Bioorganic & Medicinal Chemistry Letters 19 (2009) 3476–3480

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and structure–activity relationships of (1*H*-pyridin-4-ylidene)amines as potential antimalarials

Tiago Rodrigues [a], Rita C. Guedes [a], Daniel J. V. A. dos Santos [a], Marta Carrasco [a], Jiri Gut [b], Philip J. Rosenthal [b], Rui Moreira [a,*], Francisca Lopes [a]

[a] *iMed.UL, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal*
[b] *Department of Medicine, San Francisco General Hospital, University of California, San Francisco, Box 0811, CA 94143, USA*

ARTICLE INFO

Article history:
Received 6 April 2009
Revised 5 May 2009
Accepted 5 May 2009
Available online 9 May 2009

Keywords:
Antiplasmodial
(1*H*-Pyridin-4-ylidene)amines

ABSTRACT

(1*H*-Pyridin-4-ylidene)amines containing lipophilic side chains at the imine nitrogen atom were prepared as potential clopidol isosteres in the development of antimalarials. Their antiplasmodial activity was evaluated in vitro against the *Plasmodium falciparum* W2 (chloroquine-resistant) and FCR3 (atovaquone-resistant) strains. The most active of these derivatives, **4m**, had an IC_{50} of 1 μM against W2 and 3 μM against FCR3. Molecular modeling studies suggest that (1*H*-pyridin-4-ylidene)amines may bind to the ubiquinol oxidation Q_o site of cytochrome bc_1.

Malaria is a serious global health problem, and the problem is complicated by the rapid emergence and spread of multidrug-resistant *Plasmodium falciparum*.[1] The urgent need for new drugs triggered the search for lead compounds, preferably acting on underexploited parasite targets.[2,3] Targeting the mitochondrial electron transport chain of the human malaria parasite has proved to be a valid chemotherapeutic strategy[4,5] as shown by atovaquone (**1**, Fig. 1), which is used in combination with proguanil to treat multidrug-resistant *P. falciparum* infections.[6] Atovaquone is a potent inhibitor of the bc_1 complex, a mitochondrial multisubunit energy-transducing membrane protein.[7,8] Atovaquone binds selectively to the Q_o site of cytochrome *b*, close to the site of interaction with the Rieske iron-sulfur protein (ISP), displacing ubiquinol and substantially blocking the conformational change of the ISP that is required for electron transfer to cytochrome c_1.[8] This inhibition of electron transfer by atovaquone blocks respiration and produces a concomitant collapse of mitochondrial transmembrane potential.[8–10]

Despite its effectiveness, atovaquone resistant strains swiftly emerged when it was introduced in clinical therapy.[11] Interest in developing novel inhibitors of parasite respiration as potential antimalarials led to the re-discovery of the anticoccidial drug clopidol, **2**, a 4(1*H*)-pyridone with antiplasmodial activity that inhibits the mitochondrial respiration.[12,13] The structure of clopidol was modified based on the assumption that it could be acting as ubiquinone antagonist.[14]

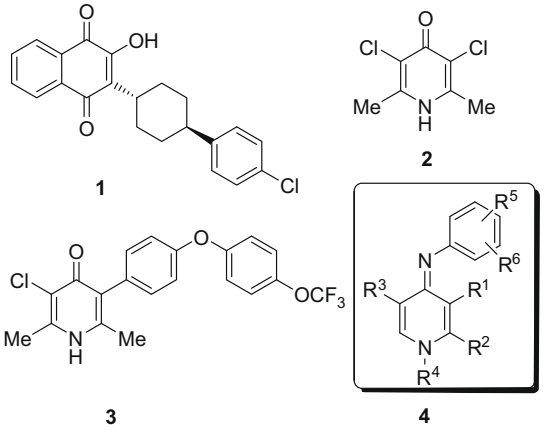


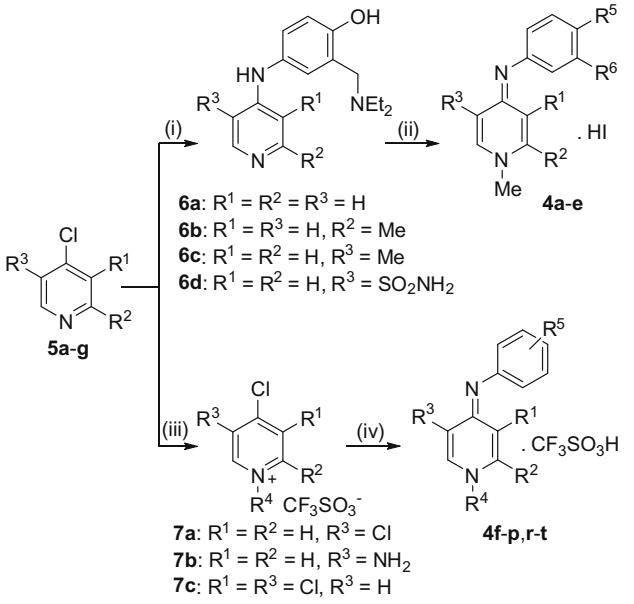
Figure 1. Structures of atovaquone, **1**, clopidol, **2**, GW844520, **3**, and (1*H*-pyridin-4-ylidene)amines, **4**.

Replacing the 5-Cl of clopidol by lipophilic side chains led to the discovery of GW844520, **3**, which displays excellent in vitro activity against multi-drug resistant *P. falciparum* strains coupled with low frequency of spontaneous in vitro resistance.[14] We now report our research on the (1*H*-pyridin-4-ylidene)amine scaffold, **4**, as a potential isostere of clopidol and as a potential antimalarial.

Based on the evidence that a lipophilic chain can improve antimalarial activity of 4(1*H*)-pyridones relative to that of clopidol,[14] we decided to incorporate an aromatic moiety at the imine nitro-

* Corresponding author. Tel.: +351 217946476; fax: +351 217946470.
E-mail address: rmoreira@ff.ul.pt (R. Moreira).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.05.017

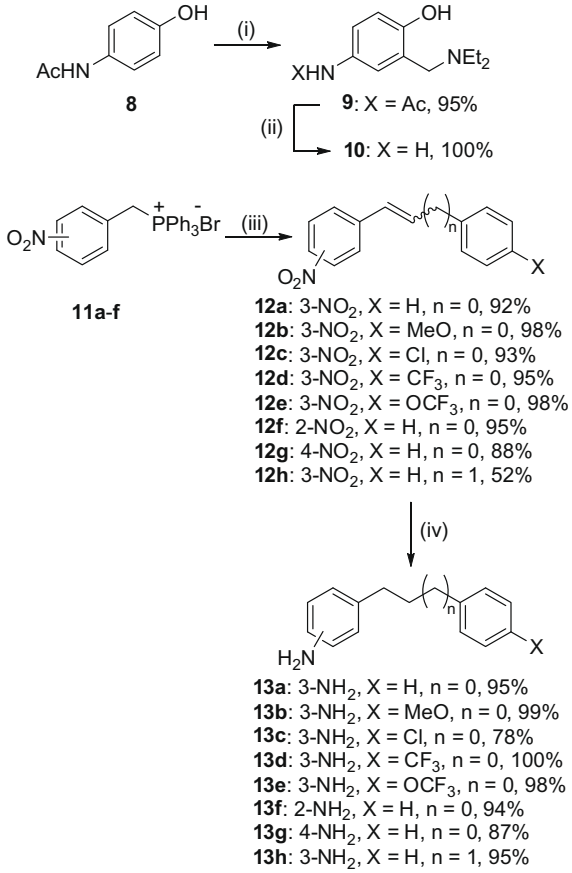


Scheme 1. Synthesis of (1*H*-pyridin-4-ylidene)amines **4a–l**. Reagents and conditions: (i) **10**, EtOH, reflux, 54–89%; (ii) (a) NaH, DMF, 10 min, rt; (b) MeI, 59–73%; (iii) MeOTf or EtOTf; (iv) **13a–h**, TEA, EtOH, reflux. See Tables 1–3 for structures of compounds **4**.

gen atom of the (1*H*-pyridin-4-ylidene)amine scaffold. A first set of compounds, **4a–e**, was prepared via the appropriate 4-(*N*-arylamino)pyridines **6a–d** (Scheme 1). Briefly, 4-chloropyridines **5a–d** were refluxed in EtOH with the Mannich base **10**, prepared using a previously described procedure (Scheme 2),[15] to give intermediates **6a–d** in good yield. Reacting compounds **6a–d** with methyl iodide and sodium hydride in DMF afforded the final products **4a–e**, as iodide salts, in 25–76% yield. For R^2 = Me, **6b**, methylation gave the desired **4b** as well as the corresponding methylamonium salt, **4c**. For **6d** ($R^3 = SO_2NH_2$), methylation occurred at the sulfonamide, phenol and diethylaminomethyl groups (i.e., giving **4e**).

The remaining compounds containing extended lipophilic side chains were prepared via the pyridinium triflates **7a–c** (Scheme 1). These were reacted with the appropriate anilines **13a–h** to give **4f–p** and **4r–t**, as triflate salts, in 11–97% yield.[16] Compounds **13a–h** were synthesised using Wittig chemistry in phase-transfer catalysis conditions (Scheme 2), which afforded alkenes **12** in very good yields.[17] Compounds **12** were then reduced in excellent yields to the corresponding phenethylanilines **13a–g** and 3-(3-phenylpropyl)aniline, **13h**, by Pd-C-catalysed hydrogenation with triethylsilane in methanol (Scheme 2).[18] The *N*-hydroxy derivative **4q** was synthesised by oxidation of the 4-(*N*-arylamino)pyridine **6e** with *m*CPBA (Scheme 3). The geometry of the imine C=N bond was established as being *E*, based on NOESY experiments, a result consistent with the reported X-ray crystal structure for **4e**.[19]

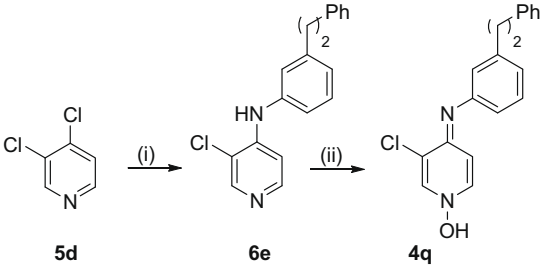
All compounds **4a–t** were tested in vitro against chloroquine-resistant W2 and atovaquone-resistant FCR3 *P. falciparum* strains.[20] For the series based on the Mannich-base moiety (Table 1), derivative **4a**, unsubstituted at the 1*H*-pyridin-4-ylidene moiety, presented IC_{50} values of 8.6 and 7.9 μM against the W2 and FCR3 strains, respectively, that is, close to those of clopidol (9.7 and 3.4 μM, respectively). Incorporation of a methyl group at C-3 of the 1*H*-pyridin-4-ylidene moiety (i.e., **4d**) led to a significant decrease in antiplasmodial activity when compared to the parent **4a**. However, a methyl group at C-2 (i.e., **4b**) led to a decrease in activity against the W2 strain but not against the FCR3 strain. Compounds with cationic side chains (**4c** and **4e**) were inactive. Increasing lipophilicity of (1*H*-pyridin-4-ylidene)amines by replacing the Mannich-base side-chain at the imine moiety for a phenyl-



Scheme 2. Synthesis of anilines required for compounds **4a–t**. Reagents and conditions: (i) DEA, CH_2O, EtOH, reflux, 75%; (ii) HCl 6 N, reflux, 100; (iii) (a) NaOH (1.2 M equiv)/CH_2Cl_2, rt; (b) ArCHO; (iv) Et_3SiH, Pd/C, MeOH, rt.

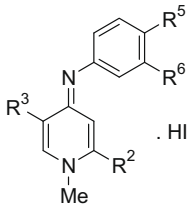
ethyl group and incorporating a chlorine atom at C-3 led to an improvement in activity (**4f** vs **4a**; Table 2). We then focused our SAR study on (1*H*-pyridin-4-ylidene)amines with a phenylethyl side-chain. Inspection of the data in Table 2 allows the following observations:

1. Exchanging the *N*-methyl for an *N*-ethyl group (i.e., R^4) had relatively little effect on activity against the W2 and FCR3 strains (3-Cl: **4f** vs **4h**; 3-NH_2: **4n** vs **4o**).
2. For the 3-Cl series, a 4-OCF_3 substituent at the terminal aromatic ring increased activity against the W2 and FCR3 strains, when compared to the unsubstituted counterpart (**4l** vs **4h**). The substituent effect on the antiplasmodial activity against both strains was 4-OCF_3 (**4l**) > 4-CF_3 (**4k**) > 4-Cl (**4j**) > H (**4h**) ≈ 4-MeO (**4i**), which is similar to that reported for the SAR of clopidol analogues.[14] In contrast, for the 3-NH_2 analogues, a 4-OCF_3 substituent at the terminal aromatic ring had



Scheme 3. Synthesis of compound **4q**. Reagents and conditions: (i) **13a**, TEA, EtOH, reflux; (ii) *m*CPBA, CHCl, reflux.

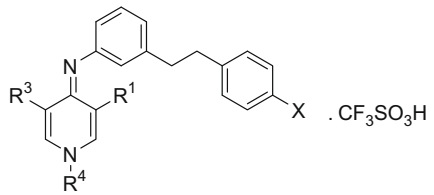
Table 1
Antiplasmodial activity of (1*H*-pyridin-4-ylidene)amines containing a Mannich-base side chain, **4a–e**



Compound	R^2	R^3	R^5	R^6	Yield (%)	IC_{50}[a] (μM)	
						W2	FCR3
4a	H	H	OH	CH_2NEt_2	59	8.61 (±0.41)	7.90 (±0.24)
4b	Me	H	OH	CH_2NEt_2	66	20.7 (±1.7)	8.09 (±0.44)
4c	Me	H	OH	$CH_2N(Me)Et_2{}^+I^-$	25	>50	9.86 (±0.28)
4d	H	Me	OH	CH_2NEt_2	73	33.0 (±0.7)	>10
4e	H	SO_2NMe_2	OMe	$CH_2N(Me)Et_2{}^+I^-$	36	>50	>10
Clopidol						9.73 (±0.07)	3.37 (±0.19)
Atovaquone						0.0012	1.89 (±0.10)
Chloroquine						0.052	0.051
3, GW844520						0.03 (T9-96 strain)[14]	

[a] Antiplasmodial activities determined as described previously.[20]

Table 2
Effect of C-3, C-5, N-1 substitution at the (1*H*-pyridin-4-ylidene)amine scaffold on the antiplasmodial activity against *P. falciparum* W2 and FCR3 strains; compound **4n** was assayed as hydroiodide; compound **4q** was obtained in the neutral form



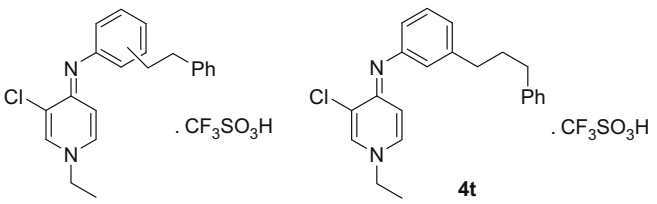
Compound	R^1	R^3	R^4	X	Yield (%)	IC_{50} (μM)	
						W2	FCR3
4f	H	Cl	Me	H	59	4.75 (±0.38)	2.89 (±0.89)
4g	H	Cl	Me	MeO	57	5.24 (±0.68)	5.12 (±1.61)
4h	H	Cl	Et	H	82	3.46 (±0.28)	3.53 (±0.29)
4i	H	Cl	Et	MeO	87	3.44 (±0.08)	3.95 (±0.93)
4j	H	Cl	Et	Cl	11	3.24 (±0.24)	2.17 (±0.33)
4k	H	Cl	Et	CF_3	76	1.60 (±0.15)	1.87 (±0.33)
4l	H	Cl	Et	OCF_3	76	1.07 (±0.07)	1.70 (±0.20)
4m	Cl	Cl	Et	OCF_3	74	0.94 (±0.12)	2.80 (±0.03)
4n	H	NH_2	Me	H	50	1.47 (±0.10)	2.21 (±0.22)
4o	H	NH_2	Et	H	97	1.67 (±0.15)	2.08 (±0.95)
4p	H	NH_2	Et	OCF_3	70	3.91 (±0.03)	1.83 (±1.27)
4q	H	Cl	OH	H	31	>8900	>8900

a detrimental effect on activity. This result is consistent with the report that electron-donating substituents at C-3 of 4(1*H*)-pyridones led to a significant drop in activity relatively to their 3-Cl analogues.[14]

3. Incorporating a second chlorine atom at C-5 of the 1*H*-pyridin-4-ylidene moiety does not affect significantly the antiplasmodial activity (**4l** vs **4m**).
4. The *N*-hydroxy derivative (**4q**; Table 2) was inactive against W2 and FCR3 strains, in line with the reduced antiplasmodial activity displayed by *N*-hydroxy-4(1*H*)-pyridones when compared to their NH counterparts.[14]
5. Regarding the position of the phenylethyl side chain (Table 3), activity against W2 was reduced both in the 2- and 4-isomers (**4r** and **4s**, respectively) when compared to the 3-analogue, **4h**. Finally, a 3-phenylpropyl side chain increased activity when compared to the 2-phenylethyl equivalent, particularly against the FCR3 strain (**4t** vs **4h**).

A DFT quantum-chemical study was performed as a first step in evaluating the stereoelectronic properties of the (1*H*-pyridin-4-ylidene)amine scaffold that might be relevant for the antiplasmodial activity. The geometries of compounds **4**, as well as those of clopidol (**2**) and GW844520 (**3**) were fully optimised at the B3LYP/6-31G(d,p) level of theory.[21,22] The molecular electrostatic potentials (MEP)[26], for compounds **2**–**4** were calculated and visually analysed. 3-D MEPs superimposed onto the total electron density provide useful information for the interpretation of long-range interaction between molecules, which helps to explain how a ligand binds to its receptor.[27] Figure 2 depicts the MEPs for **2**, **3** and **4m**, with negative electrostatic potentials (red) on the pyridone oxygen (i.e., in **2** and **3**) and imine nitrogen atoms (i.e., **4m**), suggesting that these atoms may participate in electrostatic or hydrogen bonds. For example, water-mediated hydrogen-bonding between O-3 and Glu-282 in the Q_o site has been reported for the bc_1 inhibitor atovaquone, **1**.[28] Interestingly, the heterocyclic moieties of clopidol, GW844520 and **4m** present a positive electrostatic potential, par-

Table 3
Effect of side chain orientation and length on the antiplasmodial activity of (1*H*-pyridin-4-ylidene)amines **4** against *P. falciparum* W2 strain



Compound	Isomer	Yield (%)	IC_{50} (μM)	
			W2	FCR3
4h	3-$(CH_2)_2$Ph	82	3.46 (±0.28)	3.53 (±0.29)
4r	2-$(CH_2)_2$Ph	59	6.77 (±0.38)	3.19 (±0.90)
4s	4-$(CH_2)_2$Ph	53	>10	3.74 (±0.12)
4t	3-$(CH_2)_3$Ph	33	2.43 (±0.10)	2.25 (±1.12)

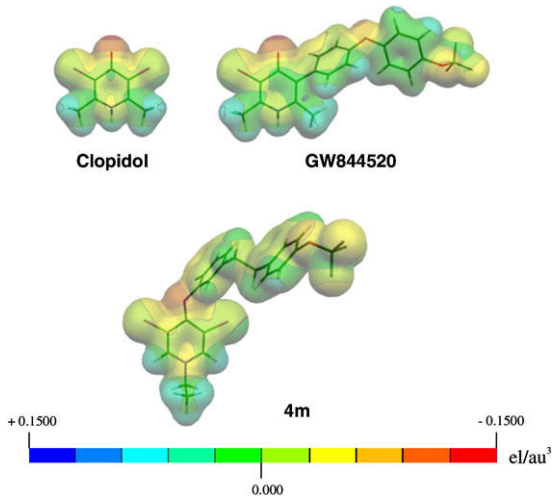


Figure 2. MEPs for clopidol, GW844520 and **4m**.

ticularly at C-2 and C-6. These observations can be extended to all active compounds **4**, thus suggesting that (1*H*-pyridin-4-ylidene)amines are likely to interact in the active site in a similar way to 4(1*H*)-pyridones.

The energies of HOMO and LUMO as well as the HOMO–LUMO gap (HLG) for compounds **4** were also calculated and those of **4m**, clopidol and GW844520 are presented in Table 4. No correlation could be found between these descriptors and antiplasmodial activity.

Presuming that cytochrome bc_1 inhibition is the likely mechanism of the antimalarial activity displayed by (1*H*-pyridin-4-ylidene)amines **4**, we undertook a docking study of these compounds as well as of clopidol and GW844520 to gain insight into their putative binding mode in the bc_1 complex using GOLD.[29] Presently, only a homology model for the cytochrome bc_1 from *P. falciparum* is available.[30] However, structures of the bc_1 complex from *Saccharomyces cerevisiae* co-crystallised with various inhibitors have been successfully used to develop a model for the binding mode of atovaquone to the Q_o site and to study cytochrome *b* mutations conferring atovaquone resistance in *Plasmodium*[31] and *Pneumocystis*.[32] In the present study we chose a bc_1 structure from *S. cerevisiae* co-crystallised with stigmatellin (PDB code: 1KYO[33]), which contains the functional homodimer with the Rieske iron-sulfur protein (ISP) in close contact with cytochrome *b*, that is, correctly oriented for electron transport. Each ligand was energy-minimised and then subjected to 5000 docking runs. The top 10 solutions (i.e., those with the highest GoldScore) were visually analysed for the hydrophobic and hydrophilic interactions between the ligand and enzyme residues.

We first validated our model by reproducing the crystallised pose of stigmatellin and studying the interaction of atovaquone in the Q_o site, which revealed that the 2-hydroxy group binds via a hydrogen bond to the nitrogen atom of His-181 of the Rieske ISP.[34] On the opposite side of the quinone system, the carbonyl carbon atom at C-4 is at 3.97 Å of the oxygen atoms of Glu-272, which can accommodate a water-mediated hydrogen bond. These interactions are consistent with the crystal structure of the yeast bc_1 complex with another hydroxyquinone bound in the Q_o binding site[34] and provide further support to our model.

Modeling the interaction of clopidol with bc_1 complex showed that the carbonyl oxygen atom is at 2.41 Å of Glu-272, which is also consistent with a water-mediated hydrogen bond (Fig. 3A, purple). In addition, only weak van der Waals contacts between the methyl and chlorine groups of clopidol with Tyr-269, Pro-271 and Leu-275 were observed. Docking GW844520 in the bc_1 complex revealed that the top ranking solutions present the 4(1*H*)-pyridone moiety almost superimposed with that of clopidol and with the side-chain at C-5 filling the hydrophobic channel leading to the Q_o centre (Fig. 3A, blue). Enhanced van der Waals contacts between the side chain with Ile-125, Ile-147, Leu-150, Phe-151, Leu-275, Met-295 and Phe-296 were observed, indicating that the bulk of intermolecular interactions of GW844520 and cytochrome *b* are essentially hydrophobic.

Docking (1*H*-pyridin-4-ylidene)amine **4l** revealed that the top ranking conformations sit in the Q_o binding site with the side chain

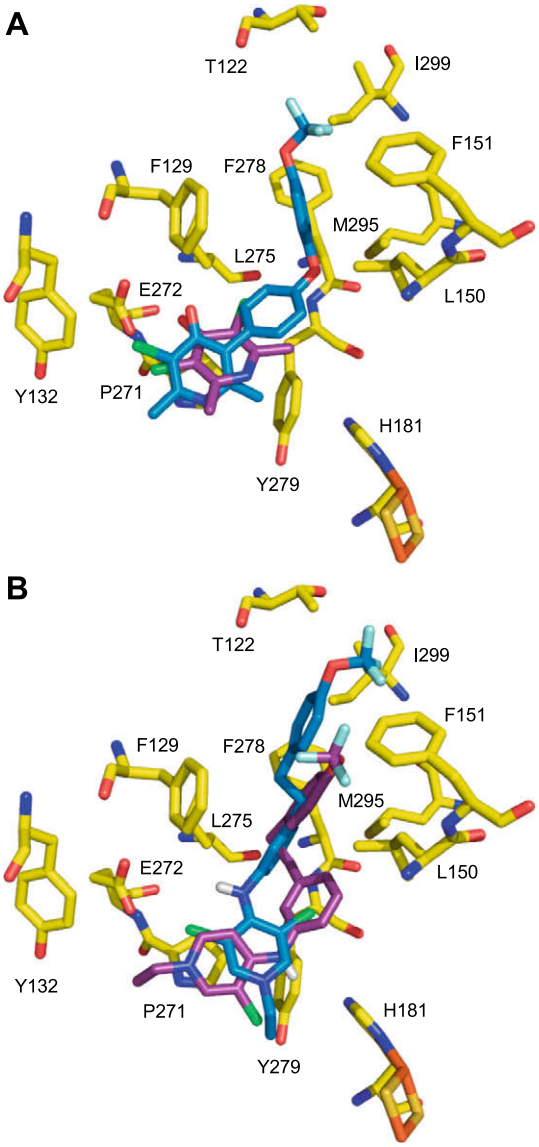


Figure 3. (A) binding modes of clopidol (purple), GW844520 (blue); (B) putative binding modes of **4l** (purple) and **4m** (blue).

Table 4
HOMO and LUMO energies, and the difference between HOMO and LUMO energy levels (HLG) for clopidol, GW844520, and **4m**

Compound	HOMO (eV)	LUMO (eV)	HLG (eV)
Clopidol	−6.03969	−0.66503	−5.37466
GW844520	−5.71534	−0.87673	−4.83861
4m	−5.26391	−0.93414	−4.32977

occupying the hydrophobic channel leading to the Q_o centre and interacting with aliphatic and aromatic side chains of Ile-125, Ile-147, Leu-150, Phe-151, Leu-275, Met-295 and Phe-296, that is, similar to GW844520 (Fig. 3B, purple). Interestingly, the iminium hydrogen atom of **4l** is in proximity (2.8 Å) to the nitrogen atom of His-181, which is compatible with an hydrogen bond. The most potent compound against the W2 strain, **4m**, presented an identical docking pose to **4l**, where the (1*H*-pyridin-4-ylidene)amine moiety is occupying the hydrophobic channel (Fig. 3B, blue). The iminium hydrogen atom of **4m** is 4.2 Å away from the oxygen atom of Glu-272, which is compatible with a water-mediated hydrogen bond. These results support the hypothesis that (1*H*-pyridin-4-ylidene)amines **4** can bind to the Q_o site in cytochrome *b*, promoting interactions with the residues that define the hydrophobic channel leading to the Q_o centre in a similar way to 4-pyridone GW844520, a known bc_1 complex inhibitor.[30]

In conclusion, a series of (1*H*-pyridin-4-ylidene)amines, **4**, containing an lipophilic side-chain at the imine nitrogen atom have been synthesised as potential isosteres of 4(1*H*)-pyridone antimalarials. Some of these (1*H*-pyridin-4-ylidene)amines display significant antiplasmodial activity against the *P. falciparum* W2 CQ-resistant and FCR3 atovaquone-resistant strains, with IC_{50} ranging from 0.9 to 7 μM. Docking studies suggest that (1*H*-pyridin-4-ylidene)amines may bind to the Q_o site of bc_1 complex, and thus are suitable scaffolds that might find further applicability in the design of antimalarials.

Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia (FCT, Portugal); T.R. acknowledges FCT for the Ph.D. grant SFRH/BD/30689/2006. P.J.R. is a Doris Duke Charitable Foundation Distinguished Clinical Scientist.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.017.

References and notes

1. Martinelli, A.; Moreira, R.; Cravo, P. V. L. *Mini-Rev. Med. Chem.* **2008**, *8*, 201.
2. Fidock, D. A.; Eastman, R. T.; Ward, S. A.; Meshnick, S. R. *Trends Parasitol.* **2008**, *24*, 537.
3. Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5274.
4. Mather, M. W.; Henry, K. W.; Vaidya, A. B. *Curr. Drug Targets* **2007**, *8*, 49.
5. Painter, H. J.; Morrisey, J. M.; Mather, M. W.; Vaidya, A. B. *Nature* **2007**, *446*, 88.
6. Bloland, P. B. *Drug Resistance in Malaria*; WHO: Geneva, 2001 (WHO/CDS/CSR/DRS/2001.4.).
7. Kessl, J. J.; Meshnick, S. R.; Trumpower, B. L. *Trends Parasitol.* **2007**, *23*, 494.
8. Mather, M. W.; Darrouzet, E.; Valkova-Valchanova, M.; Cooley, J. W.; McIntosh, M. T.; Daldal, F.; Vaidya, A. B. *J. Biol. Chem.* **2005**, *280*, 27458.
9. Hunte, C.; Palsdottir, C.; Trumpower, B. L. *FEBS Lett.* **2003**, *545*, 39.
10. Fisher, N.; Bray, P. G.; Ward, S. A.; Biagini, G. A. *Trends Parasitol.* **2007**, *23*, 305.
11. Korsinczky, M.; Chen, N.; Kotecka, B.; Saul, A.; Rieckmann, K.; Cheng, Q. *Antimicrob. Agents Chemother.* **2000**, *44*, 2100.
12. Markley, L.; Van Heertum, J.; Doorenbos, H. *J. Med. Chem.* **1972**, *15*, 1188.
13. Fry, M.; Williams, R. B. *Biochem. Pharmacol.* **1984**, *33*, 229.
14. Yeates, C. L.; Batchelor, J. F.; Capon, E. C.; Cheesman, N. J.; Fry, M.; Hudson, A. T.; Pudney, M.; Trimming, H.; Woolven, J.; Bueno, J. M.; Chicharro, J.; Fernández, E.; Fiandor, J. M.; Gargallo-Viola, D.; Heras, F. G.; Herreros, E.; León, M. L. *J. Med. Chem.* **2008**, *51*, 1845.
15. Raynes, K. J.; Stocks, P. A.; O'Neill, P. M.; Park, K.; Ward, S. A. *J. Med. Chem.* **1999**, *42*, 2747.
16. *3-Amino-4-chloro-1-ethylpyridinium triflate*, **7b**: To a solution of **5b** (0.77 mmol, 1 M equiv) in dry toluene (1.5 mL) was added ethyl triflate (0.80 mmol, 103 μL, 1.04 M equiv). The reaction mixture was left reacting at room temperature for 24 h. The precipitate was collected and washed with diethyl ether to afford **7b** as a brown solid: 95%, mp 56–58 °C. ^{1}H NMR (DMSO-d_6, 400.13 MHz) δ 1.48 (3H, t, J = 7.2, C*H*$_3$); 4.46 (2H, q, J = 7.2, C*H*$_2$); 6.92 (2H, br s, N*H*); 8.04 (1H, d, J = 6.4, Pyr-*H*3); 8.20 (1H, d, J = 6.4, Pyr-*H*2); 8.25 (1H, s, Pyr-*H*2′).
N-[(4E)-3-Amino-1-ethylpyridin-4(1H)-ylidene]-3-{2-[4-(trifluoromethoxy)phenyl]-ethyl}aniline, **4p**: Pyridinium triflate **7b** (0.80 mmol, 1 M equiv) and aniline **13e** (0.92 mmol, 1.15 M equiv) were dissolved in ethanol absolute (3.5 mL/mmol). TEA (0.92 mmol, 1.15 M equiv) was added to the solution that was kept under reflux temperature for 24 h. The solvent was evaporated under reduced pressure and the crude product purified by flash chromatography CH_2Cl_2:MeOH (9.5:0.5) to afford **4p** as a yellow oil: 70%. ^{1}H NMR (CD_3OD, 400.13 MHz) δ 1.53 (3H, t, J = 7.2, C*H*$_3$); 2.99 (4H, br s, C*H*$_2$C*H*$_2$); 4.23 (2H, q, J = 7.2, NC*H*$_2$); 6.85 (1H, d, J = 6.8, Pyr-*H*3); 7.10 (1H, s, Ar-*H*); 7.15-7.19 (4H, m, Ar-*H*); 7.27 (2H, d, J = 7.6, Ar-*H*); 7.41 (1H, t, J = 7.6, Ar-*H*); 7.78 (1H, d, J = 7.6, Pyr-*H*2); 7.82 (1H, s, Pyr-*H*2′). ^{13}C NMR (CD_3OD, 100.61 MHz) δ 15.13, 36.47, 37.00, 53.85, 105.39, 120.55, 121.57, 122.01, 124.14, 124.67, 126.68, 129.62, 129.86, 134.00, 134.20, 137.54, 140.58, 143.47, 145.05, 147.36. ESI/MS (m/z): 402.53 ($M+H^+$). Anal. Calcd for $C_{22}H_{22}F_3N_3O{\cdot}CF_3SO_3H$: C, 50.09; H, 4.20; N, 7.62; S, 5.81. Found: C, 50.34; H, 4.26; N, 7.66; S, 5.86.
17. Hwang, J.-J.; Lin, R.-L.; Shieh, R.-L.; Jwo, J.-J. *J. Mol. Catal. A: Chem.* **1999**, *142*, 125.
18. Mandal, P. K.; McMurray, J. S. *J. Org. Chem.* **2007**, *72*, 6599.
19. Rodrigues, T.; Moreira, R.; Lopes, F. *Acta Crystallogr., Sect. E* **2009**, *65*, o283.
20. Semenov, A.; Olson, J. E.; Rosenthal, P. J. *Antimicrob. Agents Chemother.* **1998**, *42*, 2254.
21. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
22. Geometries of compounds **2**–**4m** were energy-minimised using density functional theory. These calculations were performed with the B3LYP hybrid functional and the 6-31G(d,p) basis set implemented in GAUSSIAN03 software package.[23] After geometry optimisations, partial charges were included using Amber's Antechamber module[24] (included with Chimera software).[25]
23. Frisch, M. J. et al., *GAUSSIAN 03, Revision C.02*; Gaussian: Wallingford, CT, 2004.
24. Wang, J.; Wang, W.; Kollman, P. A.; Case, D. A. *J. Mol. Graph. Model.* **2006**, *25*, 247.
25. UCSF Chimera package from the Resource for Biocomputing, Visualisation, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081); Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605.
26. Portmann, S.; Lüthi, H. T. *Chimia* **2000**, *54*, 766.
27. Jones, G.; Willet, P.; Glen, R. C. *J. Mol. Biol.* **1995**, *245*, 43.
28. Kessl, J. J.; Lange, B. B.; Merbitz-Zahradnik, T.; Zwicker, K.; Hill, P.; Meunier, B.; Palsdottir, H.; Hunte, C.; Meshnick, S.; Trumpower, B. L. *J. Biol. Chem.* **2003**, *278*, 31312.
29. Molecular docking studies of compounds **2**–**4** into cytochrome bc_1 were performed with the flexible GOLD (Genetic Optimisation for Ligand Docking, v3.0.1; CCDC Software Ltd: Cambridge, UK) docking program using GoldScore scoring function. For each ligand 5000–10,000 docking runs were performed (with a population size of 100; 100,000 genetic algorithm operations; 5 islands).
30. Biagini, G. A.; Fischer, N.; Berry, N.; Stocks, P. A.; Meunier, B.; Williams, D. P.; Bonar-Law, R.; Bray, P. G.; Owen, A.; O'Neill, P. M.; Ward, S. A. *Mol. Pharmacol.* **2008**, *73*, 1347.
31. Kessl, J. J.; Ha, K. H.; Merrit, A. K.; Lange, B. B.; Hill, P.; Meunier, B.; Meshnick, S. R.; Trumpower, B. L. *J. Biol. Chem.* **2005**, *280*, 17142.
32. Kessl, J. J.; Hill, P.; Lange, B. B.; Meshnick, S.; Meunier, B.; Trumpower, B. L. *J. Biol. Chem.* **2004**, *279*, 2817.
33. Lange, C.; Hunte, C. *Proc. Natl. Acad. Sci.* **2003**, *99*, 2800.
34. Kessl, J. J.; Moskalev, N. V.; Gribblr, G. W.; Nasr, M.; Meshnick, S. R.; Trumpower, B. L. *Biochim. Biophys. Acta* **2007**, *1767*, 319.