European Journal of Medicinal Chemistry 76 (2014) 369–375

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Amino derivatives of indolone-*N*-oxide: Preparation and antiplasmodial properties

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ARTICLE INFO

Article history:
Received 12 December 2013
Received in revised form
11 February 2014
Accepted 13 February 2014
Available online 14 February 2014

Keywords:
Antimalarial drugs
Indolone-*N*-oxide
Amino-indolone-*N*-oxide derivatives

ABSTRACT

There is an urgent need for new antimalarial drugs with novel mechanisms of action on novel targets. Indolone-*N*-oxides (INODs) display antimalarial properties *in vitro* and *in vivo*, but identified leads such as 6-(4-chloro-phenyl)-5-oxy-[1,3]dioxolo[4,5-*f*]indol-7-one **1**, suffer from very poor aqueous solubility. In this study, structural modifications have been made by introducing various amino and bulky groups to produce sufficiently water soluble and active compounds for further pharmacological and pharmacokinetic studies. We report here the preparation of twelve novel amino derivatives and their antiplasmodial activities including those of two other structurally known compounds. The 5-methoxy-2-(4-morpholin-4-yl-phenyl)-1-oxy-indol-3-one, **9**, has the highest antiplasmodial activity *in vitro* (IC_{50} = 6.5 nM; FcB1 strain) and selectivity index (SI (CC_{50} MCF7/IC_{50} FcB1) = 4538.5). The 6-amino-2-(4-chloro-phenyl)-1-oxy-indol-3-one, **14**, (IC_{50} = 183 nM; SI = 60), is an excellent candidate for further mechanistic studies. Indeed, this is structurally the closest analogue to the current lead, **1**, bearing an NH_2 group at R^2 offering possibilities for functionalization and labeling.

1. Introduction

The emergence of the resistance of *Plasmodium falciparum* (*P. falciparum*) has been a major problem for the treatment of malaria in the last four decades making predictions of the future patterns of this disease difficult [1,2]. Recent reports showed a decreased susceptibility to artemisinin of *P. falciparum* in the Great Mekong region in Asia [3]. These indications prompt the need for new antimalarial drugs which ideally should act with novel mechanisms of action, on novel targets and/or should represent new chemotypes even though parasite resistance may evolve again.

We recently identified several distinct chemotypes leading to the selection of the indolone-*N*-oxide (INOD) core as a key scaffold for a structure–activity relationship (SAR) development. Our studies focused on compounds with aryl groups (R^3) at the 2-position of the indolone-*N*-oxide core (Fig. 1) [4].

These compounds penetrate rapidly into the erythrocyte where they are biotransformed into reduced metabolites [5]. This bioreduction is decisive for their antimalarial properties at the blood stage of the disease [6,7]. The mechanism of action is partially understood but the cellular targets are not yet identified [8]. A series of structural modifications showed that the groups (R^1 or R^2) at the 5- or 6-position had no major effect on the activity while variation of the substituent at the *para* position of the aryl group (R^3) led to the best activities *in vitro*. The introduction of a 4′-chloro-phenyl group at R^3 led to the generation of compound **1** with an activity (CI_{50}) of 75 nM against the FcB1 strain and a good selectivity index (SI = 212; MCF7/FcB1) (Fig. 2). *In vivo* antimalarial activity against *Plasmodium berghei* (ANKA) led to a 62.1% inhibition of parasitemia at 30 mg/kg/day. It was concluded from these *in vivo* studies that the poor aqueous solubility of **1** could explain the suboptimum *in vivo* results. We introduced the 4′-dimethylamino-phenyl at R^3 which gave a better water solubility and a good activity *in vitro* (CI_{50} < 3 nM on FcB1) but poor inhibition of parasitemia *in vivo* (15.3%) (Fig. 2). We therefore undertook the synthesis of new derivatives with amino groups varying in nature and position to obtain a better water solubility while keeping the antiplasmodial activity and log *P* values as close as possible to the current lead **1** (log P_{calc} = 2.09). This structural variation was also done to assess the impact on the antiplasmodial activity of an NH_2 group at the R^2 position. Such a function gives the possibility to link labels for mechanistic and metabolic study purposes. For this reason, the effect of bulky groups at R^1/R^2 position, mimicking the steric effect of labeled groups, was also evaluated. The consequences of these

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http://dx.doi.org/10.1016/j.ejmech.2014.02.038
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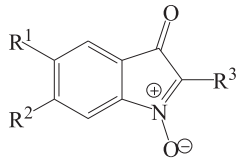


Fig. 1. Indolone-*N*-oxide series.

structural variations on the antiplasmodial activity are presented in this report and compared to the lead hit, **1**, *in vivo* and the best hit, **2**, *in vitro*.

2. Results and discussion

2.1. Chemistry

The synthetic approach previously published has been improved (Scheme 1). The synthesis of the amino-indolone-*N*-oxide derivatives is divided into two sub-steps: the first involves *Sonogashira* coupling of 2-halo-nitroaryls **d** with an alkyne **c**, followed by the nitro-alkyne cycloisomerization of the *o*-alkynylnitrobenzenes **f** in the presence of catalytic amounts of $Pd(CH_3CN)_2Cl_2$ in acetonitrile under reflux [9]. Compounds **d** were obtained by electrophilic nitration of *o*-bromoaryl aldehydes **e** using a fuming nitric acid/acetic acid mixture [4]. Synthesis of 1-alkynes **c** involved the preparation of 1-(trimethylsilyl)-alkynes **b** [10,11] *via Sonogashira* coupling, followed by a desilylation step [10,12] (Scheme 1). This reaction is very general and more functional-group-tolerant than the methods already described [4]. The new compounds are reported in Table 1.

The compounds were chemically characterized by chromatography (TLC and HPLC), infrared (IR), NMR (^{1}H and ^{13}C), and mass spectrometry as well as HRMS. The structures of INODs were determined primarily from spectroscopic data. The IR spectra showed bands in the range of 1690–1710 cm^{-1} which confirmed the presence of C=O function. In the ^{13}C NMR spectra the most significant information was the disappearance of signals at $\delta = 86$ and 95 ppm corresponding to the ethynylene group present in the intermediate reagent **f** and the appearance of signals at $\delta = 186$–188 ppm indicating the presence of the carbonyl function of the indolone-*N*-oxide.

Log P_{calc} values (Table 1) calculated from the VCCLAB software [13] range between 0.95 and 3.36. Compound **8** is the most lipophilic in the series, and compound **10**, the least. Compounds remained very poorly soluble in water except for salt forms (hydrochloride salt of compounds **2** and **4**). Compounds **7**, **9**, **11** and **14** had a log $P_{calc,}$ near 2.

2.2. Biology

The twelve compound series was evaluated for the ability to inhibit the growth of *P. falciparum in vitro* (strain FBc1), the cytotoxicity against the MCF7 cell line and the determination of the selectivity index. The present study focused on the amino derivatives of indolone-*N*-oxide, derived from their parent compound that showed a high antimalarial activity ($IC_{50} < 100$ nM) [6]. Summary of these assay results are presented in Table 1. Half of the amino derivatives were active against FcB1 *P. falciparum* strain with IC_{50} values <150 nM. Replacement of the phenyl group by either 3-pyridyl (*N* at position 4 of the indolone moiety, compound **11**, or by pyridinium-*N*-oxide (*N* at position 5 of the indolone moiety, compound **12**, led to a significant activity loss. This confirmed that the phenyl group of the indolone moiety was essential for the antiplasmodial activity. Replacement of the R^3 phenyl group by a 3′-pyridyl cycle (**10**) led to a good activity ($IC_{50} = 161$ nM). Replacement of the phenyl group (R^3) by an aliphatic chain (2-propyl-isoindole-1,3-dione, **17**) led to a significant loss of activity ($IC_{50} > 8000$ nM). The inclusion of an amino group at R^2 position (compounds **13, 15, 16, 17**) considerably decreased the activity ($IC_{50} > 1000$ nM) and the SI. One exception was observed with **14** ($IC_{50} = 183$, SI = 60) that is an analogue of the lead **1** with a 4′-chlorophenyl group at R^3. This unexpected result is very interesting in that it gives the possibility to link fluorophores on the amino group for imaging purposes and other labeled entities for metabolism studies. Hydrochloride salt of compounds **2** and **4** have a good activity (CI_{50}/nM: **6** <0.3, **5** <3). Also, the introduction of bulky groups at R^2, (ethyl ester, **18**) ($IC_{50} = 284$), and at R^1 ((1-chloro-vinyl)benzene, **19**) ($IC_{50} = 840$), decreased the activity moderately giving the possibility of using bulky labeled groups. The cytotoxicity of the amino derivatives of indolone-*N*-oxide (CC_{50} 7–44 μM) was relatively low and within the same range of that of chloroquine ($CC_{50} = 19.4$ μM) and artesunate ($CC_{50} = 9.8$ μM). The main molecule of interest resulting from this study and justifying further *in vivo* studies was compound **9**. This 4′-morpholino phenyl derivative had a high activity against the FcB1 strain ($IC_{50} = 6.5$ nM) and a low toxicity (SI = 4538).

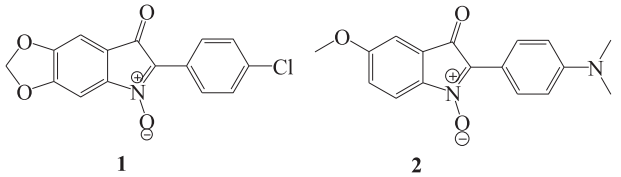


Fig. 2. Lead **1** in the indolone-*N*-oxide series (INODs). IC_{50}, nM (FcB1; 3D7): 75 ± 63; 58 ± 17. CC_{50}, μM (MCF7; KB): 15.9; 27. SI (MCF7/FcB1); (KB/3D7): 212; 450. $t_{1/2}$ (mouse and human liver microsomes) < 1 min. **Hit 2**: IC_{50}, nM (FcB1; 3D7): <3; 1.7. CC_{50}, μM (MCF7; KB): 43.9; 338. SI (MCF7/FcB1); (KB/3D7): >14 623; 198 823.

3. Conclusion

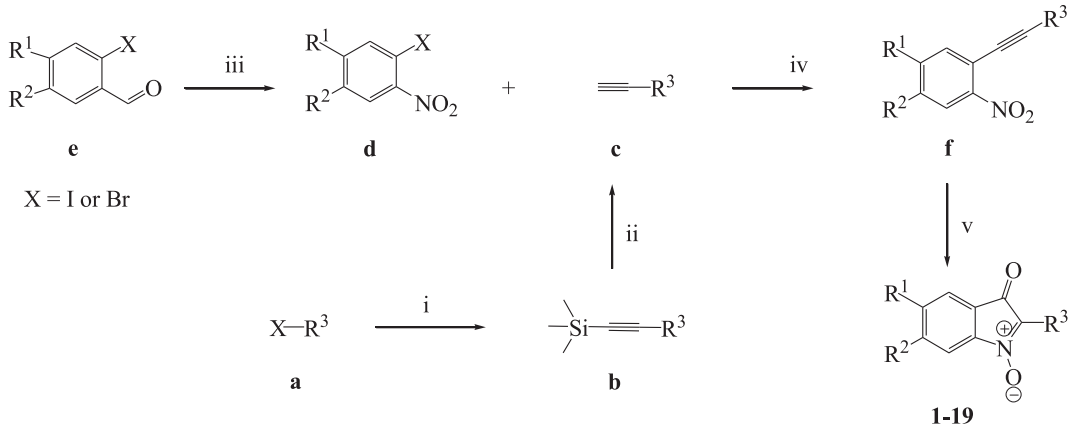
A series of twelve new amino-indolone-*N*-oxide derivatives have been prepared and fifteen compounds tested against *P. falciparum*. Half of them had IC_{50} values <150 nM. Amino groups positioned at R^3 gave increased activity while it was the reverse at R^2. There was one exception to this tendency and which was a first significant result of this work: the identification of a compound usable as tool for pharmacological and pharmacokinetic studies. A second relevant result was the very active morpholino derivative that emerged from this study, with its high antimalarial activity and very low toxicity, and appeared as an excellent candidate for *in vivo* studies.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were determined with an Electrothermal 9300 capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer PARAGON 1000 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on an AC Bruker spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C) using $(CD_3)_2SO$ and $CDCl_3$ as solvents. High resolution mass spectra (HRMS) were recorded on a Bruker Maxis spectrometer (Service Commun Toulouse, France). Silica Gel 60 (Merck 70–230) was used for column chromatography. The progress of the reactions was monitored by thin layer chromatography on using Kieselgel 60 F254 (Merck)



Scheme 1. General synthetic route for the indolone-*N*-oxide derivatives. Reagents and conditions: (*i*) $(CH_3)_3Si{-}C{\equiv}CH$, $Pd(PPh_3)_2Cl_2$, 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.

plates. Compounds **18** and **19** were obtained from MDPI (Postfach 4005 Basel, Switzerland).

*4.1.2. Synthesis of compounds **f**: general procedure for the Sonogashira coupling*

See Supporting information.

4.1.3. General procedure for the cycloisomerization

$Pd(CH_3CN)_2Cl_2$ (0.025 mmol, 6.5 mg, 5 mol-%) was added to a solution of *o*-alkynylnitrobenzene **f** (0.5 mmol) in CH_3CN (15 mL), and the mixture was refluxed for 1 h in an argon atmosphere. The reaction mixture was concentrated, and the residue obtained was purified by column chromatography (ethyl acetate in petroleum ether) to give the indolone-*N*-oxide.

*4.1.3.1. 2-(4-Amino-phenyl)-1-oxy-indol-3-one (**3**).* Violet solid, yield: 55%, mp: 161–163 °C. ^{1}H RMN (300 MHz, DMSO-d_6): δ: 6.09 (br, 2H, NH_2), 6.68 (d, J = 9 Hz, 2H), 7.50–7.60 (m, 3H), 7.71–7.77 (m, 1H), 8.47 (d, J = 9 Hz, 2H). ^{13}C RMN (75 MHz, DMSO-d_6) δ: 113.1 (×2), 113.2, 121.2, 122.6, 129.0 (×2), 129.3, 130.2, 131.6, 135.2, 147.7, 151.5, 187.6 (C=O). IR (KBr, cm^{-1}): 3473, 3377, 1703, 1621, 1598, 1534, 1492, 1459, 1379, 1301, 1281, 1178, 1139, 1073, 875, 835, 785. HRMS (DCI, CH_4) *m/z* calcd for $C_{14}H_{11}N_2O_2$ $[M+H]^+$ 239.0821. Found 239.0826.

*4.1.3.2. 2-(4-Dimethylamino-3-methyl-phenyl)-5-methoxy-1-oxy-indol-3-one (**7**).* Purple solid, yield 62%, mp: 161–163 °C. ^{1}H RMN ($CDCl_3$, 300 MHz) δ: 2.38 (s, 3H, CH_3), 2.80 (s, 6H, 2 CH_3), 3.89 (s, 3H, CH_3), 7.05 (d, J = 9 Hz, 2H), 7.13 (s, 1H), 7.54 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 6.9 Hz, 2H). ^{13}C RMN (75 MHz, $CDCl_3$) δ: 13.3 (CH_3), 40 (2 CH_3), 56.1 (CH_3), 108.1, 111.4, 112.1, 114.2, 115.7, 118.2, 125.1, 128.9, 129, 133.2, 139.9, 151.1, 161.9, 187.4 (C=O). IR (KBr, cm^{-1}): 3054, 2980, 1690, 1602, 1540, 1480, 1420, 1383, 1265, 1194. HRMS (DCI, CH_4) *m/z* calcd for $C_{18}H_{19}N_2O_3$ $[M+H]^+$ 311.1396. Found 311.1387.

*4.1.3.3. 2-(4-Butylamino-phenyl)-5-methoxy-1-oxy-indol-3-one (**8**).* Purple solid, yield 53%, mp: 187–189 °C. ^{1}H RMN (300 MHz, $CDCl_3$) δ: 1.00 (t, J = 7.2 Hz, 3H, CH_3), 1.26–1.32 (m, 2H, CH_2), 1.37–1.50 (m, 2H, CH_2), 3.19 (t, 2H, CH_2), 3.87 (s, 3H, OCH_3), 4.15 (br, 1H, NH), 6.65 (d, J = 9 Hz, 2H), 7.01–7.09 (m, 2H), 7.49 (d, J = 8.1 Hz, 1H), 8.61 (d, J = 9 Hz, 2H). ^{13}C RMN (75 MHz, $CDCl_3$) δ: 13.1 (CH_3), 19.8 (CH_2), 32.4 (CH_2), 43.1 (CH_2), 56 (CH_3), 107.8, 110.3 (×2), 114.7, 115.1, 117.9, 124.7, 128.6 (×2), 131.9, 141.5, 151.1, 162.1, 187.8 (C=O). IR (KBr) cm^{-1}: 3360, 3052, 2986, 1711, 1608, 1532, 1467, 1420, 1374, 1265, 1178, 1046, 1030, 757. HRMS (DCI, CH_4) *m/z* calcd for $C_{19}H_{21}N_2O_3$ $[M+H]^+$ 325.1552. Found 325.1541.

*4.1.3.4. 5-Methoxy-2-(4-morpholin-4-yl-phenyl)-1-oxy-indol-3-one (**9**).* Violet solid, yield 62%, mp: 224–226 °C. ^{1}H RMN (300 MHz, $CDCl_3$) δ: 3.33 (t, J = 6 Hz, 4H, 2 CH_2), 3.89 (t, J = 6 Hz, 2H, 2 CH_2), 3.91 (s, 3H, CH_3), 6.98 (d, J = 9 Hz, 2H), 7.07 (d, J = 6 Hz, 1H), 7.14 (s, 1H), 7.54 (d, J = 9 Hz, 1H), 8.68 (d, J = 9 Hz, 2H). ^{13}C RMN (75 MHz, $CDCl_3$) δ: 47.6 (CH_2), 56.2 (CH_3), 66.6 (CH_2), 107.9, 114.0 (×2), 114.9, 117.1, 118.2, 124.6, 129.0 (×2), 131.7, 141.1, 151.9, 161.9, 187.4 (C=O). IR (KBr) cm^{-1}: 3054, 2981,1701, 1602, 1540, 1480, 1420, 1381, 1265, 1183, 1087, 875, 756. HRMS (DCI, CH_4) *m/z* calcd for $C_{19}H_{19}N_2O_4$ $[M+H]^+$ 339.1345. Found 339.1332.

*4.1.3.5. 5-Methoxy-1-oxy-2-pyridin-3-yl-indol-3-one (**10**).* Red solid, yield 47%, mp: 157–157 °C. ^{1}H RMN (300 MHz, $CDCl_3$) δ: 3.92 (s, 3H, CH_3), 7.09–7.18 (m, 2H), 7.40–7.44 (m, 1H), 7.61 (d, J = 8.7 Hz, 1H), 8.66 (d, J = 4.2 Hz, 1H), 8.91 (d, J = 8.4 Hz, 1H), 9.80 (s, 1H). ^{13}C RMN (75 MHz, $CDCl_3$) δ: 55.9 (CH_3), 114.1, 116.7, 121.7, 122.2, 124.3, 126.6, 128.5, 133.2, 136.4, 137.8, 151.3, 162.1, 187.6 (C=O). IR (KBr) cm^{-1}: 3084, 3030, 1708, 1598, 1520, 1485, 1451, 1376, 1274, 1187, 1090, 871, 753. HRMS (DCI, CH_4) *m/z* calcd for $C_{14}H_{11}N_2O_3$ $[M+H]^+$ 255.0770. Found 255.0781.

*4.1.3.6. 6-amino-2-(4-diméthyaminophényl)-1-oxy-indol-3-one (**13**).* Yellow solid, yield 85%, mp: 197–199 °C. ^{1}H RMN (300 MHz, DMSO-d_6) δ: 3.09 (s, 6H, 2 CH_3), 6.12 (br, 2H, NH_2), 6.32 (d, J = 3 Hz, 1H), 6.82–6.88 (m, 3H), 7.72 (dd, J = 9 Hz, J = 1 Hz, 1H), 8.07 (d, J = 9 Hz, 2H). ^{13}C RMN (75 MHz, DMSO-d_6) δ: 40.1 (2 CH_3), 85.6, 111.5 (×2), 116.8, 122.0, 123.3, 125.4, 132.6 (×2), 150.8, 154.3, 159.0, 160.0, 187.6 (C=O). IR (KBr) cm^{-1}: 3434, 3343, 1702, 1644, 1583, 1378, 1287, 1195, 1141, 1064, 820, 740. HRMS (DCI, CH_4) *m/z* calcd for $C_{16}H_{16}N_3O_2$ $[M+H]^+$ 282.1243. Found 282.1242.

*4.1.3.7. 6-Amino-2-(4-chloro-phenyl)-1-oxy-indol-3-one (**14**).* Brown solid, yield 81%, mp: 169–171 °C. ^{1}H RMN (300 MHz, DMSO-d_6) δ: 6.56 (dd, J = 9 Hz, J = 3 Hz, 1H, Ar–H), 6.92 (br, 2H, NH_2), 7.35 (d, J = 9 Hz, 1H), 7.58–7.74 (m, 3H), 8.60 (d, J = 9 Hz, 2H). ^{13}C RMN (75 MHz, DMSO-d_6) δ: 100.1, 108.7, 112.9, 124.9, 125.7, 129.1 (×2), 129.4 (×2), 135.1, 151.4, 154.5, 156.8, 184.3 (C=O). IR (KBr) cm^{-1}: 3423, 3349, 1706, 1649, 1584, 1521, 1474, 1375, 1300, 1268, 1011, 818, 729. HRMS (DCI, CH_4) *m/z* calcd for $C_{14}H_{10}ClN_2O_2$ $[M+H]^+$ 273.0431. Found 273.0443.

Table 1
Amino derivatives of indolone-*N*-oxide: chemical characteristics and *in vitro* antiplasmodial and cytotoxic activities.

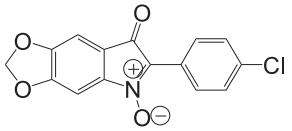
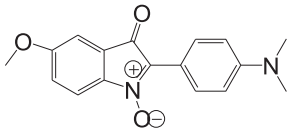
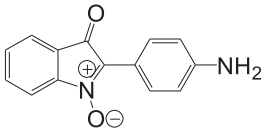
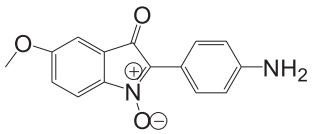
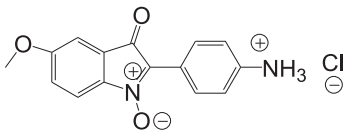
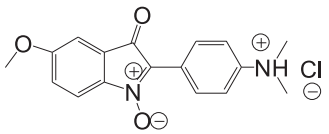
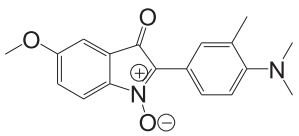
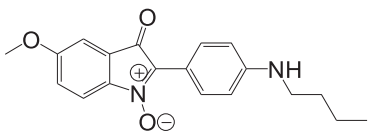
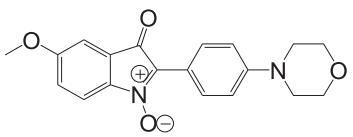
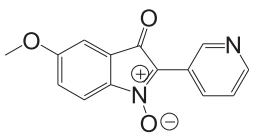
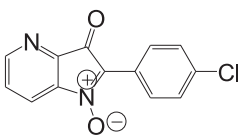
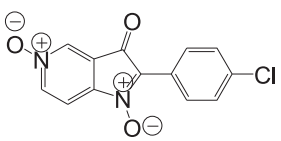
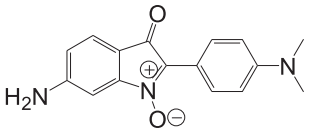
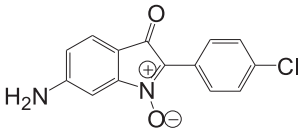
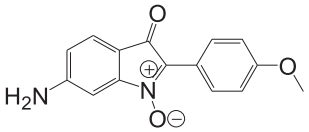
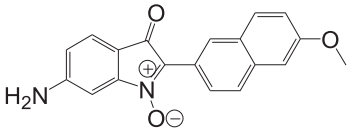
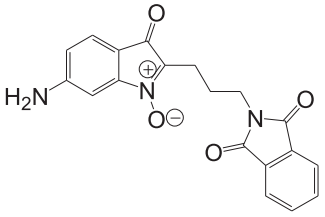
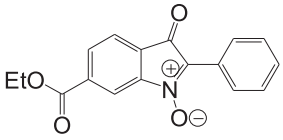
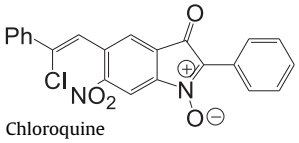
Compd	Structure	Log P_{cal}[b] (VCCLAB)	IC_{50} (nM) FcB1 strain	CC_{50} (μM)[c] MCF-7	Selectivity index MCF-7/FcB1
1[a]		2.09	75 ± 63	15.9	212
2[a]		2.24	<3	43.9	>14 623
3		1.61	168 ± 84	13	77
4[a]		1.63	24 ± 19	12.7	528
5		−0.36	<3	9.7	>3233
6		−0.60	<0.3 nM	33.7	>112 333
7		2.46	40 ± 30	35.4	885
8		3.36	21 ± 23	43.2	2057
9		2.16	6.5 ± 5	>29.5	4538.5
10		0.95	161 ± 39	17.3	107
11[a]		1.86	>2720	nd	nd
12[a]		1.02	>5440	nd	nd

Table 1 (*continued*)

Compd	Structure	Log P_{cal}[b] (VCCLAB)	IC_{50} (nM) FcB1 strain	CC_{50} (μM)[c] MCF-7	Selectivity index MCF-7/FcB1
13		1.88	12 264 ± 178	21.3	1.8
14		2.20	183	11	60
15		1.65	1565 ± 447	>37.3	>33
16		2.72	1319	7.8	6
17		1.23	8301	6.6	0.8
18		2.25	284 ± 54	26.4	91.9
19		4.35	840	59	70
	Chloroquine	5.28	151 ± 6	19.4	167
	Sodium artesunate	2.29	6 ± 3	9.8	1633

[a] Ref [4].
[b] Log P_{calc}; calculated with VCCLAB (http://www.virtuallaboratory.org/lab/alogps/start.html).
[c] The drug concentration needed to cause 50% decrease the cell viability. The CC_{50} SD were always lower than 10% and were discarded for maximum lisibility.

*4.1.3.8. 6-Amino-2-(4-methoxy-phenyl)-1-oxy-indol-3-one (**15**).* Red solid, yield 65%, mp: 228–230 °C. ^{1}H RMN (300 MHz, DMSO-d_6) δ: 3.86 (s, 3H, CH_3), 6.12 (br, 2H, NH_2), 6.35 (s, 1H), 6.82–6.89 (m, 3H), 7.46–7.51 (m, 1H), 8.05–8.08 (m, 2H). ^{13}C RMN (75 MHz, DMSO-d_6) δ: 55.3 (CH_3), 108.6, 114.2 (×2), 118.8, 122.0, 122.8, 124.8, 129.7 (×2), 134.8, 154.0, 159.1, 161.3, 187.4 (C═O). IR (KBr) cm^{-1}: 3414, 3350, 1698, 1644, 1583, 1530, 1487, 1382, 1253, 1181, 1017, 835. HRMS (DCI, CH_4) *m/z* calcd for $C_{15}H_{13}N_2O_3$ $[M+H]^+$ 269.0926. Found 269.0922.

*4.1.3.9. 6-Amino-2-(6-methoxy-naphthalen-2-yl)-1-oxy-indol-3-one (**16**).* Red solid, yield 92%, mp: 165–167 °C. ^{1}H RMN (300 MHz, DMSO-d_6) δ: 3.91 (s, 3H, CH_3), 6.56 (d, J = 9 Hz, 1H), 6.90 (br, 2H, NH_2), 7.21 (dd, J = 9 Hz, J = 3 Hz, 1H), 7.36 (m, 2H), 7.90 (d, J = 9 Hz, 1H), 7.94 (d, J = 9 Hz, 1H), 8.32 (s, 1H), 8.60 (d, J = 9 Hz, 1H), 9.21 (s, 1H). ^{13}C RMN (75 MHz, DMSO-d_6) δ: 55.8 (CH_3), 99.2, 106.5, 108.8, 112.6, 119.8, 122.2, 124.76, 124.82, 127.1, 128.1, 128.2, 131.2, 132.4, 135.4, 151.6, 156.8, 159.3, 187.9 (C═O). IR (KBr) cm^{-1}: 3437, 3352, 1699, 1650, 1625, 1587, 1521, 1495, 1469, 1370, 1330, 1264, 1228, 1124, 1017, 851. HRMS (DCI, CH_4) *m/z* calcd for $C_{19}H_{15}N_2O_3$ $[M+H]^+$ 319.1083. Found 319.1091.

*4.1.3.10. 2-(3-(6-Amino-3-oxo-1-oxy-3H-indol-2-yl)-propyl)-isoindole-1,3-dione (**17**).* Red solid, yield 82%, mp: 166–168 °C, ^{1}H RMN (300 MHz, DMSO-d_6) δ: 1.18 (t, J = 6 Hz, 2H, CH_2), 1.91 (m, 2H, CH_2), 3.60 (t, J = 6 Hz, 2H, CH_2), 6.47 (dd, J = 9 Hz, J = 3 Hz, 1H), 6.72 (d, J = 3 Hz, 1H), 6.79 (br, 2H, NH_2), 7.18 (d, J = 9 Hz, 1H), 7.81–7.83 (m, 4H). ^{13}C RMN (75 MHz, DMSO-d_6) δ: 19.3 (CH_2), 23.9 (CH_2), 37.7 (CH_2), 99.2, 108.9, 112.0, 123.4, 124.4, 132.0, 134.8, 138.7, 151.0, 156.3, 168.3, 184.4 (C═O). IR (KBr) cm^{-1}: 3449, 3355, 2929, 1706, 1643,

1583, 1541, 1501, 1398, 1367, 1260, 1109, 1016, 721. HRMS (DCI, CH_4) m/z calcd for $C_{19}H_{16}N_3O_4$ $[M+H]^+$ 350.1141. Found 350.1152.

4.1.4. General procedure for the preparation of indolone-N-oxides hydrochlorides

The hydrogen chloride solution (4 M in dioxane) (6 mmol) was added to a solution of indolone-*N*-oxide (**2** or **4**) (1 mmol) in 20 mL of 1,4-dioxane. The reaction was stirred at room temperature for 1 h and verified by CCM. The reaction mixture was filtered and the remaining solid was washed with 1,4-dioxane to afford the indolone-*N*-oxide hydrochlorides (**5** or **6**), respectively.

*4.1.4.1. 2-(4-Aminophenyl)-5-methoxy-1-oxy-indol-3-one, hydrochloride (**5**).* Red solid, yield 93%, mp: 216–218 °C. ^{1}H RMN (300 MHz, DMSO-d_6) δ: 3.87 (s, 3H, CH_3), 6.24 (br, 3H, NH_3), 6.76 (d, $J = 8.1$ Hz, 2H), 7.16–7.21 (m, 2H), 7.49 (d, $J = 8.4$ Hz, 1H, Ar–H), 8.41 (d, $J = 9$ Hz, 2H). HRMS (DCI, CH_4) m/z calcd for $C_{15}H_{13}N_2O_2$ $[M+H]^+$ 269.0926. Found 269.0915.

*4.1.4.2. 2-(4-Dimethylamino-phenyl)-5-methoxy-1-oxy-indol-3-one, hydrochloride (**6**).* Red brick solid, yield 74%, mp: 183–185 °C. ^{1}H RMN (300 MHz, DMSO-d_6) δ: 3.03 (s, 6H, 2 CH_3), 3.87 (s, 3H, CH_3), 4.02 (br, 1H, NH), 6.92 (dd, $J = 9$ Hz, $J = 3$ Hz, 2H), 7.16 (d, $J = 3$ Hz, 1H), 7.20 (dd, $J = 9$ Hz, $J = 3$ Hz, 1H), 7.49 (d, $J = 9$ Hz, 1H), 8.51 (dd, $J = 9$ Hz, $J = 3$ Hz, 2H). ^{13}C RMN (75 MHz, DMSO-d_6) δ: 40.3 (2 CH_3), 56.7 (CH_3), 108.4, 112.4 (×2), 114.6, 115.0, 118.8, 122.4, 124.9, 128.8 (×2), 141.0, 151.0, 161.7, 187.5 (C=O). HRMS (DCI, CH_4) m/z calcd for $C_{17}H_{17}N_2O_3$ $[M+H]^+$ 297.1239. Found 297.1256.

4.2. Biology

4.2.1. In vitro P. falciparum culture and inhibition assays of parasite growth

P. falciparum (FcB1, Chloroquine-resistant strain) was maintained *in vitro* in RPMI 1640 medium (BioWhittaker, Cambrex, Belgium) containing L-glutamine (BioWhittaker), 25 mM HEPES (BioWhittaker), and 10% human serum (EFS, Toulouse, France) as already described [14]. Human RBCs (group O±, Toulouse, France) were extensively washed with RPMI medium to remove remaining plasma and leukocytes. Parasitized RBCs were maintained in 25 cm^2 culture flasks (TPP, Switzerland) in a controlled atmosphere (5% CO_2, 100% relative humidity) and synchronized by a combination of magnetic enrichment followed by D-sorbitol lysis [15,16]. Chloroquine (CQ, Sigma (ref C6628)) was dissolved in culture medium and arthemether (ART, Cambrex) in ethanol (stock solutions: 10 mg/mL). The stock solutions of the tested drugs were prepared by mixing 1 mg of drug in 1 mL DMSO and adding it to a solution of 1 mg BSA in 1 mL of RPMI medium, giving a stock solution at 0.5 mg/mL. For the drug assays, serial drug dilutions were made in *P. falciparum* culture media and added to 96-well (TPP) culture plates. For the evaluation of *Plasmodium* growth inhibition, the culture was plated in 96-well plates as described elsewhere [6]. [^{3}H]-Hypoxanthine (Perkin–Elmer) was added 24 h after the beginning of incubation. At the end of incubation (48 h), the microtiter plates were frozen and thawed, and each well was harvested onto glass-fiber filter paper. The [^{3}H]-hypoxanthine incorporation was determined with a β-counter (1450-Microbeta Trilux, Wallac-Perkin–Elmer). Growth inhibition percentages were plotted as a semilogarithmic function of drug concentration. The IC_{50} values were determined by linear regression analysis on the linear segments of the curves. In each assay, drugs were tested in triplicate and assays were repeated three times. Controls were carried out to assess the background (negative control) and parasite growth (positive control).

4.2.2. In vitro cytotoxicity assay

Cytotoxicity was determined on human breast cancer cells (MCF7). The cells were cultured in the same conditions as those used for *P. falciparum*, except that 10% human serum was replaced by 10% fetal calf serum (Cambrex). After trypsinization, cells were distributed in 96-well plates at 2×10^4 cells/well in 100 μL of culture medium. After an overnight incubation 100 μL culture medium containing the tested compounds at various concentrations (the final concentrations in the wells were 1, 10, and 100 μg mL^{-1}) was added. Cell growth inhibition was estimated by a colorimetric assay based on XTT reduction after a 48 h contact between drugs and cells [17]. Experiments were performed twice in triplicate.

4.2.3. Selectivity index (SI)

The selectivity indexes presented correspond to the ratios between, respectively, the toxicity on MCF-7 human cell line and the FcB1 antiplasmodial activity. They are calculated as follows: SI FcB1 = CC_{50} (MCF-7)/IC_{50} (FcB1).

Acknowledgments

This work was supported by the French National Research Agency (ANR-10-BLAN-0726, Mechanisms of Action and Targets of new antimalarial Redox molecules, MATURE). We thank J.-P. Nallet for his scientific contribution.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.02.038.

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