as triple-stage antimalarial hits, i.e., were active against both liver- and blood-stage *Plasmodium* parasites, as well as against mature (stage V) *P. falciparum* parasites. Remarkably, blood-stage activity of these PQ-IL **3a,b** against both chloroquine-sensitive (3D7) and resistant (Dd2) *P. falciparum* strains was clearly superior to those of the respective covalent analogues (**4a,b**) and of parent primaquine, **1**.[15] This was somewhat puzzling, as **3a,b** and **4a,b** (Fig. 1) differ from each other in the nature of the bond established between the primaquine and the cinnamic acid building blocks: in **3a,b**, both motifs are bound through an ammonium carboxylate ionic bond, whereas in **4a,b**, the same motifs are linked through a covalent amide bond; in other words, **3a,b** may be regarded as a new ionic formulation of PQ, alternative to commercial primaquine phosphate, whereas **4a,b** substantially differ from the parent drug, including in aspects as relevant as the absence of an aliphatic amine group, traditionally regarded as a key feature in antimalarial 8-aminoquinolines, like PQ.[5,16] As such, one would expect PQ-IL **3a,b** to more closely mimic the antimalarial profile of PQ than **4a,b**, namely the low blood-stage activity of the parent drug.

In view of the above, we decided to further investigate a possible explanation for the increased blood-stage activity of PQ-IL **3a,b**, as compared to covalent analogues **4a,b**, whose chemical synthesis has been described elsewhere.[12,15] Although the full picture about the mechanism of action of antimalarial 8-aminoquinolines (8-AQ) like PQ remains to be elucidated,[17] PQ-IL **3a,b** are not expected to act on intraerythrocytic *Plasmodia* in a way that considerably diverges from that of the parent drug, or other antimalarial 8-AQ.

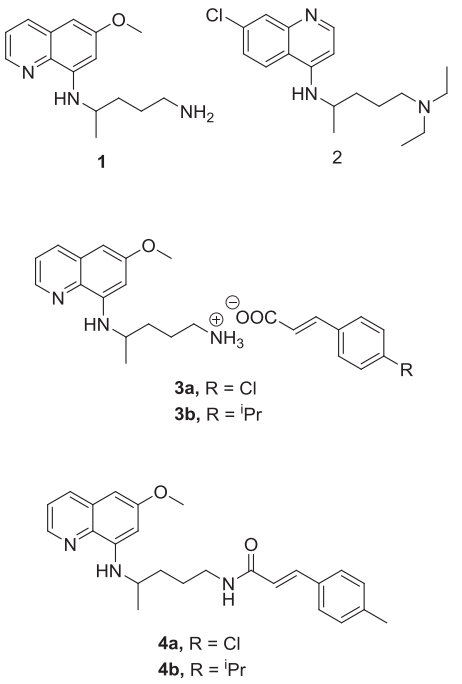

Fig. 1. Structures of classical antimalarial drugs primaquine (**1**), chloroquine (**2**), primaquine-derived ionic liquids (**3a,b**) previously found as triple stage antimalarial hits,[15] and their covalent counterparts **4a,b**.[12]

Hence, we hypothesized that **3a,b** might owe their enhanced blood-stage activity to a better ability to permeate through the membranes of *Plasmodium*-infected erythrocytes (PiRBC), and subsequently internalize the intraerythrocytic parasitophorous vacuole (PV). Accordingly, we envisaged that additional drug-membrane interaction studies would be useful to gain deeper insight, at the molecular level, into the antimalarial action of PQ-IL **3a,b**, while also relevant towards development of next-generation antimalarial API-IL with higher efficacy and safety.[18] In this connection, interactions of PQ-IL **3a,b**, and their covalent analogues **4a,b**, with lipid membrane models were investigated using differential scanning calorimetry (DSC).[19] To such end, multilamellar vesicles (MLV)[20] were used as membrane models due to their positional order, undergoing a much more cooperative gel-fluid phase transition than large unilamellar vesicles (LUV) that do not have a positional order normal to the plane of the bilayer, which results in diffuse peaks difficult to be analysed by DSC.[21] Membrane models were assembled using zwitterionic and anionic phospholipids: multilamellar vesicles (MLV) of the zwitterionic phospholipid, 1,2-dipalmitoyl-*rac*-*glycero*-3-phosphocholine (DPPC), were chosen as a simple but suitable model of the human cell membrane, mimicking the neutral surface charge of membrane of non-infected mammalian erythrocytes, mainly composed of phosphatidylcholines.[22,23] Although the parasite membrane model is much more complex containing different lipids and proteins, the charge of parasite's membrane contains an increased content in negatively charged phospholipids and for that reason the negatively charged phospholipid, 1,2-dipalmitoyl-*sn*-*glycero*-3-phospho-rac-(1-glycerol) (DPPG) was selected to prepare MLV.[24] Moreover, the composition of PiRBC is very similar to that of the parasite, which is able to traffic phospholipids between RBC and its membrane.[25] Results obtained provide support to our working hypothesis, as follows.

In vitro activity of primaquine-derived IL, **3a,b**, against blood-stage parasites of CQ-sensitive and resistant *P. falciparum* strains was recently found as superior to those of both the parent drug, **1**, and of covalent analogues **4a,b**.[15] This was an interesting finding, since (i) primaquine and similar antimalarial 8-AQ usually have no or low blood-stage activity, and (ii) the activity enhancement observed emerged from simple replacement of the usual drug's counterion (phosphate) by cinnamate ions. We rationalized that the observed effect would hardly be due to a significant change in the mode of antimalarial action of PQ-IL **3a,b** in regard to PQ itself, but instead arise from an increased permeation of this antimalarial API into PiRBC, when formulated as PQ-IL **3a,b**. As such, we studied the effect of PQ-IL on model RBC and PiRBC membranes through assessment of their influence on the thermodynamic behaviour of DPPC and DPPG MLV. Tables 1 and 2 show the calorimetric results obtained for each PQ-IL with the membrane model of DPPC and DPPG, respectively. The thermodynamic description is based on the parameters characterizing the pre-transition and the main transition temperature. The pre-transition corresponds to the transition from gel ($L\beta'$) to a rippled gel phase ($P\beta'$), whereas the main transition temperature corresponds from gel to liquid crystalline ($L\alpha$) phase transition of the liposomes.[26] Each of these phase transitions temperatures are characterized by a temperature (T_m and T_p, respectively), and an enthalpy (ΔH_m, and ΔH_p, respectively). The obtained thermodynamic parameters for the pure lipids without and with the ILs are listed in Tables 1 and 2.

The phase transition observed for MLV of pure DPPC is characterized by a T_m of 41.5 °C, a T_p of 36.2 °C with an ΔH_m of 7.3 kJ·mol^{-1} and an ΔHp of 1.14 kJ·mol^{-1} (Table 1). In the case of DPPG, the T_m obtained was 40.3 °C, the T_p 34.5 °C with an ΔH_m of 6.8 kJ·mol^{-1} and an ΔHp of 0.74 kJ·mol^{-1} (Table 2). The observed thermodynamic parameters for the lipids are in very good agreement with literature values.[27]

The calorimetric results support a pronounced interaction between PQ-IL **3a,b** and the membrane models (Tables 1 and 2), since these IL change the features of lipids thermodynamic parameters, by decreasing the melting temperatures and increasing the transition enthalpy of the lipids. Moreover, the results point to a differential interaction according to the phospholipid composition, being the interaction also influenced by the specific IL. In the presence of **3a,b**, the T_m of DPPC is 40.7 and 40.2 °C, respectively (Table 1). The main phase transition temperature decreases, which is indicative of a fluidification of the membrane induced by both compounds, and specially by **3b**. The ΔH_m obtained was 9.60 kJ·mol^{-1} and 9.30 kJ·mol^{-1} in the presence of **3a** and **3b**, respectively. The decrease in ΔH points to a destabilization of

Table 1
Thermodynamic parameters, as obtained by DSC analysis of phase pre-transition and phase transition of DPPC MLV alone and in the presence of primaquine ionic (**3a,b**) and covalent (**4a,b**) derivatives.

Lipid	Drug	T_m (°C)	T_p (°C)	ΔH_m (kJ·mol^{-1})	ΔHp (kJ·mol^{-1})
DPPC	–	41.5	36.2	7.22	1.14
DPPC	**3a**	40.7	–	9.60	–
DPPC	**3b**	40.2	–	9.30	–
DPPC	**4a**	40.1	–	8.56	–
DPPC	**4b**	40.1	–	7.91	–

Table 2
Thermodynamic parameters, as obtained by DSC analysis of phase pre-transition and phase transition of DPPG MLV alone and in the presence of primaquine ionic (**3a,b**) and covalent (**4a,b**) derivatives.

Lipid	Drug	T_m (°C)	T_p (°C)	ΔH_m (kJ·mol^{-1})	ΔH_p (kJ·mol^{-1})
DPPG	–	40.3	34.5	8.70	0.74
DPPG	**3a**	38.1	–	9.31	–
DPPG	**3b**	38.3	–	9.82	–
DPPG	**4a**	39.0	32.7	10.30	0.60
DPPG	**4b**	39.0	32.4	9.30	0.43

the model membrane. In addition, the broadening of the main transition by the presence of **3a,b** reinforces the destabilization effect induced by these IL, due to their intercalation within the lipid bilayer.[18] Moreover, PQ-IL are able to abolish the pre-transition from Lβ′ to Pβ′ of DPPC. This observation agrees with the noticed marked interaction of the drug primaquine with the zwitteronic phospholipid head groups, which seems to be related with the intercalation of this drug within the lipid bilayers.[18] The destabilization effect in the lipid bilayers depends of the chemical structure of the compounds, as **3b** seems to be responsible for a more pronounced change in the bilayers. PQ-IL **3a** is less lipophilic than **3b**, where the presence of the more hydrophobic isopropyl group is probably responsible for a deeper penetration of this compound into the lipid bilayers.[28]

The non-charged covalent analogues **4a,b** are also able to strongly interact with DPPC. In the presence of both compounds, the T_m of DPPC is 40.1 °C (Table 1). The ΔH_m obtained was 8.56 kJ·mol^{-1} and 7.91 kJ·mol^{-1} in the presence of **4a** and **4b**, respectively. Thus, the main transition temperature decreases in both cases, and therefore both compounds promote fluidification of the model membrane. In all cases, the disappearance of Lβ′ to Pβ′ transition and the increment of the ΔH reinforces the pronounced interactions of these compounds with the membrane model.

Differences are more dramatic when analyzing results presented in Table 2. Hence, it is clear that PQ-IL **3a,b** both strongly interact with the negatively charged model membrane (DPPG). In the presence of **3a** and **3b**, the T_m of DPPG is 38.1 and 38.3 °C, respectively (Table 2). The ΔH_m obtained was 9.31 kJ·mol^{-1} and 9.82 kJ·mol^{-1} in the presence of **3a** and **3b**, respectively. Thus, both compounds induce a pronounced disordering effect on the structure of DPPG bilayers. In other words, the increased ΔH_m caused by incorporation of PQ-IL reflects a large reduction in the cooperativity of the transition. Moreover, the pre-transition of the DPPG bilayers is completely abolished in the presence of PQ-IL, indicating that these compounds destabilize the gel phases of DPPG.[18]

In turn, although incorporation of the non-charged analogues **4a,b** in DPPG bilayers also leads to an alteration of the lipid mixture layer arrangement and chain packing, effects are clearly less pronounced. In the case of **4a**, the T_m obtained was 39.0 °C, the T_p 32.7 °C with an ΔH_m of 10.30 kJ·mol^{-1} and an ΔH_p of 0.60 kJ·mol^{-1} (Table 2). In the presence of **4b**, the T_m of DPPG is also 39.0 °C, a Tp of 32.4 °C with an ΔH_m of 9.30 kJ·mol^{-1} and an ΔHp of 0.43 kJ·mol^{-1} (Table 2). Thus, both compounds lead to a decrement of the transition temperatures and an increment of the ΔH, but with an overall lower destabilization effect on the negatively charged phospholipids bilayers, as compared with their ionic counterparts, **3a,b**. This difference may be explained by the more pronounced electrostatic interactions of the charged antimalarial drugs with the polar head groups of the phospholipids, which in turn may underlie a more effective permeation of PQ-IL **3a,b** into PiRBC, as compared to **4a,b**, thus explaining why the former have increased blood-stage antimalarial activity than the latter.

To summarize, the interactions of novel primaquine-derived antimalarial compounds, **3a,b** and **4a,b**, with membrane models were assessed using DSC. Drug-membrane interaction studies are gaining importance in understanding the global mechanism of action of antimalarial drugs, especially in those cases where, like in antimalarial 8-AQ, such mechanism remains to be elucidated.[18] Results herein reported point to a differential interaction dependent of the charge of the head groups of the phospholipids, and on the structure of the antimalarial compound. Globally, results show that the four compounds tested are able to interact with the model membranes, as in all cases a decrease in T_m and T_p and an increase in ΔH were observed. The compounds were all able to intercalate the zwitterionic lipid bilayers, which mimic membranes of healthy RBC, making them more fluid. However, the same type of effects were clearly more pronounced on negatively-charged lipid bilayers that mimic membranes of PiRBC, and also of intraerythrocytic parasites, especially for ionic derivatives **3a,b**, as compared to their uncharged counterparts **4a,b**. This may underlie the fact that ionic compounds **3a,b** have stronger antimalarial effects against intraerythrocytic parasites, than their covalent analogues, **4a,b**. It should be noted that the increased permeability of many drugs into PiRBC has been often ascribed to the so-called "New Permeability Pathways" (NPP), i.e., new channel proteins that are recruited (or activated) into the membranes of RBC once these become infected, allegedly to promote parasite growth and viability by facilitating uptake of nutrients and discharge of toxins;[29–34] actually, cinnamic acid derivatives had been earlier suggested to internalize PiRBC via NPP,[29] which prompted us to engage into a preliminary study of compounds **4** as NPP inhibitors; although some inhibition could be observed (data not shown), it was not strong enough to explain the low nanomolar in vitro activities of these compounds. Moreover, the higher lipophilicity of **3b** with respect to **3a** may be correlated with the more pronounced effect of the former as compared to the latter; this agrees with previous studies in our group on *N*-cinnamoylated derivatives of chloroquine[12,13] and mepacrine,[35] where the more lipophilic *p*-isopropyl-substituted derivatives were amongst the most active in vitro against blood-stage *P. falciparum*. Such behaviour might be associated to a stronger ability of those compounds to disturb membranes of PiRBC and/or of intraerythrocytic parasites, a hypothesis that is presently under investigation.

In conclusion, our results provide evidence, at the molecular level, that IL derived from antimalarial API may contribute to an increased permeation of the parent API into malaria-infected erythrocytes. As such, new API-IL derived from other classical antimalarial scaffolds are being studied in our group, and new findings thereof will be timely reported that might reveal relevant implications towards novel IL-based antimalarial approaches.

References

1. Ferraz R, Branco LC, Marrucho IM, et al. Development of novel ionic liquids based on ampicillin. *MedChemComm*. 2012;3:494–497.
2. Ferraz R, Branco LC, Prudencio C, Noronha JP, Petrovski Z. Ionic liquids as active pharmaceutical ingredients. *ChemMedChem*. 2011;6:975–985.
3. Ferraz R, Costa-Rodrigues J, Fernandes MH, et al. Antitumor activity of ionic liquids based on ampicillin. *ChemMedChem*. 2015;10:1480–1483.
4. Schlitzer M. Antimalarial drugs – what is in use and what is in the pipeline. *Arch Pharm*. 2008;341:149–163.
5. Teixeira C, Vale N, Perez B, Gomes A, Gomes JRB, Gomes P. "Recycling" classical drugs for malaria. *Chem Rev*. 2014;114:11164–11220.
6. Araujo MJ, Bom J, Capela R, et al. Imidazolidin-4-one derivatives of primaquine as novel transmission-blocking antimalarials. *J Med Chem*. 2005;48:888–892.
7. Ferraz R, Gomes JRB, de Oliveira E, Moreira R, Gomes P. Unanticipated stereoselectivity in the reaction of primaquine alpha-aminoamides with substituted benzaldehydes: a computational and experimental study. *J Org Chem*. 2007;72:4189–4197.
8. Vale N, Prudencio M, Marques CA, et al. Imidazoquines as antimalarial and antipneumocystis agents. *J Med Chem*. 2009;52:7800–7807.
9. Matos J, da Cruz FP, Cabrita E, et al. Novel potent metallocenes against liver stage malaria. *Antimicrob Agents Chemother*. 2012;56:1564–1570.
10. Matos J, Vale N, Collins MS, et al. PRIMACENES: novel non-cytotoxic primaquine-ferrocene conjugates with anti-Pneumocystis carinii activity. *MedChemComm*. 2010;1:199–201.
11. Vale-Costa S, Vale N, Matos J, et al. Peptidomimetic and organometallic derivatives of primaquine active against Leishmania infantum. *Antimicrob Agents Chemother*. 2012;56:5774–5781.
12. Pérez B, Teixeira C, Albuquerque IS, et al. PRIMACINS, N-cinnamoyl-primaquine conjugates, with improved liver-stage antimalarial activity. *MedChemComm*. 2012;3:1170–1172.
13. Perez B, Teixeira C, Gut J, Rosenthal PJ, Gomes JRB, Gomes P. Cinnamic acid/chloroquinoline conjugates as potent agents against chloroquine-resistant Plasmodium falciparum. *ChemMedChem*. 2012;7:1537–1540.
14. Perez BC, Teixeira C, Albuquerque IS, et al. N-cinnamoylated chloroquine analogues as dual-stage antimalarial leads. *J Med Chem*. 2013;56:556–567.
15. Ferraz R, Noronha J, Murtinheira F, et al. Primaquine-based ionic liquids as a novel class of antimalarial hits. *RSC Adv*. 2016;6:56134–56138.
16. Vale N, Moreira R, Gomes P. Primaquine revisited six decades after its discovery. *Eur J Med Chem*. 2009;44:937–953.
17. Baird JK, Rieckmann KH. Can primaquine therapy for vivax malaria be improved? *Trends Parasitol*. 2003;19:115–120.
18. Basso LGM, Rodrigues RZ, Naal R, Costa AJ. Effects of the antimalarial drug primaquine on the dynamic structure of lipid model membranes. *Biochim Biophys Acta-Biomembr*. 2011;1808:55–64.
19. *Multilamellar vesicles (MLV):* Each drug was mixed with DPPC and DPPG in chloroform:methanol mixture (3:1 v/v) in the molar ratio of lipid (L):drug (D) 10:1 The tested drugs with a final concentration of 150 μM were inserted in the organic solvents since were water insoluble, being this a general procedure to study lipophilic drugs.[36–39] Lipid films were formed from these solutions, dried at 60.0 ± 0.1 °C under a stream of N_2 and left overnight under reduced pressure to remove all traces of the organic solvents. The lipid films were hydrated by adding the buffer system and then alternately heated at 60.0 ± 0.1 °C, mixed by vortexing for about 10 min. This procedure was repeated three times. Finally, the samples were aged overnight at 4.0 ± 0.1 °C and shaken by vortex at room temperature.[22]
20. D*ifferential scanning calorimetry (DSC)*: Differential scanning calorimetry (DSC) was performed in a Malvern MicroCal VP-DSC (Malvern, Worcestershire, UK). Samples were run against HEPES buffer in the reference cell, and blank experiments with HEPES buffer in both cells were also performed for subsequent blank correction. The solution or suspension volume used in each cell was 0.5261 mL. Two successive heating and cooling scans were performed for each sample, the heating scan at a scanning rate of 1 °C/min and the cooling scan at 3 °C/min, over the temperature range of 30–50 °C for DPPC or 20–50 °C for DPPG. The results provided correspond to the second heating scan, as we have observed that small differences can exist between first and second scans, but not thereafter. Samples with drug-to-lipid (D:L) molar ratios of 1:10 were used. All procedures regarding sample preparation and handling (lag time at low temperature, time between mixtures, and start of the experiment) were kept constant in all experiments, to ensure that all samples had the same thermal history. The instrument was electrically calibrated for temperature and the scan rate with the SETARAM Calibration Unit. The Micro-DSCIII software was used for blank subtraction [run with buffer solution on both cells (sample and reference)]. T_m, T_p, and the ΔH_m and ΔH_p were calculated by integration of the heat capacity versus temperature curve (C_p versus Temperature). A linear baseline was used to calculate the integral areas under the curves.[39]
21. Kreutzberger MA, Tejada E, Wang Y, Almeida PF. GUVs melt like LUVs: the large heat capacity of MLVs is not due to large size or small curvature. *Biophys J*. 2015;108:2619–2622.
22. Pinheiro M, Arede M, Nunes C, et al. Differential interactions of rifabutin with human and bacterial membranes: implication for its therapeutic and toxic effects. *J Med Chem*. 2013;56:417–426.
23. Teixeira V, Feio MJ, Rivas L, et al. Influence of lysine N-epsilon-trimethylation and lipid composition on the membrane activity of the cecropin A-melittin hybrid peptide CA(1–7)M(2–9). *J Phys Chem B*. 2010;114:16198–16208.
24. Hsiao LL, Howard RJ, Aikawa M, Taraschi TF. MOdification of host-cell membrane lipid-composition by the intra-erythrocytic human malaria parasite *Plasmodium falciparum*. *Biochem J*. 1991;274:121–132.
25. Vial HJ, Eldin P, Tielens AGM, van Hellemond JJ. Phospholipids in parasitic protozoa. *Mol Biochem Parasitol*. 2003;126:143–154.
26. Chiu M, Prenner E. Differential scanning calorimetry: an invaluable tool for a detailed thermodynamic characterization of macromolecules and their interactions. *J Pharm Bioallied Sci*. 2011;3:39–59.
27. Jing WG, Demcoe AR, Vogel HJ. Conformation of a bactericidal domain of puroindoline a: structure and mechanism of action of a 13-residue antimicrobial peptide. *J Bacteriol*. 2003;185:4938–4947.
28. Figueiredo R, Moiteiro C, Medeiros MA, et al. Synthesis and evaluation of rifabutin analogs against Mycobacterium avium and H(37)Rv, MDR and NRP Mycobacterium tuberculosis. *Bioorg Med Chem*. 2009;17:503–511.
29. Elford BC, Haynes JD, Chulay JD, Wilson RJM. Selective stage-specific changes in the permeability to small hydrophilic solutes of human erythrocytes infected with Plasmodium falciparum. *Mol Biochem Parasitol*. 1985;16:43–60.
30. Kanaani J, Ginsburg H. Effects of cinnamic acid derivatives on in vitro growth of Plasmodium falciparum and on the permeability of the membrane of malaria-infected erythrocytes. *Antimicrob Agents Chemother*. 1992;36:1102–1108.
31. Hill DA, Desai SA. Malaria parasite mutants with altered erythrocyte permeability: a new drug resistance mechanism and important molecular tool. *Future Microbiol*. 2010;5:81–97.
32. Desai SA. Why do malaria parasites increase host erythrocyte permeability? *Trends Parasitol*. 2014;30:151–159.
33. Martin RW, Kirk K. Transport of the essential nutrient isoleucine in human erythrocytes infected with the malaria parasite Plasmodium falciparum. *Blood*. 2007;109:2217–2224.
34. Egee S, Lapaix F, Decherf G, et al. A stretch-activated anion channel is up-regulated by the malaria parasite Plasmodium falciparum. *J Physiol*. 2002;542:795–801.
35. Gomes A, Pérez B, Albuquerque I, et al. N-cinnamoylation of antimalarial classics: quinacrine analogues with decreased toxicity and dual-stage activity. *ChemMedChem*. 2014;9:305–310.
36. Augustynska D, Burda K, Jemiola-Rzeminska M, Strzalka K. Temperature-dependent bifurcation of cooperative interactions in pure and enriched in beta-carotene DPPC liposomes. *Chem Biol Interact*. 2016;256:236–248.
37. Kim YA, Tarahovsky YS, Yagolnik EA, Kuznetsova SM, Muzafarov EN. Integration of quercetin-iron complexes into phosphatidylcholine or phosphatidylethanolamine liposomes. *Appl Biochem Biotechnol*. 2015;176:1904–1913.
38. Mady MM, Shafaa MW, Abbase ER, Fahium AH. Interaction of doxorubicin and dipalmitoylphosphatidylcholine liposomes. *Cell Biochem Biophys*. 2012;62:481–486.
39. Abrunhosa F, Faria S, Gomes P, et al. Interaction and lipid-induced conformation of two cecropin-melittin hybrid peptides depend on peptide and membrane composition. *J Phys Chem B*. 2005;109:17311–17319.