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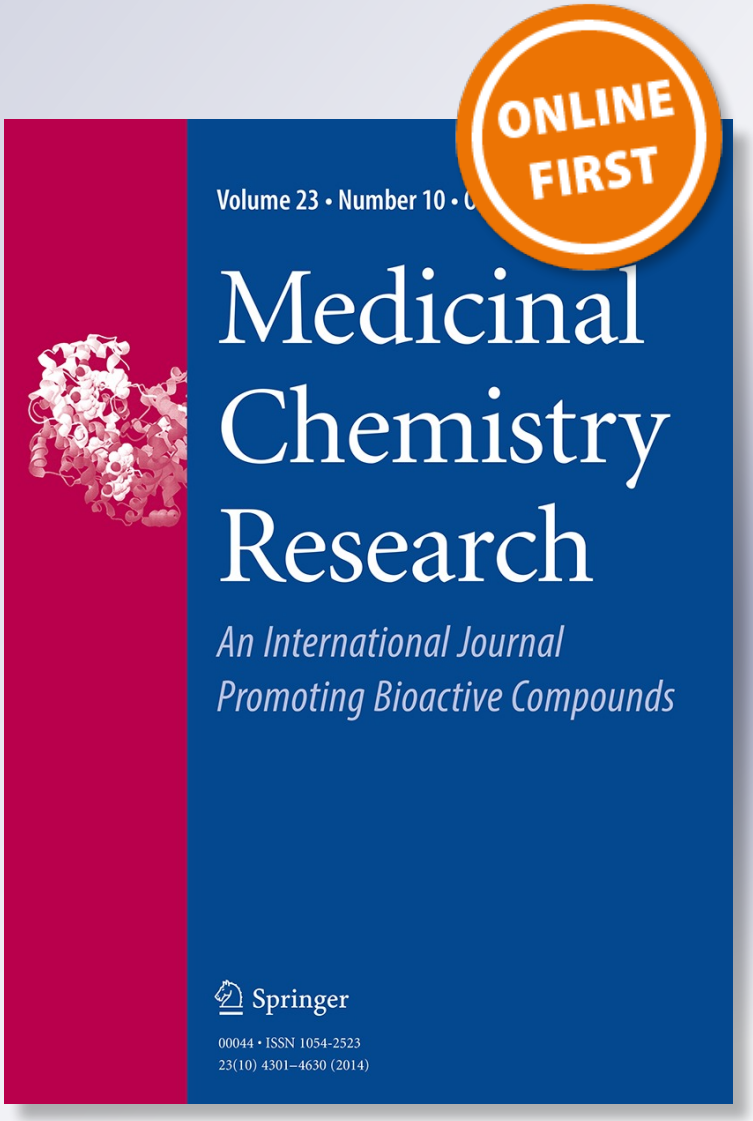

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antimalarial activity of novel imidazoisoquinolinone derivatives correlates with heme binding affinity

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Abstract A series of novel imidazoisoquinolinone derivatives were synthesized and evaluated for in vitro antimalarial efficacy against chloroquine sensitive GHA strain of *Plasmodium falciparum*. Compounds **2**, **4**, **6**, **9**, and **17** revealed moderate to good activities in the micromolar range. Binding interaction between these active compounds and heme were determined and correlated with antimalarial activity. A good correlation ($r = 0.98$) was observed between antimalarial activity and the heme dissociation constants (K_d). These suggest that antimalarial mode of action of this class of compounds appears to be similar to that of chloroquine and involves the inhibition of hemozoin formation.

Keywords Malaria · Imidazoisoquinolinones · Antiplasmodial · Hemozoin · *Plasmodium falciparum*

Introduction

Malaria is a mosquito-borne infectious disease caused by *Plasmodium falciparum*. Malaria is of major relevance in the world with a prevalence of 300–500 million clinical cases and 2 million deaths each year. The loss of efficacy of the available antimalarial compounds due to drug resistance increases the concerns about this health problem (Guantai *et al.*, 2010). The continuing spread of drug-resistant malaria parasites 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 antimalarial action (Joshi and Viswanathan, 2006; Gorka *et al.*, 2013; O'Neill *et al.*, 1997).

Heme detoxification is a crucial biochemical process of malaria parasite. The survival of the parasite is highly dependent upon proteolysis of hemoglobin of the host during its erythrocyte cycle. Digestion of hemoglobin releases toxic iron II ferroprotoporphyrin IX moiety that subsequently oxidizes to ferriprotoporphyrin IX or hematin. Hematin is then polymerized forming inert crystals of hemozoin or malaria pigment. The polymerization process is a mechanism of detoxification and can be targeted for antimalarial therapy (Acharya *et al.*, 2010). Considerable data now support the hypothesis that antimalarial quinolines like chloroquine (CQ) inhibit parasite growth by binding to hematin and preventing its aggregation to hemozoin. But CQ resistance appears to arise from changes in the food vacuole membrane protein PfCRT of the parasite, rather than changes in detoxification pathway. Thus, the discovery of chemically novel class of drugs against the same target could have immense clinical value (Fidock *et al.*, 2000; Fitch, 2004).

Isoquinolines generally constitute an important branch of heterocyclic compounds and are currently used against parasitic infections. Imidazoisoquinolinones have structural features different from those of the existing drugs and represent valuable substrates for the synthesis of biologically active compounds, as we demonstrated in our previous research. (Bollini *et al.*, 2009).

Here we report the antimalarial activity of a series of imidazoisoquinolinone (DHIIQ) derivatives against chloroquine sensitive (GHA) strain of *P. falciparum*. The compounds were also evaluated for heme interaction

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activity and correlated with their antiplasmodial activity against CQ sensitive. The mode of action for the most active compound **6** was proposed to follow heme detoxification inhibition pathway.

Experimental

Chemistry

Melting points were determined in a capillary with an Electrothermal 9100 SERIES-Digital apparatus and were uncorrected. IR spectra were recorded with an FT Perkin Elmer Spectrum One from KBr discs. UV spectra were measured with a Jasco V-570 UV/VIS/NIR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker MSL 300 spectrometer at room temperature with tetramethylsilane as an internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The mass spectrum was obtained on a Shimadzu QP 5000. Elemental analysis was carried out with a Coleman Analyser.

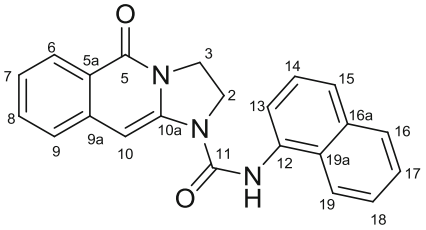
General procedure for the synthesis of isocyanates (**13a**–**13e**)

In a three-necked flask equipped with a magnetic stirrer and immersed in an ice bath is placed NaN_3 (7.59 g, 11 mmol) in 25 mL of water. A mixture of a corresponding acyl chloride (8.5 mmol) and 30 mL of acetone was slowly added drop-wise to the well-stirred solution of the azide at such a rate that the temperature remains at 0–5 °C. Then, the mixture was allowed to rise to room temperature, and the product was extracted with benzene and heated at reflux for 2 h. The organic layer was dried ($MgSO_4$), filtered, and the solution was evaporated under vacuum. The residue was purified by crystallization or simple distillation depending on the physical properties of the product obtained. The NMR and IR spectra were obtained according to literature data (Allen, 1955).

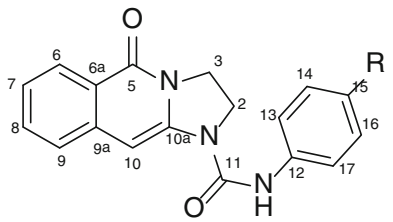
General procedure for the synthesis of compounds **14**–**17**

A mixture of 2,3-dihydroimidazo[1,2-*b*]isoquinolin-5(1*H*)-one, 1 (0.5 g, 2.7 mmol) (Bollini *et al.*, 2009), the corresponding isocyanate derivatives, namely naphthylisocyanate, phenylisocyanate, 4-nitrophenylisocyanate, and 4-methylphenylisocyanate (2.7 mmol) in CH_2Cl_2 (20 mL) was stirred at reflux for 2–6 h. Then the mixture was allowed to rise to room temperature, and the crystalline solid was filtered and crystallized from the corresponding solvent to give the title compounds **14**–**17**.

N-(1-Naphthyl)-5-oxo-2,3-dihydroimidazo[1,2-*b*]isoquinoline-1(5*H*)-carboxamide (**14**)



Reaction time: 2 h. White solid (ethanol), yield: 0.60 g, 63 %, mp 268–279 °C (d); IR (KBr) ν_{max} 3399 (N–H amide), 1739 (C=O, urea), 1699 (C=O), 1570 (N-CO), 803 and 718 (C–H Ar) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.1 (1H, s, NH urea), 8.03–8.09 (2H, m, H–Ar), 7.95–7.98 (1H, m, H–Ar), 7.86 (1 H, dd, J = 7.0, 2.3 Hz, H–Ar), 7.46–7.58 (6H, m, H–Ar), 7.29 (1H, dt, J = 6.7, 1.2 Hz, HAr), 6.9 (1H, s, H-10), 4.26–4.43 (4H, m, H-3, H-2). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 162.41 (C-5), 156.32 (C-11), 142.15 (C-10a), 137.16 (C-16a), 135.29 (C-12), 134.15 (C-8), 132.58 (C-6), 129.25 (C-9a), 128.69 (C-14; C-19a), 128.24 (C-9), 126.14 (C-19), 125.44 (C-5a), 124.77 (C-16; C-18), 124.25 (C-7), 123.26 (C-17), 116.27 (C-13; C-15), 89.79 (C-10), 50.32 (C-2), 45.25 (C-3), HRESIMS m/z (pos): 356.1354 $C_{22}H_{18}N_3O_2$ (calcd 356.1321). Anal. Calcd. For $C_{22}H_{17}N_3O_2$: C 74.35; H, 4.82; N 11.82. Found: C, 74.07; H, 5.03; N, 12.10.



5-Oxo-*N*-phenyl-2,3-dihydroimidazo[1,2-*b*]isoquinoline-1(5*H*)-carboxamide (**15**)

Reaction time: 2 h, white solid (ethyl acetate), yield: 0.33 g, 41 %, mp 189–190 °C. IR (KBr) ν_{max}: 3340 (N–H amide), 1730 (C=O, urea), 1699 (N–C=O), 748 and 705 (C–H Ar) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.93 (1H, s, NH (urea)), 8.10 (1H, d, J = 8.2 Hz, H-6), 7.57 (1H, d, J = 6.1 Hz, H–Ar), 7.28–7.38 (5H, m, H–Ar), 7.04–7.12 (2H, m, H–Ar), 7.00 (1H, s, H-10), 3.26–3.69 (4H, m, H-3, H-2). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 160.41 (C-5), 155.61 (C-11), 142.15 (C-10a), 138.24 (C-9a), 134.15 (C-12), 132.58 (C-14; C-16), 128.69 (C-6; C-9), 128.24 (C-7; C-8), 126.29 (C-5a), 124.26 (C-15), 121.23 (C-13; C-17), 89.79 (C-10), 50.32 (C-2), 45.25 (C-3). HRESIMS calcd for $C_{18}H_{15}N_3O_2$ $[M+H]^+$ 306.1164, found 306.1198. Anal. Calcd. For $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76. Found: C, 71.10; H, 4.95; N, 13.76.

5-Oxo-*N*-(4-nitrophenyl)-2,3-dihydroimidazo[1,2-*b*]isoquinoline-1(5*H*)-carboxamide (**16**)

Reaction time: 6 h, yellowish needles (ethyl acetate), yield: 0.71 g, 76 %, mp: 249–251 °C. IR ν_{max} (KBr): 3339 (N–H amide), 1739 (C=O, urea), 1701 (C=O), 1568 (N-CO), 1550 and 1350 (–NO_2), 847 and 748 (C–H Ar) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.18 (1H, s, NH (urea)), 8.20 (2H, d, $J = 9.0$ Hz, H-14, H-16), 7.92 (1H, d, $J = 8.2$ Hz, H-6), 7.72 (2H, d, $J = 9.0$ Hz, H–Ar, H-13, H-17), 7.39 (1H, dt, $J = 8.2$ Hz, $J = 1.1$ Hz, H–Ar), 7.26 (1H, d, $J = 7.1$ Hz, H–Ar), 7.19 (1H, dt, $J = 7.1$, 1.1 Hz, H–Ar,) 7.01 (1H, s, H-10), 3.34-4.08 (4H, m, H-3, H-2). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 155.09 (C-5), 144.61 (C-11), 143.25 (C-10a), 142.15 (C-15), 136.15 (C-9a), 132.58 (C-12), 128.24 (C-9), 127.25 (C-6), 126.92 (C-7; C-8), 125.40 (C-14; C-16), 124.25 (C-5a), 120.50 (C-13; C-17), 89.79 (C-10), 50.32 (C-3), 45.25 (C-2). HRESIMS calcd for $C_{18}H_{14}N_4O_4$ $[M + H]^+$ 351.1093, found 351.1105. Anal. Calcd. For $C_{18}H_{14}N_4O_4$: C, 61.71; H, 4.03; N, 15.99. Found: C, 61.48; H, 3.79; N, 15.61.

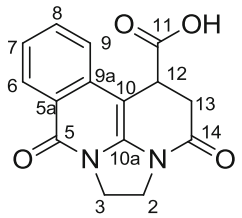
5-Oxo-*N*-(4-methylphenyl)-2,3-dihydroimidazo[1,2-*b*]isoquinoline-1(5*H*)-carboxamide (**17**)

Reaction time: 4 h, white needles (ethanol), yield: 0.18 g, 21 %, mp: 265–267 °C (d). IR ν_{max} (KBr): 3340 (N–H amide), 1725 (C=O, urea), 1699 (NC=O), 840 and 752 (C–H Ar) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.90 (1H, s, NH (urea)), 8.03 (1H, dd, $J = 8.1$ Hz, $J = 1.2$ Hz, H-6), 7.53–7.60 (2H, m, H–Ar), 7.49 (2H, d, $J = 8.9$ Hz, H-14, H-16), 7.38 (2H, d, $J = 8.9$ Hz, H-13, H17), 7.19-7.30 (1H, m, H–Ar), 6.96 (1H, s, H-10), 4.09 (4H, m, H-2, H-3), 2.22 (3H, s, C*H*$_3$). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 159.14 (C-5), 155.61 (C-11), 146.84 (C-10a), 138.42 (C-9a), 134.15 (C-12), 132.61 (C-15), 129.77 (C-14; C-16), 128.69 (C-6; C-9), 126.29 (C-7; C-8), 124.25 (C-5a), 119.90 (C-13; C-17), 89.79 (C-10), 50.32 (C-3), 45.25 (C-2), 21.13 (***C***H$_3$). HRESIMS calcd for $C_{19}H_{17}N_3O_2$ $[M+H]^+$ 320.1399, found 320.1433. Anal. Calcd. For $C_{19}H_{17}N_3O_2$: C, 71.46; H, 5.37; N, 13.16. Found: C, 71.14; H, 5.61; N, 13.01.

5-Oxo-*N*-(2-fluorophenyl)-2,3-dihydroimidazo[1,2-*b*]isoquinoline-1(5*H*)-carboxamide (**18**)

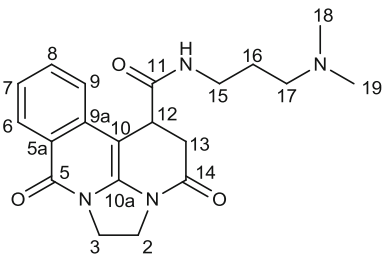
To a suspension of compound **1** (0.5 g, 2.7 mmol) and DMAP (0.6 g, 4.9 mmol) in 20 mL of CH_2Cl_2, 2-fluorophenyl isocyanate (0.33 mL, 2.7 mmol) was added over a period of several min and then was stirred at reflux for 2 h. The mixture was allowed to rise to room temperature, and the crystalline solid was collected and washed with CH_2Cl_2. It was obtained as white needles. Yield: 0.30 g, 35 %. Mp 224–227 °C. ^{1}H NMR (DMSO-d_6) δ: 8.89 (1H, s, NH (urea)), 8.08 (1H, dd, $J = 7.9$ Hz, $J = 0.5$ Hz, H-6), 7.47–7.59 (3H, m, H–Ar), 7.19–7.30 (4H, m, H–Ar), 6.96 (1H, s, H-10), 4.23 (4H, bs, H-2, H-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 159.5 (C-5), 152.1 (C-11), 140.7 (C–F), 139.3 (C-10a), 138.7 (C-9a), 132.4 (C-9), 128.5 (C-6), 126.2 (C-7; C-8), 125.9 (C-16; C-12), 123.8 (C-5a), 123.3 (C-15), 121.4 (C-17), 120.9 (C-14), 87.6 (C-10), 44.1 (C-2), 42.5 (C-3). HRESIMS calcd for $C_{18}H_{14}FN_3O_2$ $[M+H]^+$ 324.1148, found 324.1166. Anal. Calcd. For $C_{18}H_{14}FN_3O_2$: C, 66.87; H, 4.36; N, 13.0. Found: C, 66.93; H, 4.01; N, 13.12.

3,8-Dioxo-1,2,3,5,6-hexahydrobenzo[*c*]imidazo[1,2,3-*ij*][1,8]naphthyridine-1-carboxylic acid (**19**)



A mixture of compound **1** (0.5 g, 2.7 mmol) and maleic anhydride (1.6 g, 16 mmol) in 15 mL of acetone was heated at reflux with stirring for 2 h. The mixture was allowed to rise to room temperature, and the crystalline solid was collected and crystallized from ethanol to yield 0.61 g (80 %). IR ν_{max} (KBr): 2900 (O–H), 2850 (C–H), 1703 (C=O), 1669 (C=O), 1590 (–N–CO) cm^{-1}. ^{1}H RMN (DMSO-d_6, 300 MHz) δ: 12.80 (1H, s, COO*H*), 8.15 (1H, d, $J = 7.8$ Hz, H-6), 7.67 (2H, dd, $J = 7.7$ Hz, H–Ar), 7.12–7.36 (1H, m, H–Ar), 4.05–4.20 (5H, m, H-2, H-3, H-aliph), 2.87-2.90 (2H, m, H-aliph). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 174.41 (C-11), 173.67 (C-14), 170.89 (C-5), 165.73 (C-10a), 134.21 (C-8), 132.59 (C-9a), 130.94 (C-6), 125.70 (C-7), 123.86 (C-5a), 123.06 (C-9), 117.8 (C-7), 48.11 (C-2), 44.61 (C-3), 44.51 (C-12), 40.40 (C-13). HRESIMS calcd for $C_{15}H_{12}N_2O_4$ $[M+H]^+$ 285.0875, found 285.0932. Anal. Calcd. For $C_{15}H_{12}N_2O_4$: C, 63.38; H, 4.25; N, 9.85. Found: C, 63.05; H, 4.34; N, 9.79.

N-(3-(Dimethylamino)propyl)-3,8-dioxo-1,2,3,5,6,8-hexahydrobenzo[*c*]imidazo[1,2,3-*ij*][1,8] naphthyridine-1-carboxamide (**20**)



To a solution of compound **19** (0.5 g, 1.83 mmol) in 5 mL of $CHCl_3$, 0.26 mL of triethylamine was added drop-

wise with stirring at 0 °C. While maintaining the temperature of less than 5 °C, a solution of ethyl chloroformate (0.19 mL, 1.83 mmol) in 2 mL of $CHCl_3$ was added over a period of 15 min. The resulting suspension was stirred at 0 °C for 20 min. The reaction was allowed to rise to room temperature and stirred for further 15 min. A solution of 3-(dimethylamino)-1-propylamine (0.25 mL, 2.10 mmol) in 5 mL of $CHCl_3$ was added. It was heated at reflux temperature for 2.5 h. The resulting white solid was collected by filtration in vacuo and crystallized from benzene to yield 0.16 g (25 %), mp 187–188 °C. IR ν_{max} (KBr): 3401 (N–H), 2850 (C–H), 1703 (C=O), 1669 (C=O), 1590 (N-CO) cm^{-1}. ^1H RMN ($CDCl_3$, 300 MHz) δ: 8.36 (1H, ddd, $J = 8.0$ Hz, $J = 2.0$ Hz, $J = 0.4$ Hz, H-6), 7.86 (1H, s, H-15), 7.64 (1H, t, $J = 8.0$ Hz, H–Ar), 7.35–7.39 (2H, m, H–Ar), 4.31–4.40 (3H, m, H-3, H-2, H-13), 4.12–4.20 (1H, m, H-aliph), 3.90–4.01 (1H, m, H-aliph), 3.21–3.25 (3H, m, H-aliph), 2.89–3.19 (1H, m, H-aliph), 2.11–2.18 (2H, m, H-aliph), 1.86 (6H, s, H-19), 1.45–1.55 (2H, m, H-17). ^{13}C RMN ($CDCl_3$, 75 MHz) δ: 172.66 (C-11), 170.73 (C-14), 162.81 (C-5), 160.16 (C10a), 134.20 (C-9a; C-8), 131.48 (C-6), 130.91 (C-5a), 126.55 (C-7), 125.12 (C-9), 93.25 (C-10), 57.48 (C-17), 45.55 (C-2), 45.14 (C-3), 44.45 (C-18; C-19), 39.14 (C-12; C-15), 38.39 (C-13), 26.38 (C-16). HRESIMS calcd for $C_{20}H_{24}N_4O_3$ $[M+H]^+$ 369.1927, found 369.1957. MS *m/z* (%): 369.20 (M+1, 16.15), 368.15 (M^+, 29.30), 238.95 (M^+ -$CONH(CH_2)_3(CH_3)_2$, 100), 196.10 (M^+-$OCH_2CHCONH(CH_2)_3N(CH_3)_2$, 20.28), 128.95 ($CONH(CH_2)_3N(CH_3)_2^+$, 84.50), 115.10 ($CHNH(CH_2)_3N(CH_3)_2^+$, 18.81), 102.15 ($NH(CH_2)_3N(CH_3)_2^+$, 6.46), 57.90 ($NH(CH_2)_3^+$, 75.41). Anal. Calcd for $C_{20}H_{24}N_4O_3$: C, 65.20; H, 6.57; N, 15.21. Found: C, 64.99; H, 6.73; N, 15.13.

Antiparasitic activity

The evaluation of antiparasitic activity was performed at Tropical Disease Research (TDR) Program, World Health Organization (WHO, Switzerland), following the well-known in vitro disease-oriented primary antiparasitic screening against *P. falciparum*. For antimalarial activity, the chloroquine sensitive (GHA) strain was used.

The in vitro protocols and activity criteria can be found at World Health Organization website (WHO). Summarizing, for antimalarial activity if the IC_{50} is >5 μM, the compound is classified as inactive. If the IC_{50} is <5 μM, the compound is classified as active.

SAR studies

Quantum-chemical semiempirical AM1 calculations were performed for the lowest energy conformation of the compounds using the Spartan'02 suite of programs. The compounds were built with standard bond lengths and angles using the Spartan'02 1.0.1 version, and the geometry of each molecule was fully optimized by applying the semiempirical AM1 method in gas phase from the most stable conformer obtained using molecular mechanics (MMFF) methods. Then, a single point calculation was done using DFT/6-31G*. Lipophilic properties of the compounds were included into the analyses, as clogP (logP calculated by Villar method, AM1). (Muscia *et al.*, 2011; Porcal *et al.*, 2007).

Binding of DHIIQ derivatives with heme

Hemin (FP) binding assay

The interaction of the compounds with hemin (Ferriprotoporphyrin IX, FP-Fe[III]) (Sigma) was examined spectrophotometrically under reducing and non-reducing conditions by monitoring the Soret absorption band of FP. The solutions were prepared in DMSO and diluted with water to the final concentrations of 0.1, 1, 10, 100, and 1000 mM. Then they were diluted in non-reducing (159 mM FP, 0.23 M sodium phosphate pH 7.4, 1 % SDS) buffer. Chloroquine (CQ) was purchased from Parafarm, and all other reagents were analytical grade. Absorption spectra were collected using a Jasco V-570 UV/Vis/NIR spectrophotometer. Data are average values of triplicate experiments.

Results and discussion

Synthesis of the compounds **1–12** proceeded as we described previously (Fig. 1) (Bollini *et al.*, 2006). A simple reaction between substituted arylisocyanates and compound **1** was carried out to prepare additional compounds **14–18**. The arylisocyanates were obtained according to the procedures described in the literature by the corresponding reaction of acyl chloride with sodium azide (Allen, 1955).

Then, acylazides were used for the preparation of compounds **13a–e** by Curtius rearrangement reaction (Curtius, 1890; Carrër *et al.*, 2011). The coupling of compound **1** with arylisocyanates afforded the $N-1$ substituted final compounds **14–18** (Scheme 1).

We decided to explore the influence of introducing a new cycle moiety to enlarge heterocycle **1** and on the antimalarial activity. For this purpose, the synthesis of compound **19** was carried out taking advantage of the marked enaminic properties of compound **1.** (Nagarajan *et al.*, 1988) Compound **19** was synthesized by acylation of compound **1** with an excess of maleic anhydride giving a

tetracyclic lactam in good yield. In order to improve the lipohydrophilic balance and solubility in water of this derivative, we converted compound **19** to the amide **20** by the well-known method of mixed anhydrides (Scheme 2). The identity of all assayed compounds was confirmed by ^{1}H and ^{13}C NMR, and high-resolution mass spectrometry.

All products were selected for a primary in vitro screening at the Tropical Disease Research (TDR) Program, World Health Organization (WHO, Switzerland). The target compounds were evaluated for their in vitro antimalarial activity against chloroquine sensitive (GHA) strain of *P. falciparum*. Table 1 presents the in vitro antimalarial results for the compounds **1–20.**

To further explore the structure–activity relationship of *N*-1 and *C*-10 substituent of this class of compounds, a set of analogs was prepared. The introduction of *N*-benzoyl or *N*-phenylsulfonyl moiety (**2** and **4**) resulted in compounds with moderate antimalarial activity, whereas the exchange of the aromatic groups with methyl group (**3** and **5**) abolished the activity against chloroquine sensitive (GHA) strain of *P. falciparum.* The C-10 phenylsulfonyl group analogs exhibited reduced antimalarial activity (**4** vs **11**) but the 4-dichloro-5-fluorophenyl analog **6** elicited excellent activity.

All the carboxamide analogs (**14–16** and **18**) had reduced activity, but replacing the phenyl by 4-methylphenyl resulted in analog **17** with high activity and selectivity. The introduction of a new cyclic moiety to enlarge compound **1** showed no improvement in antimalarial activity (**19** and **20**).

Molecular modeling studies were performed on the developed DHIIQ derivatives by calculating the stereoelectronic properties in order to understand the mechanism

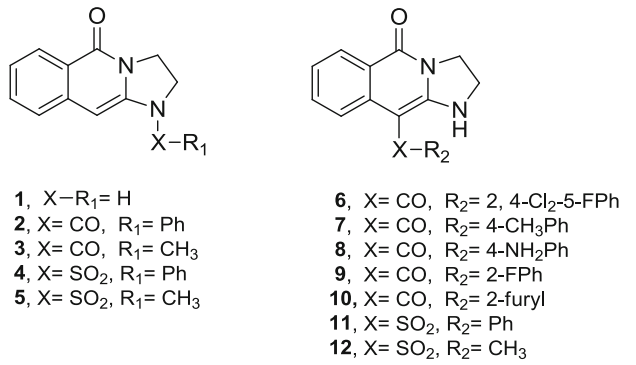
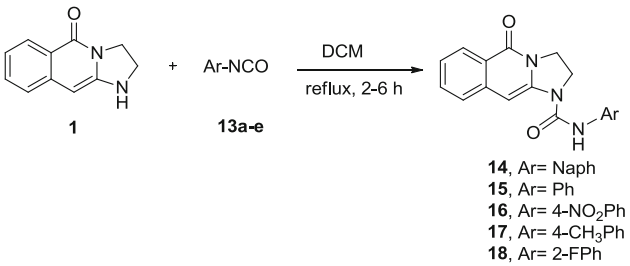


Fig. 1 Parents imidazoisoquinolinones

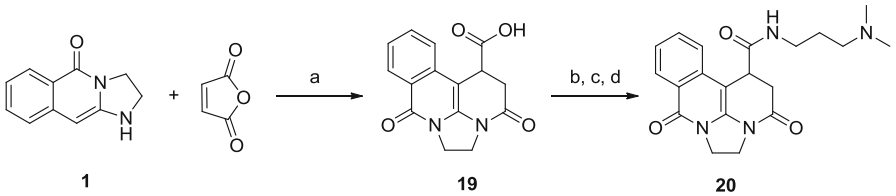


Scheme 1 Synthesis of compounds **14–18**

Table 1 In vitro antimalarial activity against *P. falciparum* GHA strain and cytotoxicity in L-6

Compounds	IC_{50} (μM)	CC_{50} (μM)	SI
1	>25	>100	–
2	4.60	192	16
3	>25	>90	–
4	1.60	169	34
5	14	16	–
6	0.13	89	329
7	>19	>23	–
8	>19	11	–
9	1.3	>90	>11
10	>19	>51	–
11	>16	>90	–
12	>16	73	–
14	19	>20	–
15	19.50	>90	–
16	ND	>90	–
17	1.20	73	61
18	16	>19.50	–
19	>19	>90	–
20	>19	>90	–
CQ	0.09	–	–

IC_{50} concentration that produces 50 % inhibitory effect, *SI* selectivity index = CC_{50} L-6/IC_{50} *P. falciparum*, *ND* not determined



Scheme 2 Synthesis of compounds **19** and **20**. Conditions: *a* Compound **1** (1 equiv), maleic anhydride (6 equiv), acetone, reflux, 2 h. *b* Compound **19** (1 equiv), triethylamine (5.4 equiv), $CHCl_3$ *c* Ethyl chloroformate, $CHCl_3$, 0 °C, 20 min. *d* 3-(dimethylamino)-1-propylamine, $CHCl_3$, reflux, 2.5 h

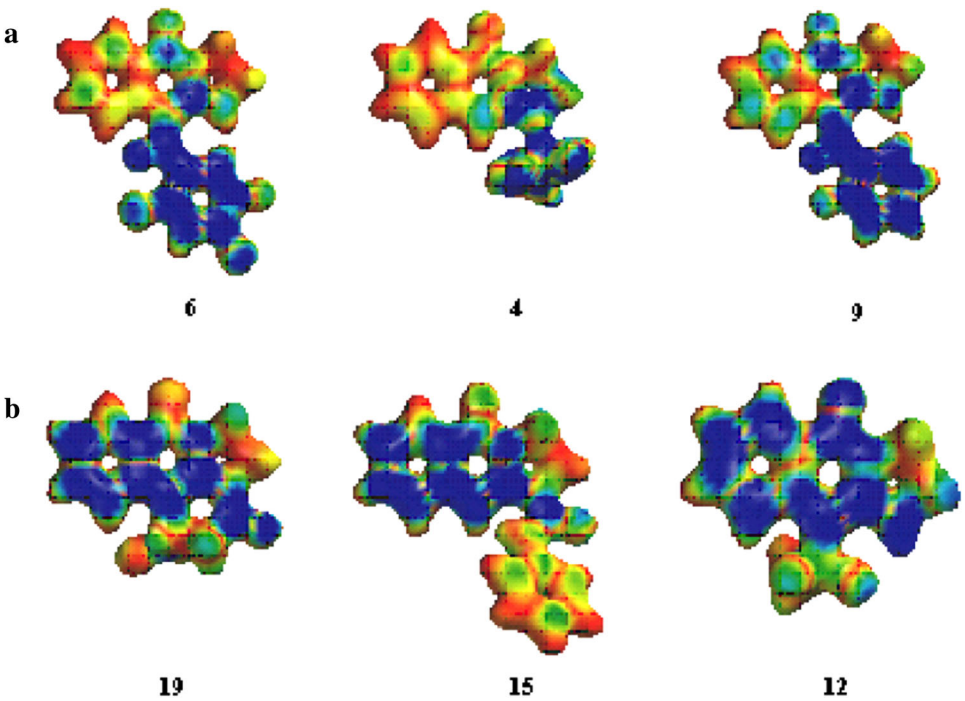


Fig. 2 LUMO mapped onto density surface maps in some relevant DHIIQ. **a** Active compounds **b** Inactive compounds. *Blue* areas correspond to the regions of the molecules more susceptible to nucleophilic attack (Color figure online)

of action. These properties were determined using DFT/B3LYP calculations. A detailed conformational search for each of the molecules was performed using MM methods to find the minimum energy and highest abundance conformer. The geometry of this conformer was fully optimized by applying B3LYP/6-31G*, and density functional calculation was performed in the gas phase. The determined and examined properties in this study were volume, total energy, solvation (water), heat formation energy, magnitude of dipolar moment, HOMO's and LUMO's energies, gap (E_{LUMO}–E_{HOMO}), and the logarithm of the partition coefficient of the non-ionized molecules (logP). Theoretical logP (*c*logP) was calculated using the Villar method, implemented in PC SPARTAŃ 02 package at the B3LYP/6-31G* density functional (Wavefunction) (Wiberg, 1986). The compounds were divided into two groups, active ($IC_{50} < 5$ μM; **2**, **4**, **6**, **9**, **17**) and inactive ($IC_{50} > 5$ μM; **1**, **3**, **5**, **7**, **8**, **10**, **11**, **12**, **14**, **18**, **19**, **20**). The activity criterion was based on WHO/TDR protocols (WHO). The difference in each variable between groups was analyzed using a nonparametric test (Meann-Whitney *U* test). (Porcal *et al.*, 2007), Active compounds displayed LUMO energy and topological polar surface area (TPSA) variables statistically different at the 0.01 level compared to inactive compounds. The two-population analysis showed that the active compounds had lower E_{LUMO} values ($E_{LUMO} = -3.25$ eV) than the inactive compounds ($E_{LUMO} = -1.18$ eV), thus implying that the reaction with a biological nucleophile would be kinetically more favorable for the first group. Figure 2 shows the distribution of the LUMO onto density surface maps in some relevant DHIIQ. In these graphics, it is possible to observe the differences in LUMO distributions between active and inactive derivatives against the *P. falciparum* GHA strain. In addition, the active compounds had better transport properties (lower TPSA) than the inactive compounds (Fig. 2).

In an effort to determine the probable mode of action of these novel imidazoisoquinolinones, we examined their interaction with oxidized ferroprotophorphyrin IX (FP). The binding of different quinoline antimalarial drugs to FP-Fe(III) has been previously shown to be important for the activity of these drugs. The binding to FP of drugs such as CQ modifies the spectral characteristics of FP, thereby producing a decrease in the absorption and a broadening of the main Soret band at 400 nm, even when it is present in stoichiometric quantities (Taylor *et al.*, 2004).

Optical spectroscopy studies were undertaken to determine the affinity of DHIIQ for heme and to explore the binding stoichiometry of the drug to target molecule. The addition of DHIIQs clearly perturbed the heme spectrum (Fig. 3A, e.g., compound **6**), indicative of an interaction between the drugs and the heme units.

Scatchard analysis of the heme-DHIIQ drugs interaction from this series of optical spectra is presented in Fig. 3b. The straight line with slope $1/K_d$ indicates that heme binding sites(s) for the drug is (are) equivalent and independent.

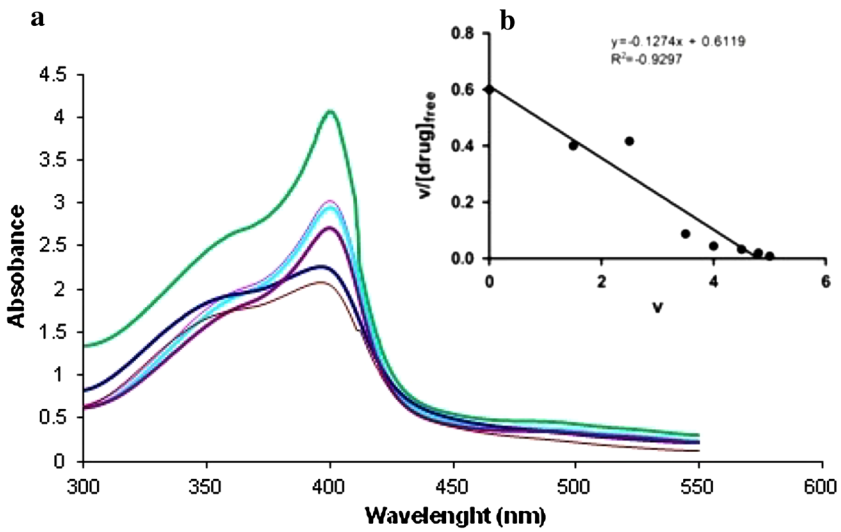


Fig. 3 Interaction of compound **6** with heme **a** Optical Soret spectroscopy for compound **6**-hemin interaction at different concentration of compound **6** (50 to 1000 μM). **b** Scatchard plot of heme-compound **6** association at 405 nm, 50 μM heme, pH 5.8, 25 °C

Table 2 Dissociation constants (K_d) and IC_{50} values of DHQII derivatives against *P. falciparum* in vitro

Compounds	K_d (μM)	IC_{50} (μM)
CQ[a]	7.1	0.09
2	40	4.60
4	16	1.60
5	81	14
6	8.3	0.13
9	21	3.10
14	93	19
17	14	1.20
19	>120	>19
20	>120	>19

The literature values of K_d for chloroquine is 2.5, 5.7 μM (Xu Kelly *et al.*, 2001)

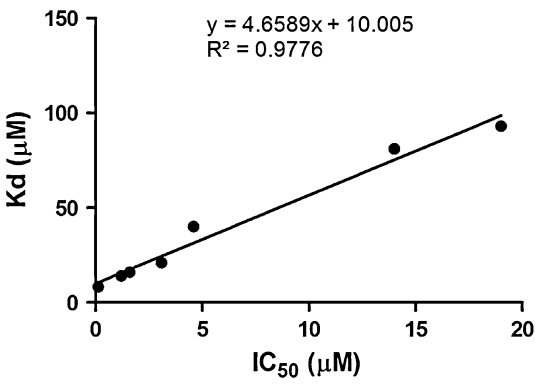


Fig. 4 Relation between dissociation constants (K_d) and compounds **2, 4**, **5**, **6, 9**, **14, 17** against *P. falciparum* in vitro

The dissociation constants (K_d) of DHIIQ and FP were calculated using a simple binding model by plotting the spectral change in dependence on the concentration (Kumar *et al.*, 2008; Chevli and Fitch, 1982). The dissociation constant for CQ binding to heme under identical conditions was also determined in the same manner and is presented in Table 2 for comparison. Compound **6** shows a binding affinity comparable to the standard antimalarial agent, CQ.

The correlation between antimalarial activity of **2**, **4**, **5**, **6**, **9**, **14**, **17** and their dissociation constants from FP indicates that this interaction may be biologically significant ($r = 0.9776$, Table 2, Fig. 3). FP is released from hemoglobin in the food vacuole after the parasite has digested the globin component. Normally, it would proceed to become ferriprotoporphyrin IX and subsequently hemozoin. Interaction with imidazoisoquinoline derivatives disrupts this normal process and may give rise to potentially toxic agents (Fig. 4).

In conclusion, new DHIIQ derivatives were synthesized, characterized, and evaluated for their in vitro antimalarial activity. Compounds **2**, **4**, **9,** and **17** displayed moderate activity against the *P. falciparum* whereas compound **6** exhibited an excellent activity (0.13 μM) similar to that of CQ (0.09 μM). A strong correlation between FP binding affinity and inhibition of malaria parasite growth in culture was observed. These results support the concept that these compounds may exert their antimalarial activity through binding to FP. These findings provide supporting evidence to stimulate further in vivo studies of these compounds in appropriate animal models of malaria disease.

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