Bioorganic Chemistry 48 (2013) 16–21

Contents lists available at SciVerse ScienceDirect

Bioorganic Chemistry

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

Synthesis and biological evaluation of new *bis*-indolone-*N*-oxides

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ARTICLE INFO

Article history:
Received 30 November 2012
Available online 6 April 2013

Keywords:
Bis-indolone-*N*-oxides
Plasmodium falciparum
Bacteria
Cytotoxicity

ABSTRACT

A series of *bis*-indolone-*N*-oxides, **1a–f**, was prepared from *bis*(ethynyl)benzenes and *o*-halonitroaryls and studied for their *in vitro* antiplasmodial activities against *Plasmodium falciparum* and representative strains of bacteria and *candida* as well as for their cytotoxicity against a human tumor cell line (MCF7). They did not cause any haemolysis (300 μg mL^{-1}). Of the synthesized *bis*-indolones, compound **1a** had the most potent antiplasmodial activity (IC_{50} = 0.763 μmol L^{-1} on the FcB1 strain) with a selectivity index (CC_{50} MCF7/IC_{50} FcB1) of 35.6. No potency against the tested microbial strains was observed.

1. Introduction

Malaria remains a major cause of morbidity and mortality throughout the world despite many efforts in recent years to control it. The number of malaria cases has fallen over the past decade and it is estimated that nearly 750,000 lives have been saved in Africa alone (Global Plan for ARTs resistance containment, WHO, Report, 2011), but this progress is very fragile. Natural and hemisynthetic artemisinins (ARTs) are used in artemisinin-based combination therapies (ACTs), which are one of the reference treatments for *Plasmodium falciparum* malaria. However, resistance to ARTs is becoming a major challenge in malaria control [1]. Resistance to artemisinins (ARTs resistance) has spread to new locations in Asia, out of the Greater Mekong sub-region (Thailand, Myanmar, China) [2]. No other antimalarial drugs with the same level of efficacy and tolerability of ARTs are available and they are essential also for the treatment of severe malaria to decrease its mortality [3]. No other drugs are ready for deployment, and drug development efforts are not expected to yield new antimalarials until the end of this decade [4]. Thus, there is an urgent need for new molecules to prepare and develop drugs for the near future.

Recently, we have developed a series of indolone-*N*-oxides (INODs) with potent antimalarial activity [5] affording a new antimalarial molecular scaffold [5,6]. We have shown that INODs cause a membrane destabilization and vesiculation of *P. falciparum*-infected erythrocytes, through a mechanism triggered by a redox signal that activates a tyrosine kinase leading to a hyperphosphorylation of the band 3 protein. The infected erythrocyte becomes not only less hospitable for parasite maturation, but also less mechanically stable and capable of harboring the developing parasite [7]. We also demonstrated the rapid uptake and biotransformation of INODs into an active metabolite in human erythrocytes [8]. This biotransformation is a bioreduction of the indolone-*N*-oxide structure, which is thiol and enzyme dependent. These reduction reactions are highly probable in erythrocytes where molecular reducers (glutathione, L-cysteine) and reductases are concentrated. The nature of the reductases responsible for this biotransformation is still not identified nor is the contribution of the chemical versus enzymatic bioreduction that is the starting point of the bioactivity of the indolone-*N*-oxides. In order to better understand the role played by the reducible scaffold of these molecules we decided to design and prepare new molecules with two indolone-*N*-oxide cores to examine if their increased H and electron pumping properties would increase the antiplasmodial activity. We also tested the impact of these redox properties on bacteria and fungi (*Pseudomonas aeruginosa*, *Cronobacter sakazakii*, *Bacillus subtilis*, *Staphylococcus aureus*, *Geotrichum candidum*, and *Candida albicans*). The effect on the biological properties of the *meta* or *para* position of the second indolone-*N*-oxide core in respect to the 2-phenyl indolone-*N*-oxide was examined and compared to the activities of representative *mono*-indolone-*N*-oxide. Six new compounds were synthesized using the *Sonogashira* coupling reaction of *bis*(ethynyl)benzene with different *o*-halo-nitroaryls followed by a cycloisomerization of *bis*(2-(2-nitrophenyl)ethynyl)benzene systems by an electrophilic palladium(II) complex.

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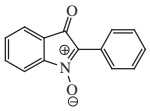
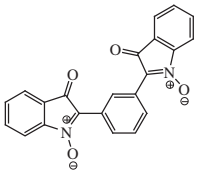
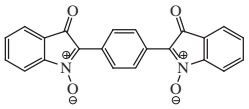
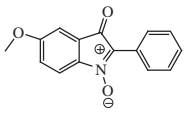
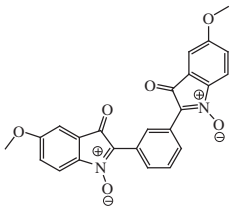
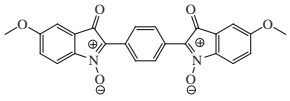
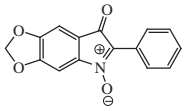
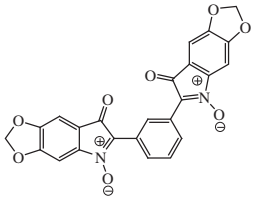
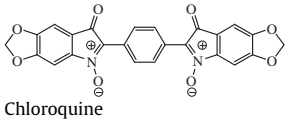
http://dx.doi.org/10.1016/j.bioorg.2013.03.001

Table 1
Structure, antiplasmodial and cytotoxic activities of *bis*-indolone-*N*-oxide derivatives.

Compounds	Structures	MW (g mol^{-1})/logP_{calc}[c]	IC_{50} (μmol L^{-1}) FcB1	CC_{50} (μmol L^{-1})[d] MCF7	Selectivity index MCF7/FcB1
I[a]		223.23/2.06	0.889	19.5	22
1a		368.34/2.19	0.763	>27.2	>35.6
1b		368.34/2.22	2.720	>27.2	>10
II[a]		253.26/2.02	0.184	13.4	73
1c		428.39/2.11	3.738	>10	>3
1d		428.39 /2.17	6.074	>10	>1.6
III[a]		267.24/1.51	1.272	[b]	[b]
1e		456.36/1.47	3.508	>21.9	>6
1f		456.36/1.48	4.324	>21.9	>5
	Chloroquine	319/5.28	0.202	19.4	96
	Sodium artesunate	406/2.29	0.006[d]	9.8	1633

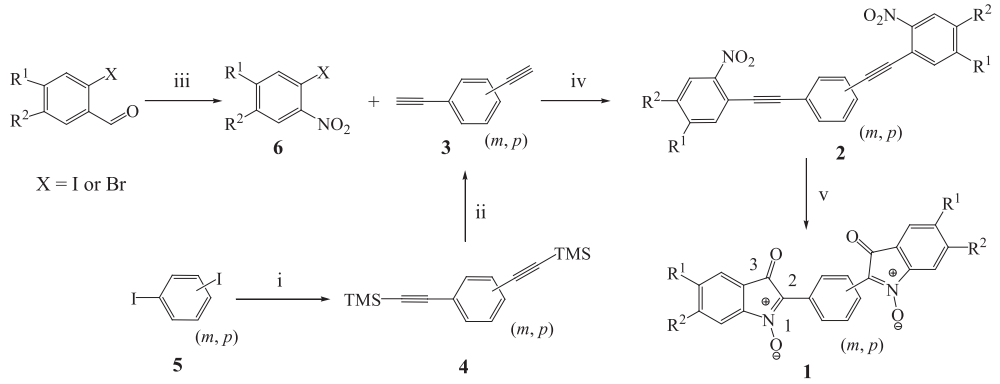
[a] Ref. [5].
[b] Not determined.
[c] LogP_{calcd}; calculated with VCCLAB (http://www.virtuallaboratory.org/lab/alogps/start.html).
[d] The drug concentration needed to cause a 50% decrease in cell viability. The IC_{50} SD were always lower than 10% and were discarded for maximum lisibility.

2. Results

2.1. Chemistry

A series of six analogues of *bis*-indolone-*N*-oxide **1a–f** (Table 1) was prepared. The synthesis of the *bis*-isatogens **1** was divided into two sub-steps: the first involved *Sonogashira* coupling of 2-halo-nitroaryls **6** with diynes **3**, followed by the nitro-alkyne cycloisomerization of the *bis*(2-(2-nitrophenyl)ethynyl)benzenes **2** in the presence of catalytic amounts of $Pd(CH_3CN)_2Cl_2$[9] in acetonitrile under reflux (Scheme 1). Compounds **6** were obtained by electrophilic nitration of *o*-bromoaryl aldehydes using a fuming nitric acid/acetic acid mixture [5]. *Di*-iodobenzenes **5** were coupled with two equivalents of trimethylsilylacetylene to provide **4**[10,11] in high yield. Then, the alkynes were deprotected to afford the diyne **3**[10,12] in quantitative yield (Scheme 1).

The structures of *bis*-isatogens were determined from spectroscopic data. The IR spectra showed bands in the range of 1690–1710 cm^{-1} which confirmed the presence of C=O functions. In the ^{13}C NMR spectra the most significant information was the disappearance of signals at δ = 86 and 95 ppm corresponding to the



Scheme 1. Synthesis of *bis*-indolone-*N*-oxides derivatives. Reagents and conditions: (i) $(CH_3)_3Si{-}C{\equiv}H$, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, N_2, rt; (ii) CH_3OH, CH_2Cl_2, K_2CO_3; (iii) AcOH, HNO_3 fuming; (iv) $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, N_2, rt; and (v) $Pd(CH_3CN)_2Cl_2$, CH_3CN, N_2, reflux 86 °C.

Table 2
Antimicrobial activity of *bis*-indolone-*N*-oxide derivatives.

Compounds	*Pseudomonas aeruginosa* MIC[a]	*Cronobacter sakazakii* MIC	*Bacillus subtilis* MIC	*Staphylococcus aureus* MIC	*Geotrichum candidum* MIC	*Candida albicans* MIC
1a	>814.46	>814.46	430.31	>814.46	85.79	534.83
1b	430.28	341.78	341.8	304.07	84.16	271.49
1c	>700.3	>700.3	>700.3	[b]	95.71	[b]
1d	>700.3	>700.3	>700.3	[b]	91.04	[b]
1e	437.22	245.86	275.66	250.9	70.12	304.58
1f	437.22	245.86	275.86	246.73	67.93	309.62
Tetracycline	56.52	17.86	71.15	2.25	–	–
Ampicillin	71.89	14.34	14.48	0.56	–	–
Amphotericin[c]	–	–	–	–	6.77	0.32
DMSO	[b]	[b]	[b]	[b]	[b]	[b]

[a] MICs: minimum inhibitory concentrations (μmol L^{-1}).
[b] Inactive.
[c] Ref. [14].

ethynylene group present in the intermediate reagent **2a–f** and the appearance of signals at δ = 186–188 ppm indicating the presence of the carbonyl functions in the compounds **1a–f**. The color of the *bis*-isatogens was dark orange to dark red.

The log*P* values (Table 1) calculated with the VCCLAB software are in the range 1.47–2.22 and show a slight increase in the lipophilicity for compounds **1a–d**, and a slight decrease for compounds **1e–f**, compared to those of the parent *mono*-indolone-*N*-oxides. Traditionally, the values of the n-octanol/water partition coefficient, $\log P_{o/w}$, are measured using the "shake-flask" method. However, the reducibility character of the *N*-oxide derivatives in solution makes the use of this time-consuming classical method limited. The prediction of log*P* by the RP-HPLC analysis can replace the shake-flask method and is appropriate for the *N*-oxide derivatives as recently demonstrated by Moreno et al. [13]. The log*P* values of the new synthesized derivatives **1a–f** (1.47–2.22) are in the same range as those determined by RP-HPLC for 3-methylquinoxaline-2-carboxylic acid benzylamide 1,4-di-*N*-oxide derivatives (1.76–2.72) [13].

2.2. Pharmacology

2.2.1. In vitro haemolytic, cytotoxic and antiplasmodial activities

The haemolytic assay determined on human erythrocytes was the first step used to determine a potential cytotoxicity. It is also a classical test to evaluate the membrane perturbation power of compounds. No haemolysis was found, even with high concentrations (300 μg mL^{-1}). It shows that the antiplasmodial activities observed (see later) were not correlated with a potential haemolytic activity and are presumed to be the result of an intracellular action. This was confirmed by the SYTOX Green assay which shows no membrane destabilization against micro-organisms. Cytotoxicity was assayed on the mammalian MCF7 cell line and was higher than 10 μmol L^{-1} (Table 1).

By comparing the new *bis*-analogues with the *mono*-analogues (2-phenyl-3*H*-indol-3-one-*N*-oxide, **I**, 5-methoxy-1-oxy-2-phenyl-indol-3-one, **II**, and 5-oxy-6-phenyl-[1,3]dioxolo-[4,5-*f*]indol-7-one, **III**) previously reported [5], it appears that mono parent compounds present better antiplasmodial activity (IC_{50}/μmol L^{-1}: 0.184–1.27) than the *bis*-derivatives **1a–f**, (IC_{50}/μmol L^{-1}: 0.76–6.07) (Table 1). It should be noted that the activity of the *bis*-analogue **1a** (IC_{50} = 0.76 μmol L^{-1}) was moderate but comparable to that of the *mono*-analogue, **I** (IC_{50} = 0. 89 μmol L^{-1}), **1a** and **I** bearing not substituted cycles. When substituents are introduced, the *meta* substitution gave always better results than the *para* position but a strong decrease of the activity is observed. Replacement of R^1 (H) by a methoxyl group leads to a significant decrease or loss of activity (CI_{50}/μmol L^{-1}: **II** = 0.184, **1c** = 3.74, **1d** = 6.07) as well as the replacement of the H ($R^1 = R^2$) by a dioxymethylene group (O—CH_2—O) (CI_{50}/μmol L^{-1}: **III** = 1.272, **1e** = 3.50, **1f** = 4.32). Selectivity index defined as the ratio of the CC_{50} value obtained with MCF7 cells to the IC_{50} value with CQ-resistant *P.f.* strain FcB1 were in the μmolar range, one log higher than the antiplasmodial IC_{50} value varying from 10 to 27.2 μmol L^{-1} (Table 1).

2.2.2. Antimicrobial activities

Compounds **1a–f** were evaluated for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative bacteria and against two fungi using standard techniques. Mini-

mum inhibitory concentrations (MICs) were calculated to express the antimicrobial activity and were defined as the concentration of the compound required to give complete inhibition of bacterial growth (Table 2). The data show that the compounds have no considerable potency against all the tested micro-organisms. The best activities observed were against *B. subtilis.*

3. Discussion

New *bis*-analogues had the same antiplasmodial activities as *mono*-analogues when no substituents were present at R^1, R^2 and at R^3 (meta or para positions of the phenyl group). Introducing substituents led to a strong decrease in the antiplasmodial activities. For example, when the methoxy group was introduced at the R^1 position in the *mono*-indolone series, the IC_{50} value decreased by about 0.7 μmol units from compound **I** to compound **II**, while the introduction of this substituent in the *bis*-analogue series increased the IC_{50} value by about 3.35 μmol units from compound **1a** to compound **1c.** The pharmacomodulation on the *mono*-indolone-*N*-oxide series gave hits with IC_{50} values in the nanomolar range, by introducing various substituents at R^1, R^2 and/or R^3 positions [5]. The results obtained in this current study show that the same substituents had the opposite effect, increasing the IC_{50} values. The activity of the *bis*-indolone-*N*-oxides seems limited by steric effects. The IC_{50} value variation (0.7–6.0 μmol L^{-1}) is more dependent on substituent and steric effects than on log*P* values (1.74–2.22). We previously reported that the indolone-*N*-oxides were bio-reducible in red blood cells, through thiol and enzyme dependent reactions [8]. However, the twofold increase in the reducible character of the molecule obtained in this study did not improve the antiplasmodial activity. This result strongly suggests that an enzymatic reduction of these molecules is necessary to induce a redox signal that activates the syk tyrosine kinase leading to a hyperphosphorylation of band 3 of the erythrocyte [7]. Moreover, a decrease in the antiplasmodial activity was observed with the more bulky derivatives (**1a**–**b**; **1e**–**f**) supporting the idea that indolone-*N*-oxides might be the substrates of some reductases present in the erythrocyte, and that the steric hindrance generated in *bis*-analogues may impair the enzymatic bioreduction.

Concerning the other antimicrobial activities, the introduction of a second indolone-*N*-oxide moiety did not increase the antibacterial and antifungal activities [15].

4. Conclusion

Six novel *bis*-indolone-*N*-oxide compounds have been prepared in comparison with *mono*-indolone-*N*-oxides analogues. The increased number of reducible functions does not improve the antimicrobial activities of the indolone-*N*-oxide derivatives.

5. Experimental

5.1. General

Commercially reagent grade chemicals were used as received without additional purification. All reactions were followed by TLC (E. Merck Kieselgel 60 F-254), with detection by UV light at 254 nm. Column chromatography was performed on silica gel (60–200 mesh E. Merck). IR spectra were recorded on a Perkin–Elmer PARAGON 1000 FT-IR spectrometer. ^1H and ^{13}C NMR spectra were recorded on an AC Bruker spectrometer at 300 MHz (^1H) and 75 MHz (^{13}C) using $(CD_3)_2SO$ as solvent with $(CD_3)_2SO$ (δ_H 2.5) or $(CD_3)_2SO$ (δ_C 39.5). Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (0 ppm). The mass spectra were recorded on an ion trap mass spectrometer (Finnigan LCQ Deca XP Max) using electrospray as an ionization source. High-resolution mass spectra (HRMS) were recorded on a Bruker Maxis spectrometer (Service Commun, ICT, Toulouse, France). Melting points were determined with an Electrothermal 9300 capillary melting point apparatus and are uncorrected. The purity of all compounds was determined by LC–PDA–MS methods and was found to be in the range 96–99%.

5.2. General procedure for the Sonogashira coupling

$Pd(PPh_3)_2Cl_2$ (0.2 mmol) was added to a solution of bis(ethynyl)benzene, **3** (1 mmol) and 2-halonitroaryl, **6** (2.2 mmol) in freshly distilled triethylamine (20 mL) and DMF (10 mL), and the mixture was de-aerated with argon for 30 min. CuI (0.2 mmol) was added, and the mixture was de-aerated with argon for 10 min and stirred at room temperature for 6 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried ($NaSO_4$), and concentrated, and the residue obtained was purified by column chromatography (cyclohexane in dichloromethane) to afford an intermediate, **2**.

5.2.1. 1,3-Bis(2-(2-nitrophenyl)ethynyl)benzene ***2a***

Yellow solid, yield: 53%, mp: 134–137 °C. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.36–7.40 (m, 3H), 7.45–7.52 (m, 2H), 7.58–7.63 (m, 5H), 8.07 (d, J = 1.2, 7.9 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 86.1 (2C≡C), 95.1 (2C≡C), 117 (2C), 122.8 (2C), 125.4 (2CH), 128.4 (CH), 129.7 (2CH), 130.9 (2CH), 133 (2CH), 134.3 (2CH), 135.5 (CH), 149.7 (2C). IR (KBr, cm^{-1}): 3019, 2412, 1605, 1579, 1523, 1465, 1343.

5.2.2. 1,4-Bis(2-(2-nitrophenyl)ethynyl)benzene ***2b***

Yellow solid, yield: 68%, mp: 151–153 °C. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.35–7.41 (m, 2H), 7.43–7.52 (m, 2H), 7.64–7.75 (m, 6H), 8.06 (d, J = 1.2, 7.9 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 86.1 (2C≡C), 95.1 (2C≡C), 117.2 (2C), 122.8 (2C), 125.3 (2CH), 131.1 (2CH), 131.1 (2CH), 129.6 (2CH), 133 (2CH), 134.3 (2CH), 149.7 (2C). IR (KBr, cm^{-1}): 3019, 2412, 1607, 1579, 1524, 1465, 1343.

5.2.3. 1,3-Bis(2-(5-methoxy-2-nitrophenyl)ethynyl)benzene ***2c***

Yellow solid, yield: 47%, mp: 131–133 °C. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.91 (s, 6H, 2 CH_3), 7.14–7.43 (m, 4H), 7.58–7.67 (m, 4H), 8.15 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.3 ($2CH_3$), 87 (2C≡C), 97.1 (2C≡C), 116.1 (2CH), 117.9 (2C), 118.5 (2CH), 123.6 (2C), 124.9 (2CH), 128.1 (CH), 131 (2CH), 134.7 (CH), 145.5 (2C), 162.1 (2C). IR (KBr, cm^{-1}): 3054, 2936, 1653, 1610, 1578, 1520, 1477, 1447, 1385, 1357, 1260.

5.2.4. 1,4-Bis(2-(5-methoxy-2-nitrophenyl)ethynyl)benzene ***2d***

Yellow solid, yield: 54%, mp: 156–158 °C. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.90 (s, 6H, 2 CH_3), 7.14–7.42 (m, 6H), 7.62–7.68 (m, 2H), 8.17 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.3 ($2CH_3$), 86.94 (2C≡C), 97.1 (2C≡C), 116.2 (2CH), 118 (2C), 118.5 (2CH), 123.7 (2C), 125.1 (2CH), 131.2 (2CH), 131.2 (2CH), 145.7 (2C), 161.4 (2C). IR (KBr, cm^{-1}): 3054, 2950, 1653, 1611, 1576, 1520, 1476, 1447, 1385, 1356, 1260.

5.2.5. 1,3-Bis(2-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethynyl)benzene ***2e***

Pale brown solid, yield: 58%, mp: 134–136 °C. ^1H NMR (300 MHz, DMSO-d_6) δ: 6.14 (s, 4H, $2CH_2$), 7.04 (s, 2H), 7.35–7.59 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 86.7 (2C≡C), 96.4 (2C≡C), 97.3 ($2CH_2$), 105.4 (2CH), 112.4 (2CH), 114.7 (2C), 122.5 (2C), 128.5 (CH), 129.7 (2CH), 132.1 (CH), 144.4 (2C), 147.8 (2C), 151.6 (2C). IR (KBr, cm^{-1}): 3077, 3020, 2400, 1615, 1557, 1522, 1493, 1443, 1342, 1297, 1210.

*5.2.6. 1,4-Bis(2-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethynyl)benzene **2f***

Pale brown solid, yield: 61%, mp: 162–164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.15 (s, 4H, 2 CH_2), 7.04 (s, 1H), 7.34–7.60 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 86.7 (2C≡C), 96.3 (2C≡C), 97.3 ($2CH_2$), 105.6 (2CH), 112.4 (2CH), 114.7 (2C), 122.5 (2C), 130.9 (2 CH), 131 (2CH), 144.6 (2C), 147.9 (2C), 151.6 (2C). IR (KBr, cm^{-1}): 3077, 3019, 2400, 1614, 1557, 1524, 1493, 1443, 1342, 1297, 1211.

5.3. General procedure for the cycloisomerization

$Pd(CH_3CN)_2Cl_2$ (0.025 mmol, 6.5 mg 5 mol-%) was added to a solution of diethynylene benzene **2** (0.5 mmol) in CH_3CN (15 mL), and the mixture was refluxed for 3 h in an argon atmosphere. The precipitate formed was isolated by filtration and purified by column chromatography (ethyl acetate in petroleum ether) to give compound **1**.

*5.3.1. Bisisatogen **1a***

Dark orange solid, yield: 53%, mp: 251–253 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.55–7.77 (m, 8H), 8.48–8.81 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 114.3, 114.4, 122,4, 122.6, 123.9, 125.1, 127.5, 128.3, 128.5, 129.3, 130.7, 130.9, 131.1, 131.3, 134,4, 134.5, 147.4, 147.7, 186.8 (C-3), 186.9 (C-3′). IR (KBr, cm^{-1}): 3054, 2986, 1702, 1602, 1570, 1527, 1511, 1498, 1422, 1375, 1273, 1178, 1046, 1029, 736, 705. MS-(+)ESI, *m*/*z*: 369 $[M+H]^+$. HR-MS $[M+H]^+$ calcd for $C_{22}H_{13}N_2O_4$ 369.0875 found: 369.0888.

*5.3.2. Bisisatogen **1b***

Dark orange solid, yield: 71%, mp: 252–254 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.45–7.70 (m, 8H), 8.53–8.61 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 114.3, 114.9, 121.7, 122.1, 122.3, 124.8, 125.2, 127.7, 127.9, 128.4 (2C), 130.3, 131.1, 131.3, 134.4, 134.5, 147.9, 148, 186.4 (C-3), 186.7 (C-3′). IR (KBr, cm^{-1}): 3054, 2986, 1706, 1601, 1569, 1527, 1512, 1496, 1422, 1372, 1261, 1180, 1045, 1029, 741, 705. MS-(+)ESI, *m*/*z*: 369 $[M+H]^+$. HR-MS $[M+H]^+$ calcd for $C_{22}H_{13}N_2O_4$ 369.0875 found: 369.0884.

*5.3.3. Bisisatogen **1c***

Dark orange solid, yield: 47%, mp: 255–257 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.86 (s, 3H), 3.89 (s, 3H), 7.11–7.59 (m, 6H), 8.01–8.56 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.6 (CH_3), 56.8 (CH_3), 107.9, 108.1, 114.7, 115.2, 118.3, 118.9, 124.3, 125.1, 126.8, 128.8, 129.6, 130.8, 131.1, 140.8, 141.1, 149.8, 161.8, 161.9, 186.2 (C-3), 186.9 (C-3′). IR (KBr, cm^{-1}): 3032, 2997, 1704, 1597, 1523, 1481, 1447, 1386, 1283, 1182, 1020, 875, 758, 685. MS-(+)ESI, *m*/*z*: 429 $[M+H]^+$. HR-MS $[M+H]^+$ calcd for $C_{24}H_{17}N_2O_6$ 429.1087 found: 429.1098.

*5.3.4. Bisisatogen **1d***

Dark orange solid, yield: 51%, mp: 257–259 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.88 (s, 3H), 3.90 (s, 3H), 7.16–7.60 (m, 6 H), 8.12–8.58 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.6 (CH_3), 56.7 (CH_3), 108.2, 108.4, 113.8, 114.8, 118.7, 119.1, 124.3, 124.5, 129.1, 131.6, 131.9, 141, 141.1, 151.6, 151.7, 159.9, 160.7, 186.6 (C-3), 186.8 (C-3′). IR (KBr, cm^{-1}): 3032, 2998, 1704, 1581, 1531, 1483, 1447, 1386, 1281, 1185, 1020, 876, 756. MS-(+)ESI, *m*/*z*: 429 $[M+H]^+$. HR-MS $[M+H]^+$ calcd for $C_{24}H_{17}N_2O_6$ 429.1087 found: 429.1096.

*5.3.5. Bisisatogen **1e***

Red solid, yield: 45%, mp: 266–268 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.13 (s, 2H), 6.15 (s, 2H), 7.03–7.18 (m, 4H), 7.47–7.78 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 97.3 (CH_2), 97.4 (CH_2), 102.3, 102.7, 103.5, 117.7, 118.2, 125.7, 126.3, 127.4, 127.9, 128.1, 128.4, 130.6, 131.2, 143.9, 144.6, 149.7, 151.7, 152.4, 186.8 (C-3), 187.1 (C-3′). IR (KBr, cm^{-1}): 3100, 3092, 2922, 1702, 1632, 1586, 1506, 1490, 1445, 1392, 1350, 1277, 1168, 1029, 924, 775. MS-(+)ESI, *m*/*z*: 457 $[M+H]^+$. HR-MS $[M+H]^+$ calcd for $C_{24}H_{13}N_2O_8$ 457.0672 found: 457.0695.

*5.3.6. Bisisatogen **1f***

Red solid, yield: 54%, mp: 268–261 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.13 (s, 2H), 6.14 (s, 2H), 7.02–7.15 (m, 4H), 7.53–786 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 97.2 (CH_2), 97.4 (CH_2), 104.5, 105.1, 105.5, 105.7, 116.3, 117.1, 126.4, 126.7, 128.6, 130.3, 130.7, 144.8, 145.1, 147.5, 147.9, 149.6, 150.1, 186 (C-3), 186.3 (C-3′). IR (KBr, cm^{-1}): 3100, 3090, 2917, 1702, 1632, 1587, 1505, 1489, 1445, 1390, 1350, 1273, 1168, 1029, 919, 775. MS-(+)ESI, *m*/*z*: 457 $[M+H]^+$. HR-MS $[M+H]^+$ calcd for $C_{24}H_{13}N_2O_8$ 457.0672 found: 457.0691.

5.4. Methodology for in vitro biological evaluation

5.4.1. Haemolytic activity and SYTOX Green uptake assays

The haemolytic activity of the different compounds was determined against human erythrocytes from healthy donors. Details of both assays are given in Supplementary data.

5.4.2. In vitro antiplasmodial and cytotoxicity assays

The *in vitro* antiplasmodial assay was carried out against the chloroquine-resistant strain (FcB1) of *P. falciparum* and the *in vitro* cytotoxicity was determined on human breast cancer cells (MCF7) [16,17]. All these assays were performed as previously reported [5] and details are given in the Supplementary data.

5.4.3. Antimiocrobial assays

All microorganisms were grown in broth medium with continuous shaking (200 rpm). *B. subtilis* (CIP 5262), *S. aureus* (CIP 53156) were used as Gram-positive strains. The Gram-negative strains used were *P. aeruginosa* (CIP 82118), and *C. sakazakii* (CIP 103183). Fungal strains used were *C. albicans* (CIP 4872) and *G. candidum* (ATCC 204307). The minimal inhibitory concentrations (MICs) were obtained as previously reported [15] and details are given in the Supplementary data.

Acknowledgment

This work was supported by the French Research National Agency (ANR-10-BLAN-0726, Mechanisms of Action and Targets of new antimalarial Redox molecules, MATURE).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2013.03.001.

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