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Synthesis and biological evaluation of new imidazo[1,2-*a*]pyridine derivatives designed as mefloquine analogues

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Abstract

This paper describes the synthesis and the in vitro antimalarial profile of two new imidazo[1,2-*a*]pyridine derivatives 4·HCl and 13·HCl, structurally proposed as mefloquine (**1**) analogues, by exploring bioisosterism and molecular simplification tools. The synthetic route employed to access the title compounds used, as starting material, the previously described ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate derivative (**5**). These novel heterocyclic derivatives 4·HCl and 13·HCl presented modest antimalarial activity against the W-2 and D-6 clones of *Plasmodium falciparum* as well as inhibitors of in vitro heme polymerization compared to mefloquine.

Keywords: Antimalarial agents; Mefloquine analogues; Imidazo[1,2-*a*]pyridine; Bioisosterism; Molecular symplification approach

1. Introduction

Malaria is a serious infectious disease caused by four species of protozoan parasites of the genus Plasmodium [1], that affects ca. 300 million people worldwide. Human malaria promoted by *Plasmodium falciparum* is the most widespread and dangerous type, being responsible for annual mortality of between 1.5 and 2.7 million people, including 1 million young children [1]. Many factors influence the effectiveness of malaria chemotherapy, with regional economics figuring prominently in affordability of medications. In the last years, the situation of malaria has become even more complex due to the rising resistance of *P. falciparum* to conventional antimalarial agents such as chloroquine [2].

Mefloquine [*d*,*l*-*erythro*-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline methanol (**1**)] was designed by applying a molecular simplification approach to the quinuclidine nucleus of the natural antimalarial quinine (**2**) (Fig. 1), and was launched in 1985, it has been employed in multidrug-resistant *P. falciparum* malaria treatment [3]. Notwithstanding, in spite of great expectations, increasing resistance to mefloquine (**1**) has been observed since its initial use [4]. Neuropsychiatric [5,6], cardiovascular [5,7] and hepatic [8] side-effects associated with the use of **1** have also been reported since its introduction. Additionally, **1** is photochemically unstable, promoting light induced toxic reactions in melanin-rich tissues [9].

Quinoline antimalarials, such as mefloquine (**1**), have a mechanism of action related to heme metabolism [10]. According to the most prevalent theory, quinoline derivatives bind to the toxic ferriprotoporphyrin IX, interfering with its detoxifying polymerization process [10,11]. Recently, Bachhawat et al. [12] suggested that molecular interaction between quinoline antimalarials and heme involves the initial electrostatic interaction of

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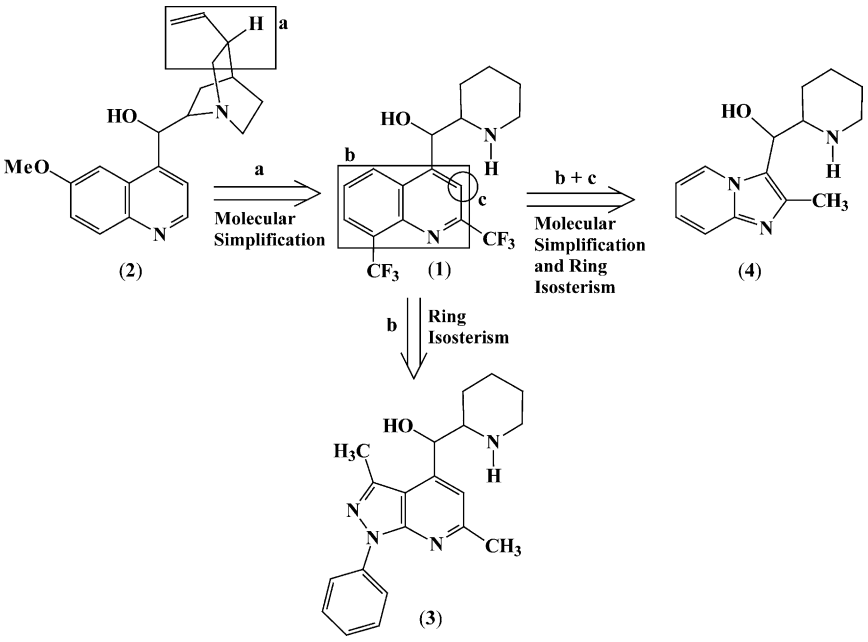


Fig. 1. Design concept of new imidazo[1,2-*a*]pyridine carbinolamine derivative **4**.

the quaternary nitrogen atom of antimalarials and an oxygen atom of the carboxylate group on heme, followed by a typical hydrophobic interaction of the quinoline nucleus and the planar porphyrin system. The protonated nitrogen atom, a consequence of the acidic internal pH of the food vacuole of the parasite (pH 5.2), is recognized as the locus of action of quinoline antimalarials [13].

In order to obtain new, effective and safe drugs to combat resistant strains of *P. falciparum* [14] we described previously the antimalarial profile of new 1*H*-pyrazolo[3,4-*b*]pyridine carbinolamine derivatives (**3**) [15]. These derivatives were planned as mefloquine (**1**) analogues and, in fact, did possess in vitro activity against mefloquine-resistant (D-6 clone) and chloroquine-resistant (W-2 clone) strains of *P. falciparum* (Fig. 1). The similar steric and electronic properties of **1** and **3** were, evidenced by molecular modeling studies which confirmed the bioisosteric relationships between a quinoline ring and a pyrazolo[3,4-*b*]pyridine nucleus [16]. Following these efforts, we report in this paper, the synthesis and antimalarial profile of the new imidazo[1,2-*a*]pyridine carbinolamine derivative **4**, designed by applying the molecular simplification of the quinoline ring of (**3**), through removal of one methine group (**c**, Fig. 1). To achieve this goal we explored the previously related isosteric relationships of quinoline and imidazo[1,2-*a*]pyridine rings [17] in order to select a heterocyclic ring pattern which would avoid formation of phototoxic 'quinonoid' species. The target compound **4** (Fig. 1) was such a molecule as will be described herein.

2. Experimental

2.1. Chemistry

M.p.s were determined with a Quimis 340 apparatus and uncorrected. ^{1}H NMR spectra were obtained in $CDCl_3$, using Me_4Si as internal standard with a Brucker AC 200 and Brucker/spectrospin 400MHz spectrometers. Splitting patterns were as follows: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; dt, double triplet; td, triple doublet; ddd, double double doublet; ddt, double double triplet. ^{13}C NMR spectra were obtained in the same spectrometers described above at 50 and 100 MHz, respectively, using $CDCl_3$ as internal standard. Infrared spectra were obtained with a Nicolet-505 Magna spectrophotometer using sodium chloride cells. High resolution mass spectra (HRMS) were measured with a Bruker BioApex FTMS system by direct injection using an electron spray interface (ESI+). HPLC analyzes were performed on a Lachrom HPLC system Merck with a L-7100 pump and a L-7450A diode array detector (set at 240 nm), equipped with a Rexchrom 5 μm RP-18 column (250 × 10 mm). Separation were done in the isocratic mode, using MeOH–water (v/v) at a flow rate of 1.5 ml/min. The HPLC grade solvents were purchased from Tedia, filtered through a Millipore filter (0.45 μm) and degassed in an ultrasonic bath prior to use.

The progress of all reactions was monitored by TLC which was performed on 2.0 × 5.0 cm aluminum sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were visualized under ultraviolet light. Merck silica gel

(70–230 mesh) was used for column chromatography. Solvents used in reactions were dried and redistilled prior to use.

2.1.1. Ethyl 2-methylimidazo[1,2-a]pyridine-3-carboxylate (*5*)

A solution of 2-aminopyridine (1 g, 10.6 mmol) and ethyl 2-chloroacetoacetate (1.60 g, 1.35 ml, 9.7 mmol) in absolute EtOH (50 ml) was refluxed for 20 h. The solvent was removed under reduced pressure and the brown oil residue was extracted with C_6H_{14} (5 × 40 ml). Combined organic layers were concentrated at half volume and placed at 0 °C to start the precipitation. The filtration of yellow crystals formed furnished ethyl ester **5** (1.74 g, 87%), m.p. 42–43 °C (literature [18] 42–44 °C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.20 (dt, 1H, H_5, $J = 7.0$ and 1.1 Hz), 7.61 (dt, 1H, H_8, $J = 8.8$ and 1.6 Hz), 7.36 (ddd, 1H, H_7, $J = 9.2$, 6.8 and 1.2 Hz), 6.98 (td, 1H, H_6, $J = 7.2$ and 1.6 Hz), 4.44 (q, 2H, –OCH_2CH$_3$, $J = 6.7$ Hz), 2.72 (s, 3H, Im–CH_3), 1.45 (t, 3H, –OCH$_2$CH_3, $J = 6.7$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.7 (*C*=O), 153.0 (C5), 147.1 (C2), 128.2 (C8a), 127.8 (C3), 116.9 (C7), 113.8 (C8), 112.8 (C6), 60.5 (O*C*H$_2$CH$_3$), 17.0 (OCH$_2$CH$_3$), 14.8 (Im–*C*H$_3$); IR (/cm): 1683 ν(C=O), 1557 ν(C=C), 1520 and 1501 ν(C=N), 1223 ν(C–O), 762 δ(N=C–H); HRMS *m/z* (ES+) *Anal*. Calc. for $C_{11}H_{12}N_2O_2$ $[M+H]^{+1}$: 205.0899, Found: 205.0785.

2.1.2. 2-Methy1imidazo[1,2-a]pyridine-3-carbaldehyde (*10*)

Preparation of reductive solution: a solution of 65% Red-Al® [bis(2-methoxyethoxy)aluminum hydride] in $C_6H_5CH_3$ (20 ml, 55 mmol) was diluted with dry $C_6H_5CH_3$ (20 ml) at 0 °C under nitrogen atmosphere. After homogenizing, a solution of morpholine (5 g, 60 mmol) in dry $C_6H_5CH_3$ (30 ml) was added dropwise. The solution was used immediately.

To a solution of ester **5** (1 g, 6 mmol) in dry $C_6H_5CH_3$ (60 ml) at −40 °C was added 46 ml of the reductive solution prepared above. The reaction mixture was stirred for 30 min between −40 and −55 °C, then quenched by addition of 1 ml of H_2O. The solvent was evaporated to dryness on a rotary vacuum evaporator, leading to the formation of a crude residue that was extracted with EtOAc (4 × 50 ml). The organic extracts were dried over anhydrous Na_2SO_4, evaporated and the resulting yellow solid was chromatographed on silica gel with CH_2Cl_2–MeOH (99:1) as eluent to furnish aldehyde derivative **10** (0.6 g, 60%), as a yellow solid, m.p. 115–116 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 10.00 (s, 1H, Im–C*H*O), 9.52 (d, 1H, H_5, $J = 6.8$ Hz), 7.67 (d, 1H, H_8, $J = 9.0$ Hz); 7.52 (dt, 1H, H_7, $J = 8.9$, 7.3 and 1.3 Hz), 7.06 (dt, 1H, H_6, $J = 6.9$, 6.9 and 1.0 Hz), 2.71 (s, 3H, Im–CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 177.0 (*C*=O), 157.3 (C5), 147.8 (C2), 130.3 (C8a), 128.5 (C3), 121.4 (C7), 116.9 (C8), 115.1 (C6), 14.1 (Im–*C*H$_3$); IR (/cm): 1638 ν(C=O), 1534 and 1495 ν(C=N), 1253 ν(C–O), 764 δ(N=C–H); HRMS *m/z* (ES+) *Anal.* Calc. for $C_9H_8N_2O$ $[M+H]^{+1}$: 161.0637, Found: 161.0636.

2.1.3. (±)-2-Methylimidazo[1,2-a]pyridin-3-yl)(pyridin-2-yl)methanol (*11*)

To a solution of aldehyde **10** (0.4 g, 2.5 mmol) in dry THF (5 ml) was added, at −78 °C, a 0.15 M solution of 2-lithiopyridine [18 ml, 2.7 mmol (prepared from reaction of 0.1 M solution of 2-bromopyridine in dry THF with 1.2 equiv. of a 1.6 M solution of *n*-BuLi in hexanes] and then stirred for 2.5 h. Next, a saturated aqueous solution of NH_4Cl (10 ml) was added to the reaction mixture, followed by extraction with EtOAc (3 × 15 ml). The organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting dark brown oil was crystallized by addition of C_3H_6O (3 ml), yielding alcohol **11** (0.37 g, 60%), as dark yellow cubic crystals, m.p. 173–175 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.41 (ddd, 1H, H'_6, $J = 5.0$, 1.7 and 0.9 Hz), 8.30 (dt, 1H, H_8, $J = 6.8$, 1.1 and 1.1 Hz), 7.84 (td, 1H, H'_4, $J = 7.7$, 7.7 and 1.7 Hz), 7.76 (d, 1H, H'_3, $J = 7.8$ Hz), 7.40 (dt, 1H, H_5, $J = 8.9$, 1.1 and 1.1 Hz), 7.25 (ddd, 1H, H'_5, $J = 7.1$, 5.0 and 1.5 Hz), 7.13 (ddd, 1H, H_6, $J = 8.9$, 6.8 and 1.3 Hz), 6.74 (td, 1H, H_7, $J = 6.7$, 5.6 and 1.3 Hz), 6.29 (d, 1H, –O*H*, $J = 4.0$ Hz), 6.22 (d, 1H, C*H*(OH), $J = 3.9$ Hz), 2.23 (s, 3H, Im–CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 158.0 (C2′), 147.8 (C6′), 144.7 (C5), 142.0 (C3), 137.2 (C8a), 124.7 (C7), 124.2 (C4), 122.7 (C2), 120.5 (C6), 119.1 (C8), 116.3 (C3′), 111.3 (C5′), 66.1 (*C*H–OH), 13.2 (Im–*C*H$_3$); IR (cm^{-1}): 3440 ν(O–H), 3079 ν(Ar–C–H), 1587, 1571 and 1502 ν(C=N), 1050 ν(C–O), 752 δ(N=C–H); HRMS *m/z* (ES+) *Anal.* Calc. for $C_{14}H_{13}N_3O$ $[M+H]^{+1}$: 240.1131, Found: 240.1130.

2.1.4. (±)-erythro/threo-2-Methylimidazo[1,2-a]pyridin-3-yl)(piperidin-2-yl)methanol (*4*)

To a hydrogenation vessel containing a solution of alcohol **11** (0.100g, 0.42 mmol) in absolute EtOH (16 ml) containing concentrated HCl (0.05 ml) was added catalytic amounts of PtO_2 (0.004 g, 0.0176 mmol). The vessel was then pressurized to 3 atm. with hydrogen, and shaken at r.t. for 4 h. The resulting suspension was filtered through celite, and the filtrate was concentrated at reduced pressure. The crude yellow oil was neutralized by addition of 10% aq. $NaHCO_3$ solution (10 ml) and extracted with EtOAc (3 × 10 ml). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure to give a 3:1 mixture of diastereomers *erythro*/*threo* of the alcohol **4** (0.072 g, 70% yield), as a light yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.50 (d, 1H, H_8, $J = 7.0$ Hz), 7.39 (d, 1H, H_5, $J = 8.9$ Hz), 7.09 (t, 1H, H_6, $J = 6.8$ Hz), 6.70 (t, 1H, H_7, $J = 6.8$ Hz), 4.90 (d, 1H, –C*H*(OH) *erythro*,

$J = 7.1$ Hz), 4.80 (d, –C*H*(OH) *threo*, $J = 9.6$ Hz), 3.71 (d, 1H, H'_2, $J = 7.0$ Hz), 2.97 (m, 4H, –O*H*, –N*H* and H'_6), 2.50 (m, 2H, H'_3), 2.21 (s, 3H, Im–CH_3 *erythro*), 2.16 (s, 3H, Im–CH_3 *threo*), 1.92 (m, 2H, H'_5), 1.54 (m, 2H, H'_4); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 144.6 (C5), 140.9 (C3), 126.0 (C8a), 125.8 (C7), 123.9 (C2), 118.9 (C6), 116.1 (C8), 69.4 (*C*HOH), 59.5 (C2′ *erythro*), 58.5 (C2′ *threo*), 46.6 (C6′ *erythro*), 46.8 (C6′ *threo*), 28.3 (C5′), 25.5 (C3′), 24.0 (C4′), 13.4 (Im–*C*H_3); IR (cm^{-1}): 3395 ν(O–H), 3263 ν(N–H), 1558 and 1499 ν(C=N), 1136 ν(C–N), 1195 ν(C–O), 745 δ(N=C–H); HRMS *m/z* (ES+) *Anal.* Calc. for $C_{14}H_{19}N_3O$ $[M+H]^{+1}$: 246.1600, Found: 246.1596.

2.1.5. 4·HCl

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.51 (d, 1H, H_5 *erythro*, $J = 6.9$ Hz), 8.46 (d, 1H, H_5 *threo*, $J = 6.9$ Hz), 7.35 (d, 1H, H_8, $J = 9.9$ Hz), 7.06 (t, 1H, H_6, $J = 8.5$ Hz), 6.67 (t, 1H, H_7, $J = 6.5$ Hz), 4.91 (d, 1H, C*H*(OH) *erythro*, $J = 6.8$ Hz), 4.79 (d, 1H, C*H*(OH) *threo*, $J = 9.7$ Hz), 3.62 (br, 3H, O*H* and NH_2^+), 2.97 (m, 2H, H'_2), 2.66 (t, 2H, H'_6 *threo*, $J = 11.8$ Hz), 2.50 (t, 2H, H'_6 *erythro*, $J = 11.6$ Hz), 2.17 (s, 3H, Im–CH_3 *erythro*), 2.12 (s, 3H, Im–CH_3 *threo*), 1.87 (dd, 2H, H'_3, $J = 11.9$Hz), 1.40 (m, 4H, H'_4 and H'_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 144.9 (C8a), 141.4 (C2), 126.0 (C5), 124.3 (C7), 116.7 (C8), 114.3 (C3), 112.0 (C6), 68.2 (*C*HOH), 60.8 (C2′), 46.1 (C6′), 27.8 (C3′), 24.2 (C4′), 23.5 (C5′), 14.3 (Im–*C*H_3).

2.1.6. (2-Methylimidazo[1,2-a]pyridin-3-yl)(pyridin-2-yl)methanone (8)

Active MnO_2 (0.545 g, 6.27 mmol) was added in portions to a solution of previously prepared alcohol **11** (0.100 g, 0.42 mmol) in CH_2Cl_2 (15 ml). The suspension was stirred at r.t. for 2.5 h and then filtered through celite and washed with CH_2Cl_2. Combined organic extracts were evaporated under reduced pressure to furnish **8** (0.086 g, 87%) as a yellow solid, m.p. 72–75 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.50 (dt, 1H, H_5, $J = 6.9$, 1.2 and 1.2 Hz), 8.72 (ddd, 1H, H'_6, $J = 5.2$, 1.6 and 1.0 Hz), 8.08 (td, 1H, H'_4, $J = 7.7$, 7.7 and 1.7 Hz), 7.81 (dt, 1H, H'_3, $J = 7.8$, 1.1 and 1.1 Hz), 7.79 (dt, 1H, H'_5, $J = 8.9$, 1.2 and 1.2 Hz), 7.65 (m, 2H, H_7 and H_8), 7.29 (td, 1H, H_6, $J = 6.8$ and 1.6 Hz), 1.98 (s, 3H, Im–CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 184.4 (*C*=O), 156.9 (C2′), 154.9 (C6′), 148.8 (C5), 147.6 (C2), 137.1 (C8a), 129.3 (C3 and C4′), 128.6 (C7), 125.6 (C5′), 122.8 (C3′), 116.4 (C8), 114.4 (C6), 17.3 (Im–*C*H_3); IR (/cm): 1619 ν(C=O), 1583 ν(C=C), 1564 and 1493 ν(C=N), 758 δ(N=C–H); HRMS *m/z* (ES+) *Anal.* Calc. for $C_{14}H_{11}N_3O$ $[M+H]^{+1}$: 238.0974, Found: 238.0971.

2.1.7. 2-Methyl-3-(pyridin-2-ylmethyl)imidazo[1,2-a]pyridine (12)

To a solution of the ketone derivative **8** (0.150 g, 0.6 mmol) in diethyleneglycol (2 ml) containing KOH (0.085 g, 2.2 mmol) was added 80% hydrazine monohydrate (0.08 ml, 2.6 mmol). The reaction mixture was heated at 195 °C for 2 h, quenched by addition of water (5 ml) and extracted with EtOAc (3 × 10 ml). The organic layers were evaporated and the crude oil obtained was chromatographed on silica gel using CH_2Cl_2–MeOH (99:1) as eluent, furnishing **12** (0.128 g, 96%) as an orange oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.56 (dd, 1H, H'_6, $J = 4.9$ and 0.9 Hz), 7.98 (d, 1H, H_5, $J = 6.8$ Hz), 7.56 (m, 2H, H_8 and H'_4), 7.06 (m, 2H, H'_3 and H'_5), 6.94 (d, 1H, H_7, $J = 7.9$ Hz), 6.72 (td, 1H, H_6, $J = 6.8$ and 0.8 Hz), 4.42 (s, 2H, CH_2), 2.47 (s, 3H, Im–CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 157.4 (C2′), 149.1 (C6′), 144.1 (C5), 140.2 (C2), 136.8 (C8a), 123.3 (C4′), 123.7 (C3), 121.7 (C7), 121.9 (C3′), 117.1 (C5′), 116.2 (C8), 111.6 (C6), 29.5 (*C*H_2), 13.0 (Im–CH_3); IR (/cm): 2922 ν(C–H), 1593, 1571 and 1503 ν(C=N), 753 δ(N=C–H); HRMS *m/z* (ES+) *Anal.* calc. for $C_{14}H_{13}N_3$ $[M+H]^{+1}$: 224.1109, Found: 224.1177.

2.1.8. (±)-2-Methyl-3-(piperidin-2-ylmethyl)imidazo[1,2-a]pyridine (13)

To a hydrogenation vessel containing a solution of deoxygenated derivative **12** (0.100 g, 0.45 mmol) in absolute EtOH (16 ml) containing concentrated HCl (0.05 ml) was added catalytic amounts of PtO_2 (0.004 g, 0.0176 mmol). The vessel was then pressurized to 3 atm. with hydrogen, and shaken at r.t. for 2 h. The resulting suspension was filtered through celite, and the filtrate was concentrated at reduced pressure. The crude yellow oil was neutralized by addition of 10% aq. $NaHCO_3$ solution (10 ml) and extracted with EtOAc (3 × 10 ml). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure to afford piperidinyl derivative **13** (0.073 g, 73%), as a light yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.90 (d, 1H, H_5, $J = 6.8$ Hz), 7.93 (d, 1H, H_8, $J = 8.9$ Hz), 7.54 (t, 1H, H_7, $J = 6.8$ Hz), 4.08 (d, 2H, CH_2, $J = 7.0$ Hz), 3.48 (t, 1H, H'_2, $J = 7.0$ Hz), 3.01 (m, 4H, –N*H*, H'_2 and H'_6), 2.97 (m, 4H, H'_5 and H'_4), 2.50 (s, 3H, Im–CH_3), 1.98 (m, 2H, H'_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 144.4 (C5), 141.2 (C3), 123.2 (C8a), 123.1 (C7), 117.2 (C2), 116.9 (C6), 111.7 (C8), 55.8 (C2′), 47.1 (C6′), 32.9 (C3′), 31.1 (C5′), 29.8 (*C*H_2), 24.7 (C4′), 13.7 (Im–*C*H_3); IR (/cm): 3382 ν(N–H), 2926 ν(C–H), 1570 and 1504 ν(C=N), 1113 ν(C–N), 756 δ(N=C–H); HRMS *m/z* (ES+) *Anal.* Calc. for $C_{14}H_{19}N_3$ $[M+H]^{+1}$: 230.1651, Found: 230.1642.

2.1.9. 13·HCl

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05 (s, 1H, H_5, $J = 6.8$ Hz), 7.47 (d, 1H, H_8, $J = 8.9$ Hz), 7.07 (dd, 1H, H_7,

$J = 8.9$ and 6.9 Hz), 6.74 (t, H_6, 1H, $J = 6.4$ Hz), 3.00 (d, 2H, CH_2, $J = 11.7$ Hz), 2.90 (d, 2H, H'_6, $J = 6.6$ Hz), 2.51 (ddt, 1H, H'_2, $J = 11.8$, 11.8 and 2.6 Hz), 2.44 (s, 3H, Im–CH_3), 1.80–1.78 (m, 2H, H'_3), 1.66–1.55 (m, 2H, H'_4), 1.30–1.25 (m, 2H, H'_5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 144.4 (C8a), 141.2 (C2), 123.2 (C5), 123.1 (C7), 117.2 (C3), 116.9 (C8), 111.7 (C6), 55.8 (C2′), 47.1 (C6′), 32.9 (CH_2), 31.9 (C3′), 26.0 (C4′), 24.7 (C5′), 13.7 (Im–CH_3).

2.2. Biological assays

2.2.1. Antimalarial activity

The in vitro antimalarial assay [28] used for evaluation of the new imidazo[1,2-*a*]pyridine derivatives **4**·HCl and **13**·HCl was an adaptation of the parasite lactate dehydrogenase (pLDH) based assay developed originally by Makler et al. [29]. The assay was set up in 96 well microplates. Antimalarial action of each compounds was tested at least at six different concentration on two *P. falciparum* clones, i.e. a chloroquine sensitive D6-Sierra Leone clone and a chloroquine resistant W2-Indochina clone. The quinoline antimalarial drugs mefloquine (**1**) and quinine (**2**) were used as the positive controls and DMSO was tested as the negative control with each assay. Citotoxicity was conducted on Vero cell line, using the method previously described by Skehan et al. [30].

2.2.2. Heme polymerization assay

Heme polymerization i.e. conversion of heme to β-hematin in the presence of oleolglycerol was assayed in vitro up according to the methods described earlier [31,32]. Briefly, the reaction mixture (200 μl) with 100 μl acetate buffer (100 mM, pH 4.8), 100 μM heme, 0.5 mg oleolglycerol and the test compound was incubated over night at 37 °C. Nonpolymerized heme was removed by washing with Tris/SDS buffer and sodium bicarbonate. β-Hematin was solubilized in 0.1 N NaOH and OD was recorded at 405 nm.

3. Results and discussion

3.1. Chemistry

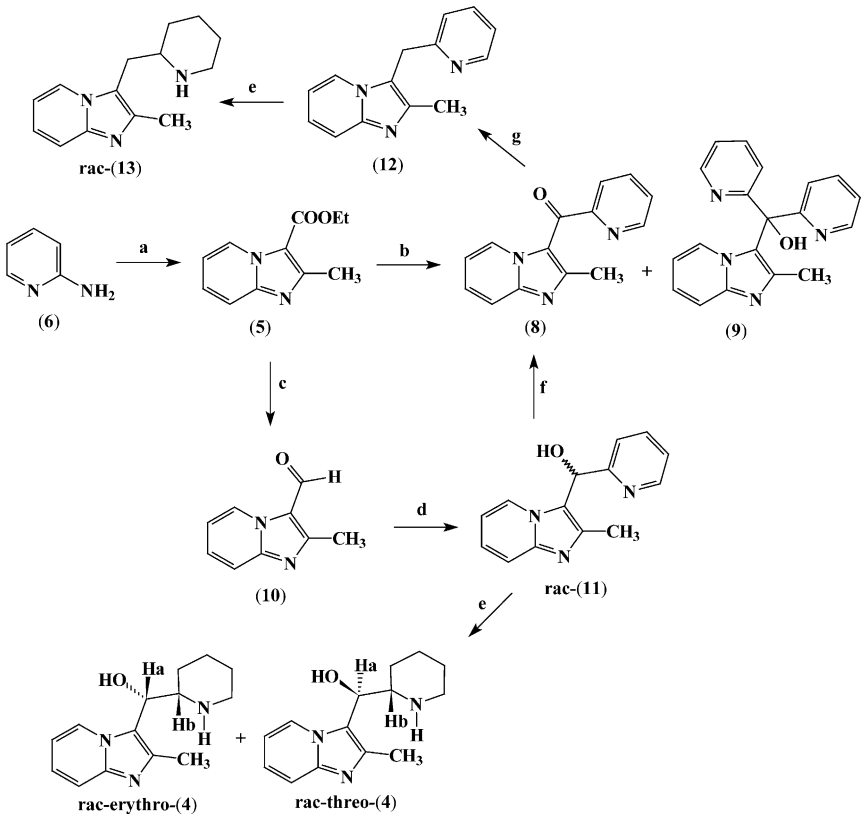
The synthesis of the new imidazo[1,2-*a*]pyridine carbinolamine derivative **4** is outlined in Scheme 1. The starting material was the imidazo[1,2-*a*]pyridine-3-carboxylic acid ethyl ester (**5**) obtained in 87% yield by condensation between 2-aminopyridine (**6**) and ethyl 2-chloroacetoacetate (**7**) [18]. The initial approach used to achieve the construction of the piperidine side-chain consisted of exploring the controlled nucleophilic addition of 1 equiv. of 2-lithiopyridine to the carbonyl group of ester **5**. However, under these conditions the desired ketone derivative **8** was obtained in only 15% yield while the major product formed was characterized as the tertiary alcohol **9** (entry 3, Table 1). Despite several experimental variations in the number of equivalents of lithium reagent used (Table 1), we were unable to improve the formation of diarylketone derivative **8**. Indicating that the kinetics of the addition of 2-lithiopyridine to **8**, generating the tertiary alcohol **9**, is faster than its formation from nucleophilic addition on carbonyl ester group of **5**. To circumvent this limitation, we turned to an alternative pathway exploring the chemoselective conversion of the ester group of **5** to the more reactive aldehyde group. Thus, ester **5** was reduced to the aldehyde **10** in 60% yield using RedAl® partially deactivated by addition of 1 equiv. of morpholine [19] (Scheme 1).

Next, aldehyde **10** was condensed with 1 equiv. of 2-lithiopyridine at −78 °C to furnish the pyridinyl alcohol **11** in 62% yield [15,20,21] (Scheme 1). The final step of the synthetic route consisted of the selective heterogeneous catalytic hydrogenation [15] of the pyridine ring, furnishing the piperidine ring of the target imidazo[1,2-*a*]-pyridine derivative **4**. Treatment of *rac*-alcohol (**11**) with molecular hydrogen in ethanol containing 4 mol% of PtO_2 giving a mixture of *erythro*-**4** and *threo*-**4** diastereomers, in 70% yield (Scheme 1). The diastereomeric *erythro*/*threo* ratio was determined to be 75:25, respectively, by reversed-phase HPLC analysis.

The assignment of the relative configuration of the major diastereomer of **4** was made by ^{1}H NMR through comparative analysis of coupling constants (J) of carbinolic hydrogen signal (Ha) of *erythro* and *threo* stereoisomers (Fig. 2). Is well known [22] that the *threo* isomer of mefloquine (**1**) and analogues presented a vicinal coupling constant between the carbinolic hydrogen (Ha) and 2-piperidinyl hydrogen (Hb) of greater magnitude than the corresponding *erythro* isomer (Fig. 2). The doublet signal of Ha of the major isomer of **4** appeared at 4.90 ppm ($J = 6.5$ Hz), and was attributed to the *erythro* diastereomer, while the minor doublet signal at 4.80 ppm ($J = 9.7$ Hz) was attributed to the *threo* isomer (Fig. 2).

In order to evaluate the antimalarial profile of the dehydroxylated analogue of **4**, we oxidized the pyridinyl alcohol **11** with MnO_2 [23] to the corresponding diheteroarylketone derivative **8**, which was subsequently deoxygenated in 96% yield by applying classical Wolf–Kishner methodology [24,25]. Next, the desired piperidinyl derivative **13** was obtained in 73% yield by PtO_2 catalyzed hydrogenation [15]. Before testing target compounds **4** and **13**, they were quantitatively converted to the corresponding hydrochlorides **4**·HCl and **13**·HCl by treatment with gaseous HCl in dichloromethane to facilitate solubilization in aqueous bioassay conditions.

Considering that the internalization of antimalarial 4-aminoquinoline derivatives in the parasite acidic vacuole



Scheme 1. Synthesis of new imidazo[1,2-*a*]pyridine derivative **4**.

Table 1
Experimental variations of the reaction of the imidazo[1,2-*a*]pyridine ethyl ester **5** with 2-lithiopyridine

Entry	Experimental conditions [a]		Product [b,c]	
	2-Lithiopyridine (equiv.)	Time (h)	8 (%)	9 (%)
1	0.5	0.5	10	15
2	0.5	1.0	14	23
3	1.0	2.5	15	38
4	1.5	2.5	20	45
5	2.0	2.5	23	50

[a] All procedures were made in THF at −78 °C.
[b] Physical and spectroscopic data of the compound **8** are described in Section 2.
[c] Spectroscopic data of the compound **9**: ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.57 (dt, 2H, H_2', $J = 4.8$, 1.2 Hz), 7.77–7.65 (m, 6H, H_3', H_4' and H_5'), 7.48 (d, 1H, H_5), 7.30–7.23 (m, 3H, H_8 and H_6'), 7.07 (ddd, 1H, H_6, $J = 9.0$, 6.7 and 1.1 Hz), 6.55 (s, 1H, O*H*), 6.47 (td, 1H, H_7, $J = 6.9$, 1.0 Hz), 1.50 (s, 3H, Im–CH_3); IR (/cm): 3425 ν(O–H), 3054 and 3004 ν(Ar–C–H), 1637 and 1430 ν(C=N), 1195 ν(C–O), 747 δ(N=C–H); HRMS *m*/*z* (ES+) *Anal.* Calc. for $C_{19}H_{16}N_4O$ $[M+H]^{+1}$: 3 17.0761, Found: 317.1405.

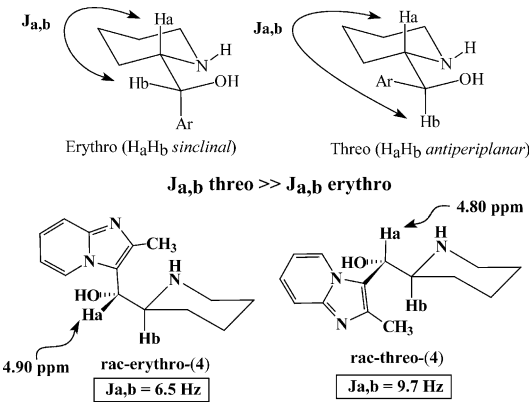


Fig. 2. Characterization of the relative configuration of the diastereomers of **4** by ^{1}H NMR.

is largely dependent of it's ionization constants [2], we determined the pK_a of **4** using the classical UV–Vis spectrophotometric method [26]. The ionization constant values found for the compound **4** in water were 5.80 (pK_{a1}) and 8.85 (pK_{a2}). The pK_{a2} value of **4**, referring to the ionization of the piperidinyl group, is very similar to mefloquine (**1**), i.e. p$K_{a2} = 8.6$ (water) [27], suggesting that the drug accumulation rate in the parasite vacuole would also be similar.

3.2. Biological activity

The in vitro antimalarial profile [28] of the diastereomeric mixture of the novel imidazo[1,2-*a*]pyridine derivatives **4**·HCl and **13**·HCl was evaluated in vitro against two *P. falciparum* clones designated as Indochina (W-2, mefloquine-sensitive and chloroquine-resistant) and Sierra Leone (D-6, mefloquine-resistant and

Table 2
Antimalarial activities of the novel imidazo[1,2-*a*]pyridine derivatives **4**·HCl and **13**·HCl

Comp.	IC_{50} (μg/l) D-6 clone [a]	S.I. [b]	IC_{50} (μg/l) W-2 clone [a]	S.I. [b]	TC_{50} (μg/l) Vero cells [c]
4·HCl	3466 ± 503	> 14	25 000 ± 5000	> 1.88	NC
13·HCl	3000 ± 500	> 16	25 000 ± 5000	> 1.88	NC
Mefloquine·HCl	10 ± 3	> 4760	26 ± 6	> 1830	NC
Quinine·HCl	13 ± 4	> 3660	238 ± 31	> 200	NC

[a] Values are mean ± SD of three observations.
[b] S.I. = selectivity index = IC_{50} (Vero Cells)/IC_{50} (*P. falciparum*).
[c] NC = no cytotoxicity up to the highest dose tested (47 000 ng/ml).

chloroquine-sensitive), using the protocol developed by Makler et al. [29] (Table 2). Compounds **4**·HCl and **13**·HCl were inactive against both clones of *P. falciparum* when compared with mefloquine (**1**) and quinine (**2**). In the spite of the removal of the pharmacophoric hydroxyl group of the carbinolamine derivative **4**·HCl, it's dehydroxylated analogue **13**·HCl presented a similar bioactivity profile, indicating that the mechanism of action against the parasite seems to be mainly dependent of the nature of the aromatic heterocyclic ring. Compounds **4**·HCl and **13**·HCl did not show Vero cell toxicicity at the concentrations tested [30].

Additionally, the imidazo[1,2-*a*]pyridine mefloquine analogues **4**·HCl and **13**·HCl reported in this communication did not show a noticeable effect on heme polymerization activity [31,32] in vitro up to a 200 μM concentration. Mefloquine (**1**) inhibited the heme polymerization process with an IC_{50} value of 27 ± 6 μM.

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