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Original article

Design and preparation of aza-analogues of benzo[c]phenanthridine framework with cytotoxic and antiplasmodial activities

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ABSTRACT

Benzo[c]phenanthridine alkaloids represent interesting lead for the discovery of new potential antiplasmodial and/or anticancer drugs. In this field, a novel library of aza-analogs of benzo[c]phenanthroline framework derivatives was designed and prepared. Although these compounds did not have specific antiplasmodial activities, some of them displayed specific *in vitro* activity against two cancer lines especially compound **24** with an IC_{50} against the MCF7 line of 0.6 μM.

1. Introduction

Benzo[c]phenanthridine bases are distributed in Papaveraceous and Rutaceous plants. They have a long history, dating back to their first report in 1839 [1]. Among them, nitidine **1** an alkaloid isolated from Rutaceous plants, was reported to exhibit a strong antimalarial activity against a range of chloroquine-sensitive and -resistant strains of *P. falciparum* and showed no cross-resistance with chloroquine [2]. Concurrently, nitidine as well as others benzo[c] phenanthridine alkaloïds, is well known to exhibit anticancer activities by acting as topoisomerases inhibitors [3,4]. As a consequence of this, structure–activity relationships were developed in order to enhance the selectivity of such compounds. In this way, synthetic benzo[c]phenanthridines were tested *in vitro* for antimalarial activity showing that fagaronine **2** was relatively inactive while O-Methyl fagaronine **3** was 6–30 times more potent than **2** [5]. In contrast, the fully synthetic analogue **4** was equally active and this gave encouragement that analogue design could give compounds with increased biological potency (Fig. 1).

In this field too, works were conducted with the aim of designing new analogues by studying the impact of the position and number of nitrogen atoms. As an example, 5-aza-analogues of benzo[c]phenanthridines were designed as new topoisomerase I-targeting anticancer agents [6], while, in the same time, aza-analogues of the marine pyrroloquinoline alkaloids wakayin and tsitsikammamines were also reported as potential antitumor agents, and many works were conducted around ellipticine skeleton showing the influences of additional nitrogen atoms [7,8]. Concurrently, in the field of antiplasmodial compounds, we have previously reported some studies concerning the synthesis and biological activities of azaphenanthridines showing the impact of the position of the nitrogen atom [9,10], and these results finally led us to design new conformationally restricted analogs of primaquine with a 1,10-phenanthroline framework [11].

As a continuation of these investigations, we initiated a project aimed to design new libraries of azaphenanthridines related to the benzo[c]phenanthridine class. In this context, we were interested in developing an efficient and general strategy for the preparation of series A, B, C from diverses amino(iso)quinolines as starting materials. Among the different routes, we focused our attention in the intramolecular aryl–aryl coupling reaction involving palladium reagents which has been already used for the preparation of many condensed heteroatomic compounds including benzo[c]phenanthridines [12–15].

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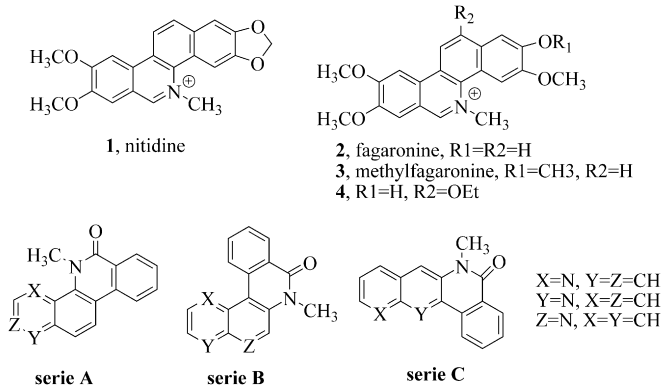


Fig. 1. Structure of nitidine **1**, fagaronine derivatives **2**–**4** and targeted libraries A, B, C.

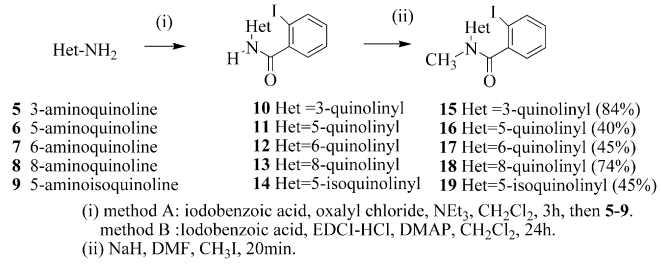
2. Results and discussion

2.1. Chemistry

Conversion of amino(iso)quinolines **5**–**9** listed in Scheme 1 to tertiary amides **15**–**19** was investigated using two methods A and B (Scheme 1, Table 1). Using method A, 2-iodobenzoic acid was first converted *in situ* into the corresponding acyl chloride which was then allowed to react with the amines **5**–**9** in presence of triethylamine. This method was efficient only for the preparation of **11**, **13** and **14**. Preparation of **10**–**14** was finally optimized by using the alternative method B (2-iodobenzoic acid, EDCI·HCl, DMAP). Structural elucidations of **10**–**14** were easily achieved by ^{1}H and ^{13}C NMR experiments, in particular because of the presence of the quaternary carbons of the amide function near 170 ppm.

Subsequently, the secondary amides **10**–**14** were alkylated by treatment with sodium hydride followed by methyl iodide. The resulting tertiary amides **15**–**19** were obtained in moderate to good yields (40–84%) as mixtures of rotamers. In each case, one rotamer was preponderant but not identified. These mixtures were used for the subsequent steps.

The palladium catalyzed reaction of **15**–**19** was evaluated using $Pd(OAc)_2$, PBu_3, Ag_2CO_3 in refluxing DMF. The reaction smoothly proceeded to give regioselectively the corresponding tetracycles with **16**, **19** to give **22**, **26** while in the case of **18**, low yield was obtained and finally the reaction was optimized by using PPh_3 to give **25** in 74% yield. Concerning the two last amides **15**, **17** reactions were found to lead to the two regioisomers (respectively **20**, **21** for **15** and **23**, **24** for **16**). Discrimination between the two regioisomers **20**, **21** was achieved by ^{1}H NMR showing for **20** a deshielded singlet at 9.22 ppm corresponding to H-6 while in the case of **23**, **24**, the ^{1}H NMR spectrum of **24** showed two singlets at 7.79 and 8.92 ppm, respectively attributed to H-5 and H-12. In addition all structures were confirmed by ^{13}C NMR and mass spectroscopy data (Scheme 2).



Scheme 1. Preparation of tertiary amides; (i) method A or method B, (ii) NaH, CH_3I.

Table 1
preparation of secondary amides.

Compound	Method A	Method B
10	44%	72%
11	90%	10%
12	22%	85%
13	82%	82%
14	47%	28%

Method A: iodobenzoic acid, oxalyl chloride.
Method B: 2-iodobenzoic acid, EDCI, DMAP.

2.2. Biology

2.2.1. Antiplasmodial activities

Two strains of *P. falciparum* were used to evaluate the *in vitro* antiplasmodial activities of compounds **20**–**26**: The chloroquine (CQ)-resistant FcB1-Columbia and F32 CQ-sensitive strains. Parasites were cultured according to the method originally described by Trager and Jensen [16–18]. For each *in vitro* test, the parasite culture was incubated with the tested compound at growing dilutions for 48 h. Results summarized in Table 2 showed that all tetracyclic compounds exhibited lower *in vitro* antiplasmodial activities than primaquine or chloroquine.

2.2.2. Cytotoxic activities [19]

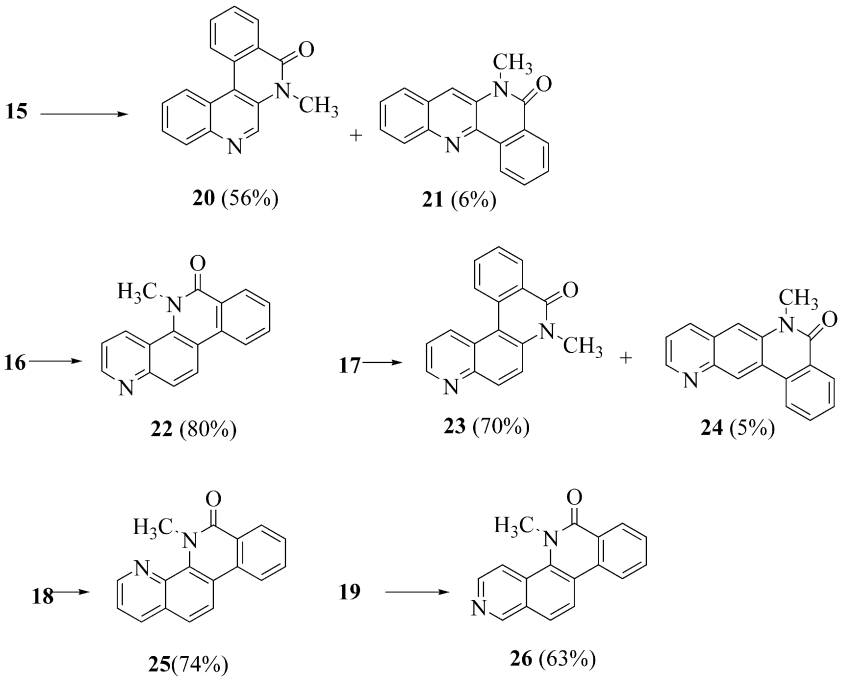
Two cell lines were used MCF7 (human breast carcinoma) and the Vero cells (monkey kidney epithelial cells). Except compounds **20**, **21**, all compounds exhibited interesting activities. Furthermore, it can be highlighted that compound **22** is not selective (similar activities against both *Plasmodium* and cancer cell lines).

Although interpretation of these results in terms of SAR is difficult at this stage, some elements could be highlighted when comparing geometries of compounds **20**–**26**. Interestingly, two groups of compounds came out. The first group constituted of twisted compounds **20**, **22**, **23**, **26** (Fig. 2) exhibiting mild antiplasmodial activities, while the second group is constituted of flat compounds **21**, **24**, **25** (Fig. 3). Among them, **21**, **24** were the less active against the two strains of *P. falciparum* tested, while compound **25** was ten fold more active. However, the latter represented a particular case in which the conformation was twisted after protonation. In addition, when considering the less active flat structures **21**, **24** against *P. falciparum*, one **21** was not cytotoxic against the cell lines tested, while a particular interest arised when considering **24**. This compound exhibited better activities than doxorubicin against the two cell lines and in a similar way (more active on MCF7 than on Vero cell line, Table 3).

As these compounds are potential intercalating as well as anti-topoisomerases agents, it can be highlighted that the position of the nitrogen atom is crucial for the activity. At this stage only few structural features of the molecules can be taken in account: repartition of electron densities (Fig. 4) and localisation of H-bonding site (nitrogen atom). These two properties may have a great impact on targeting and on the stabilization of biological complexes DNA/drug and/or DNA/topo/drug. In the case of **24**, the electron rich domain related to the quinolinic nitrogen and located on the nitrogen appeared more available to implicate this position in H-bonding, while in the case of **21**, this domain is more enclosed inside the aromatic framework. Further theoretical studies as well as experimental studies concerning the interaction of **21** and **24** with DNA and topoisomerases will be necessary in order to refine such hypothesis.

3. Conclusion

A library of benzophenanthridine-like compounds have been synthesized. Although these compounds did not have



Scheme 2. Synthesis of compounds **20**–**26** through Heck coupling strategy.

Table 2
IC_{50} (μM) of compounds **20**–**26** on two *P. falciparum* strains tested.

Compounds	F32	FcB1
20	32.7 ± 2.7	14.8 ± 0.9
21	354.6 ± 1.5	180.7 ± 16.0
22	17.6 ± 4.8	22.3 ± 8.8
23	96.1 ± 16.3	80.7 ± 10.8
24	123.0 ± 8.0	153.8 ± 5.4
25	36.3 ± 1.2	38.2 ± 1.0
26	61.0 ± 5.4	54.8 ± 5.2
PMQ	6.83 ± 1.66	6.87 ± 1.69
CQ	0.046	0.155
DOX	NT	NT

PMQ, primaquine; CQ, chloroquine; DOX, doxorubicine; NT, not tested.

specific antiplasmodial activities, further studies are now needed in order to confirm the potentiality of compound **24** for designing new leads as potential anticancer drugs. For this purpose, regioselective access to this class of compounds permitting structure–activity relationships studies are under investigation.

4. Experimental protocols

4.1. Chemistry

All column chromatographies were performed with Merck neutral aluminum oxide 90 standardized (63–200 μm) or silica gel (Acros organic, 60 Å, 35–70 μm). All thinlayer chromatographies were performed on Fluka aluminum oxide plates (with fluorescent indicator 254 nm) or Merck silica gel $60F_{254}$ plates. Melting points were determined on a Reichert-Jung–Koffler apparatus and are not corrected. NMR spectra (300 MHz for ^{1}H and 75 MHz for ^{13}C) were recorded on a Bruker Avance 300 instruments using $CDCl_3$ or MeOD as solvent. Chemical shifts were reported in ppm (δ) downfield

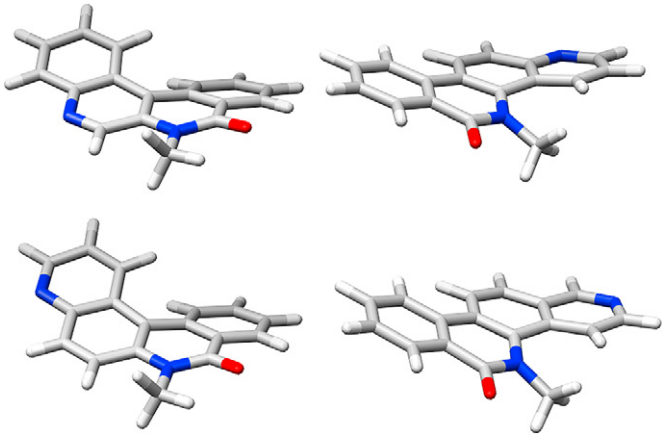


Fig. 2. lowest energy of the twisted conformations of compounds **20** (up left), **22** (up right), **23** (down left), **26** (down right).

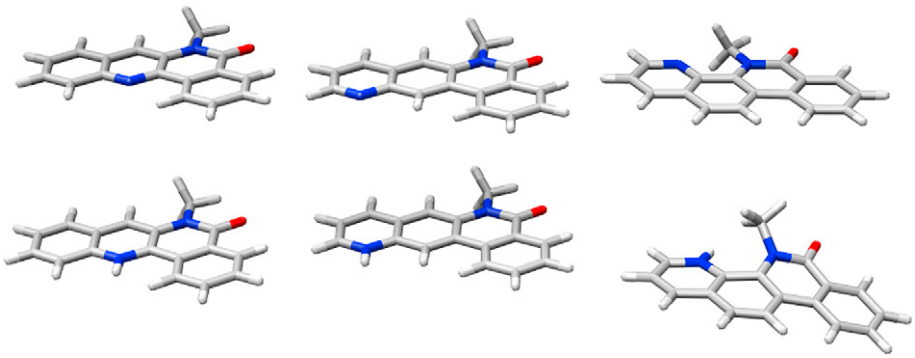


Fig. 3. lowest energy of the flat conformations of compounds **21** (up left), **24** (up middle), **25** (up right) and their protonated forms (down).

Table 3
IC_{50} (μM) of compounds **20**–**26** on MCF7 and vero cell line.

Compounds	MCF7	Vero
20	30.9 ± 4.6	22.3 ± 1.15
21	153.8 ± 4.2	42.0 ± 4.24
22	12.5 ± 8.8	3.5 ± 0.1
23	11.5 ± 1.7	1.75 ± 0.78
24	0.6 ± 0.15	2.85 ± 0.35
25	16.5 ± 0.5	3.35 ± 0.64
26	11.9 ± 1.6	2.3 ± 0.2
PMQ	NT	NT
CQ	NT	NT
DOX	0.41 ± 0.05	7.4 ± 1.6

PMQ, primaquine; CQ, chloroquine; DOX, doxorubicine; NT, not tested.

from TMS. All the coupling constants (J) are in hertz. ESI-MS analyses were performed on a Bruker Esquire 6000 instrument. Analytical analysis were not reported because amides were not stable enough, and results of these analysis were not significant, especially for the iodo derivatives. However, all compounds were successfully analyzed by SM (2 decimals), NMR and checked for their degrees of purity by HPLC before proceeding biological tests.

*4.1.1. Typical procedure for preparation of N-(iso)quinolyl-2-iodobenzamides **10**–**14***

Procedure **A**: Oxalyl chloride (2.30 g, 18.1 mmol) was added to a mixture of 2-iodobenzoic acid (1.50 g, 6.05 mmol) in dry dichloromethane (40 mL), and the mixture was heated to reflux under nitrogen with stirring for 3 h. The mixture was concentrated to dryness under vacuum. The acid chloride was redissolved in dry dichloromethane (40 mL) and the solution was taken and cooled to 0 °C. A solution of amino(iso)quinoline **5**–**9** (0.72 g, 5.0 mmol) in dry dichloromethane (20 mL) was added dropwise and distilled triethylamine (0.76 g, 7.5 mmol) was added at the same temperature. The reaction mixture was stirred for another 1 h at 0 °C. The reaction was gradually allowed to reflux. After completion of the reaction (TLC, Al_2O_3, CH_2Cl_2), the product was then diluted with a Na_2CO_3 saturated solution (35 mL). The product was then four times extracted with dichloromethane/2-propanol (3:1, 40 mL). The product was then dried with anhydrous sodium sulphate, filtered and evaporated. The residue was purified by column chromatography (Al_2O_3, CH_2Cl_2) to give the pure corresponding compound.

Procedure **B**: A mixture of amino(iso)quinoline **5**–**9** (0.72 g, 5.0 mmol), 2-iodobenzoic acid (1.86 g, 7.5 mmol), EDCI–HCl (1.44 g, 7.5 mmol) and DMAP (92 mg, 0.75 mmol) in dichloromethane (30 mL) was stirred at room temperature for 24 h. The precipitate was then washed with 5% HCl solution (5 mL) and dissolved in Na_2CO_3 saturated solution (10 mL).

The product was then extracted with dichloromethane (3 × 30 mL), the organic layer was then dried with anhydrous sodium sulphate, filtered and evaporated. The residue was purified by column chromatography (Al_2O_3, CH_2Cl_2) to give the title compound.

*4.1.1.1. N-(3-quinolinyl)-2-iodobenzamide (**10**).* Prepared from 3-aminoquinoline by the procedure **A** (44%) or by procedure **B** (72%), mp 204–206 °C; R_f 0.20 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$/MeOD, 300 MHz) δ 7.20 (m, 1H), 7.50 (m, 2H), 7.60 (t, 1H, J = 7.5 Hz), 7.69 (t, 1H, J = 7.5 Hz), 7.89 (d, 1H, J = 7.5 Hz), 7.94 (d, 1H, J = 7.5 Hz), 7.99 (d, 1H, J = 7.5 Hz), 8.86 (s, 1H), 8.97 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 92.4, 124.5, 127.4, 127.9, 128.2, 128.4, 128.6 (2C), 129.0, 131.2, 131.8, 140.2, 141.4, 143.8, 145.5, 167.9; MS (ESI, m/z) 397.03 ($M + Na^+$), 375.09 ($M + H^+$)

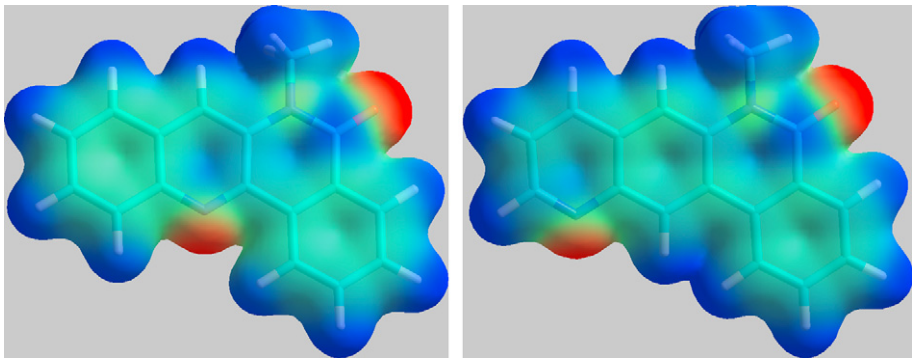


Fig. 4. Electrostatic potential surfaces for compounds **21** (left) and **24** (right). The values are color-coded onto the total electron density surface, with colors toward red indicating electron rich regions of the molecule (charge range from −0.08327 (red) to +0.08327 (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

*4.1.1.2. N-(5-quinolinyl)-2-iodobenzamide (**11**).* Prepared from 5-aminoquinoline by the procedure **A** (90%) or by procedure **B** (10%); mp 226–228 °C; R_f 0.18 (Al_2O_3, CH_2Cl_2); ^{1}H NMR (MeOD, 300 MHz) δ 7.16 (t, 1H, J = 8 Hz), 7.49 (m, 2H), 7.56 (d, 1H, J = 8 Hz), 7.79 (m, 2H), 7.90 (m, 2H), 8.60 (d, 1H, J = 7.5 Hz), 8.80 (d, 1H, J = 4 Hz); ^{13}C NMR (MeOD, 75 MHz) δ 93.3, 122.5, 124.9, 125.7, 128.0, 129.2, 129.5, 130.7, 132.4, 134.0, 134.4, 140.9, 144.1, 149.3, 151.5, 171.8; MS (ESI, m/z) 397.04 (M + Na^+), 375.10 (M + H^+).

*4.1.1.3. N-(6-quinolinyl)-2-iodobenzamide (**12**).* Prepared from 6-aminoquinoline by the procedure **A** (22%) or by procedure **B** (85%); mp 200–202 °C; R_f 0.22 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.13 (t, 1H, J = 7.5 Hz), 7.42 (m, 2H), 7.55 (d, 1H, J = 7.5 Hz), 7.69 (d, 1H, J = 9 Hz), 7.89 (d, 1H, J = 7.5 Hz), 8.09 (d, 1H, J = 9 Hz), 8.19 (m, 2H), 8.55 (s, 1H), 8.82 (d, 1H, J = 4.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 92.4, 116.6, 121.8, 123.2, 128.4, 128.6, 128.9, 130.3, 131.7, 135.5, 136.2, 140.1, 141.8, 145.6, 149.5, 167.6; MS (ESI, m/z) 397.01 (M + Na^+), 375.10 (M + H^+).

*4.1.1.4. N-(8-quinolinyl)-2-iodobenzamide (**13**).* Prepared from 8-aminoquinoline by the procedure **A** (82%) or by procedure **B** (82%); mp 157–159 °C; R_f 0.90 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17 (t, 1H, J = 7.5 Hz), 7.45 (m, 2H), 7.61 (m, 3H), 7.96 (d, 1H J = 7.5 Hz), 8.17 (d, 1H, J = 7.5 Hz), 8.77 (s, 1H,), 8.94 (s, 1H, J = 6 Hz), 10.15 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 92.8, 116.9, 121.8, 122.2, 127.4, 128.1, 128.3, 128.4, 131.4, 134.3, 136.4, 138.6, 140.3, 142.3, 148.4, 167.4; MS (ESI, m/z) 397.04 (M + Na^+), 375.10 (M + H^+).

*4.1.1.5. N-(5-isoquinolinyl)-2-iodobenzamide (**14**).* Prepared from 5-aminoisoquinoline by the procedure **A** (47%) or by procedure **B** (28%); mp 205–207 °C; R_f 0.18 (Al_2O_3, CH_2Cl_2); ^{1}H NMR (MeOD, 300 MHz) δ 7.27 (t, 1H, J = 8 Hz), 7.57 (t, 1H, J = 8 Hz), 7.67 (d, 1H, J = 8 Hz), 7.79 (t, 1H, J = 8 Hz), 8.01 (d, 1H, J = 8 Hz), 8.08 (d, 1H, J = 8 Hz), 8.14 (m, 2H), 8.50 (d, 1H, J = 6 Hz), 9.30 (s, 1H); ^{13}C NMR (MeOD, 75 MHz) δ 92.1, 116.0, 125.9, 126.7, 127.3, 127.8, 127.9, 129.0, 131.0 (2C), 131.6, 139.4, 141.7, 142.0, 151.9, 169.6; MS (ESI, m/z) 397.04 (M + Na^+), 375.10 (M + H^+).

*4.1.2Typical procedure for preparation of N-methyl-N-(iso)quinolyl-2-iodo-benzamides (**15**–**19**)*

A solution of iodobenzamide (0.30 g, 0.80 mmol) in sodium hydride (64 mg, 1.61 mmol) (60% dispersed in mineral oil), and dry DMF (5 mL) was prepared at 0 °C. The iodomethane (136 mg, 0.96 mmol) was then added, slowly, to the reaction mixture at 0 °C. The reaction mixture was stirred at room temperature for 20 min. The solution was diluted with dichloromethane (10 mL) and then washed with water (3 × 20 mL). The organic layer was dried with anhydrous sodium sulphate, filtered, and evaporated. The product was then purified using column chromatography on silica gel (CH_2Cl_2: MeOH; 95:5) to give two rotamers.

*4.1.2.1. N-methyl-N-(3-quinolinyl)-2-iodobenzamide (**15**).* Prepared from **10**. (84%); mp 156–158 °C; R_f 0.40 (SiO_2, CH_2Cl_2: MeOH; 95:5); ^{1}H NMR (major rotamer) ($CDCl_3$, 300 MHz) δ 3.62 (s, 3H), 6.82 (t, 1H, J = 7.5 Hz), 7.16 (m, 2H), 7.64 (m, 4H), 7.98 (m, 2H), 8.71 (s, 1H); MS (ESI, m/z) 411.05 (M + Na^+), 389.10 (M + H^+).

*4.1.2.2. N-methyl-N-(5-quinolinyl)-2-iodobenzamide (**16**).* Prepared from **11**. (40%); mp 117–119 °C; R_f 0.36 (SiO_2, CH_2Cl_2: MeOH; 95:5); ^{1}H NMR (major rotamer) ($CDCl_3$, 300 MHz) δ 3.58 (s, 3H), 6.78 (m, 3H), 7.55 (m, 2H), 7.62 (m, 2H), 7.95 (d, 1H, J = 7.5 Hz), 8.40 (d, 1H, J = 8.5 Hz), 8.95 (d, 1H, J = 4 Hz); MS (ESI, m/z) 411.09 (M + Na^+), 389.09 (M + H^+).

*4.1.2.3. N-methyl-N-(6-quinolinyl)-2-iodobenzamide (**17**).* Prepared from **12**. (45%); oil; R_f 0.30 (SiO_2, CH_2Cl_2: MeOH; 95:5); ^{1}H NMR (major rotamer) ($CDCl_3$, 300 MHz) δ 3.57 (s, 3H), 6.76 (t, 1H, J = 7.5 Hz), 7.06 (m, 2H), 7.33 (m, 1H), 7.49 (d, 1H, J = 7.5 Hz), 7.58 (m, 2H), 7.89 (d, 1H, J = 7.5 Hz), 7.98 (d, 1H, J = 7.5 Hz), 8.82 (m, 1H); MS (ESI, m/z) 411.04 (M + Na^+), 389.12 (M + H^+).

*4.1.2.4. N-methyl-N-(8-quinolinyl)-2-iodobenzamide (**18**).* Prepared from **13**. (74%); mp 100–102 °C; R_f 0.30 (SiO_2, CH_2Cl_2: MeOH; 95:5); ^{1}H NMR (major rotamer) ($CDCl_3$, 300 MHz) δ 3.59 (s, 3H), 6.65 (m, 2H), 6.89 (d, 1H, J = 7.5 Hz), 7.30 (t, 1H, J = 7.5 Hz), 7.41 (m, 1H), 7.55 (d, 1H, J = 7.5 Hz), 7.61 (d, 1H, J = 7.5 Hz), 7.73 (d, 1H, J = 7.5 Hz), 8.06 (d, 1H, J = 7.5 Hz), 9.00 (m, 1H); MS (ESI, m/z) 411.04 (M + Na^+), 389.10 (M + H^+).

*4.1.2.5. N-methyl-N-(5-isoquinolinyl)-2-iodobenzamide (**19**).* Prepared from **14**. (45%); mp 180–182 °C; R_f 0.30 (SiO_2, CH_2Cl_2: MeOH; 95:5); ^{1}H NMR (major rotamer) ($CDCl_3$, 300 MHz) δ 3.58 (s, 3H), 6.75 (m, 3H), 7.43 (t, H, J = 7.5 Hz), 7.64 (d, 1H, J = 7.5 Hz), 7.77 (d, 1H, J = 7.5 Hz), 7.83 (m, 2H), 8.69 (m, 1H), 9.25 (s, 1H); MS (ESI, m/z) 411.10 (M + Na^+), 389.10 (M + H^+).

4.1.3. Typical procedure for Heck cyclisation

To a solution of N-methyl-N-((iso)quinolinyl)-2-iodo-benzamides **15**–**19** (110 mg, 0.28 mmol) in dry DMF (4 mL) were successively added $Pd(OAc)_2$ (12 mg, 0.054 mmol), Bu_3P (23 mg, 0.11 mmol) and Ag_2CO_3 (154 mg, 0.56 mmol). The mixture was refluxed for 30 min and the reaction mixture was diluted with ether and the precipitate was removed by filtration. The filtrate was diluted with dichloromethane (30 mL) and washed with water (3 × 30 mL) and brine (30 mL). The organic layer was then dried with anhydrous sodium sulphate, filtered and evaporated. The mixture was purified using column chromatography (Al_2O_3, CH_2Cl_2).

*4.1.3.1. 7-Methyl-7H-dibenzo[c,f][1,7]naphthyridin-8-one (**20**).* Prepared from compound **15** (56%); mp 238–240 °C; R_f 0.30 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.02 (s, 3H), 7.73 (m, 3H), 7.88 (t, 1H, J = 7.5 Hz), 8.23 (m, 1H), 8.69 (d, 1H, J = 7.5 Hz), 8.77 (m, 2H), 9.22 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 30.1, 119.5, 123.4, 125.3, 127.5, 127.7, 128.0, 129.2, 129.4, 130.3, 130.8, 131.9, 132.2 (2C), 139.8, 144.8, 161.2; MS (ESI, m/z) 283.19 (M + Na^+).

*4.1.3.2. 6-Methyl-6H-dibenzo[b,h][1,5]naphthyridin-5-one (**21**).* Prepared from compound **15** (6%); mp 220–222 °C (lit [20]. 219–220 °C); R_f 0.70 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 3H), 7.56 (t, 1H, J = 7.5 Hz), 7.72 (m, 2H), 7.85 (m, 3H), 8.19 (d, 1H, J = 7.5 Hz), 8.51 (d, 1H, J = 7.5 Hz), 9.06 (d, 1H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 29.7, 118.1, 124.5, 127.1, 127.3, 127.5, 128.1, 128.3 (2C), 129.3, 130.4, 132.4, 132.8, 134.4, 139.6, 144.0, 161.1; MS (ESI, m/z) 283.05 (M + Na^+).

*4.1.3.3. 5-Methyl-5H-benzo[c][1,7]phenanthrolin-6-one (**22**).* Prepared from **16** (80%); mp 159–161 °C; R_f 0.80 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.02 (s, 3H), 7.43 (m, 1H), 7.63 (t, 1H, J = 9 Hz), 7.80 (t, 1H, J = 9 Hz), 7.99 (d, 1H, J = 8.5 Hz), 8.30 (d, 1H, J = 8.5 Hz), 8.47 (d, 1H, J = 9 Hz), 8.55 (d, 1H, J = 9 Hz), 8.69 (d, 1H, J = 9 Hz), 8.93 (d, 1H, J = 4.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 40.7, 117.3, 119.3, 119.8, 122.2, 123.6, 125.2, 125.4, 128.4, 128.6, 132.9, 133.4 (2C), 135.9, 149.3, 150.3, 164.3; MS (ESI, m/z) 283.04 (M + Na^+), 283.20 (M + H^+).

*4.1.3.4. 6-Methyl-6H-benzo[a][4,7]phenanthrolin-5-one (**23**).* Prepared from **17** (70%); mp 249–251 °C; R_f 0.22 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.91 (s, 3H), 7.52 (m, 1H), 7.64 (t, 1H, J = 8 Hz), 7.79 (t, 1H, J = 8 Hz), 7.85 (d, 1H, J = 9 Hz), 8.21 (d, 1H, J = 9 Hz), 8.43

(d, 1H, $J = 9$ Hz), 8.61 (d, 1H, $J = 8$ Hz), 8.88 (d, 1H, $J = 4$ Hz), 9.10 (d, 1H, $J = 8$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 30.7, 113.7, 118.6, 121.4, 126.0, 126.6, 126.8, 127.8, 129.1, 131.2, 131.9, 133.1, 134.2, 136.5, 145.1, 148.5, 161.1; MS (ESI, m/z) 283.17 ($M + Na^+$).

*4.1.3.5. 6-Methyl-6H-quinolino[6,7-b]isoquinolin-7-one (**24**).* Prepared from **17** (5%); mp 225–226 °C; R_f 0.40 (Al_2O_3, CH_2Cl_2); ^{1}H NMR (MeOD, 300 MHz) δ 3.85 (s, 3H), 7.52 (m, 1H), 7.65 (t, 1H, $J = 8$ Hz), 7.79 (s, 1H), 7.84 (t, 1H, $J = 8$ Hz), 8.34 (d, 1H, $J = 8$ Hz) 8.43 (d, 1H, $J = 8$ Hz), 8.50 (d, 1H, $J = 8$ Hz), 8.83 (d, 1H, $J = 4.5$ Hz), 8.92 (s, 1H); ^{13}C NMR ($CDCl_3$/MeOD, 75 MHz) δ 30.2, 122.2, 122.8, 123.3, 123.9, 126.1, 129.3, 129.4, 129.8, 133.5, 133.8, 137.0, 137.1, 143.7, 150.3, 162.5; MS (ESI, m/z) 283.09 ($M + Na^+$).

*4.1.3.6. 5-Methyl-5H-benzo[c][1,10]phenanthrolin-6-one (**25**).* Prepared from **18** (30% with PBu_3, 74% with PPh_3); mp 164–166 °C; R_f 0.40 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.34 (s, 3H), 7.46 (m, 1H), 7.65 (m, 2H), 7.81 (t, 1H, $J = 8$ Hz), 8.17 (d, 1H, $J = 8$ Hz), 8.35 (m, 2H), 8.62 (d, 1H, $J = 8$ Hz), 8.93 (d, 1H, $J = 4$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.6, 119.2, 121.2, 121.5, 122.2, 122.6, 126.0, 128.4, 128.8, 129.4, 132.5, 133.4, 135.8, 135.9, 140.8, 147.2, 163.8 (CO); MS (ESI, m/z) 283.09 ($M + Na^+$).

*4.1.3.7. 5-Methyl-5H-benzo[c][1,8]phenanthrolin-6-one (**26**).* Prepared from **19** (63%); mp 148–150 °C; R_f 0.18 (Al_2O_3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.09 (s, 3H), 7.70 (t, 1H, $J = 8$ Hz), 7.86 (m, 1H), 7.89 (d, 1H, $J = 9$ Hz), 8.19 (d, 1H, $J = 6$ Hz), 8.36 (d, 1H, $J = 8$ Hz), 8.43 (d, 1H, $J = 9$ Hz), 8.59 (d, 1H, $J = 6$ Hz), 8.61 (d, 1H, $J = 8$ Hz), 9.32 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 40.3, 118.0, 120.3, 121.7, 122.5, 123.1, 126.0, 127.6, 128.8, 129.1, 129.4, 133.0, 133.1, 135.0, 142.6, 153.0, 164.0 (CO); MS (ESI, m/z) 283.16 ($M + Na^+$).

Computational methods: Structure of compounds (**20**–**26**) was built in HyperChem Release 8.0.5 pro for Windows (Hypercube Inc. Gainesville, Florida).

The geometries were optimized using the semi-empirical AM1 programme in singly-excited configuration interaction. (RHF [Restricted Hartree–Fock], charge 0, spin multiplicity 1, lowest state, orbital criterion, five occupied and five unoccupied orbitals.) To an rms (root mean square) gradient of 0.01 in vacuo (Polak–Ribière method). For the protonated forms, the system was first optimized in MM+ to an rms gradient of 0.5. Then a molecular dynamics program was run for 1 ps, with 0.001 ps steps, relaxation time 0.1 ps, to a simulation temperature of 300 K. This was followed by MM+ geometry optimization to an rms gradient of 0.2. The molecular dynamics run was repeated and a further MM+ protocol was carried out to a gradient of rms 0.04. Finally, the geometries were optimized using the semi-empirical AM1 programme in singly-excited configuration interaction. (RHF [Restricted Hartree–Fock], charge 1, spin multiplicity 1, lowest state, orbital criterion, five occupied and five unoccupied orbitals.).

4.2. Biology

P. falciparum was cultured according to the method described by Trager and Jensen [16] with modifications [17]. Cultures were synchronized by 5% D-sorbitol lysis (Merck, Darmstadt, Germany) [17]. *In vitro* antimalarial activity was evaluated by [3H]hypoxanthine (ICN, France) incorporation as already described [17,18]. Incubation time between parasite culture and the drugs was 48 h.

Cytotoxicity of the drugs was estimated with MCF7 and Vero cell lines which were cultured in the same conditions as *P. falciparum*, except for the 5% human serum which was replaced by 5% fetal calf serum (Boehringer). After addition of drugs at various concentrations, cell growth was estimated by [^{3}H]hypoxanthine incorporation after 48 h incubation [19].

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