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Original article

Synthesis and antiprotozoal activity of 4-arylcoumarins

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ABSTRACT

A large series of 4-arylcoumarins was synthesized by Suzuki-Miyaura cross-coupling reaction and evaluated for antiprotozoal activity against *Plasmodium falciparum* and *Leishmania donovani*. Several compounds were found to strongly inhibit the proliferation of human cell line and/or parasites. The 4-(3,4-dimethoxyphenyl)-6,7-dimethoxycoumarin exhibit a potent activity on *L. donovani* amastigotes with a selectivity index (SI = 265) twice than amphotericin B (SI = 140).

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1. Introduction

Malaria and leishmaniasis are the most important parasitic diseases in the world. Malaria is the most threatening, with an annual incidence of 250 million clinical cases and an annual mortality of 1 million deaths, mainly in children in Sub-Saharan countries [1]. Its therapeutic management has become problematic since the emergence of multidrug-resistant strains of *Plasmodium falciparum* [2]. The challenge is to find new active compounds structurally different to current antiplasmodial molecules for which resistance arises [3].

Leishmaniasis is endemic in 88 countries, leading to 500 000 new cases and 50 000 deaths each year for its visceral form caused by *Leishmania donovani* [4]. The therapeutic is based on antimonial derivatives as first-line drugs and amphotericin B and pentamidine as second-line drugs. However, resistant parasites have emerged in several endemic areas in India and Sudan, underlying the urgent need of new antileishmanial drugs [5].

Protozoan parasites share several important features with cancer cells regarding their proliferative properties and their capacity to escape the host immune responses [6]. These similarities allow to consider the potential antiparasitic activity of anticancer compounds. For instance, miltefosine, an anticancer alkyllysophospholipid has a potent activity on *Leishmania* amastigotes and other trypanosomatid parasites [7].

We previously reported the synthesis and evaluation of 4-arylcoumarins as potent anticancer agents [8–10]. Two poly-methoxylated and hydroxylated derivatives have shown very interesting antiproliferative activity on human cancer cell lines. It is therefore relevant to consider their potential activity towards protozoan parasites. Moreover among their various biological properties 4-arylcoumarins exhibited interesting pharmacological activity *in-vitro* against chloroquine resistant strains of *P. falciparum*[11] and *in-vivo* against *P. berghei* [12]. Antileishmanial activity of natural 4-arylcoumarins was also reported on promastigote and amastigote forms of *Leishmania amazonensis*[13] and *L. braziliensis* [14]. Overall, the mechanisms of action of such molecules remain unclear in particular on the amastigote form of *Leishmania* parasites.

In this purpose, we prepared a large series of 4-arylcoumarins variously substituted on phenyl rings and investigated their potential antiproliferative properties towards *P. falciparum* and *L. donovani*.
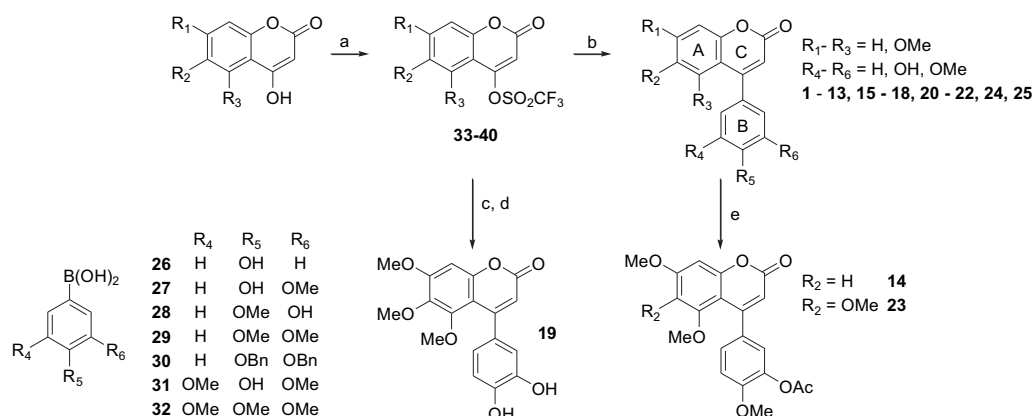
2. Chemistry

The 4-Arylcoumarins were synthesized by ligand coupling reaction of two aromatic units, the preformed coumarin ring and

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Scheme 1. Synthesis of 4-arylcoumarins **1–25** by palladium-catalysed coupling between triflates **33–40** and boronic acids **26–32**. Reagents and conditions: (a) $(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2; (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, CuI, Na_2CO_3, toluene, 110 °C; (c) **30**, $Pd(PPh_3)_4$, CuI, Na_2CO_3, toluene, 110 °C; (d) aqueous HBr, 50 °C, then H_2O; (e) $(CH_3CO)_2O$, CH_3CO_2Na.

a conveniently substituted phenyl derivative. The 4-trifluoro-methylsulfonyloxycoumarins were easily prepared in 73–97% yield by treatment of the appropriate 4-hydroxycoumarins with triflic anhydride [15]. In the modified Suzuki conditions, the C-4 arylation of the 4-activated coumarins was performed with arylboronic acids in presence of a palladium catalyst and copper (I) iodide as a co-catalyst (Scheme 1) [8,15–17]. The resulting neoflavones were isolated in good to excellent yields (59–99%, Table 1). Use of the benzyl-protected arylboronic acid **30** in the palladium-copper catalyzed Suzuki reaction led to the corresponding protected hydroxycoumarin. Deprotection of the benzyloxy groups was

performed by treatment with hydrobromic acid [8], leading to the expected dihydroxylated 4-arylcoumarin **19** in 77% yield. Acetyla-tion of **12** and **21** was performed with acetic anhydride in presence of sodium acetate [10], and led to esterified compounds **14** and **23** in 97% and 88% yield respectively (Table 1).

3. Pharmacology

The cytotoxicity of 4-arylcoumarins **1–25** was evaluated against human monocytes THP1. Antiplasmodial activity was assessed against the reference strain of *P. falciparum* type W2 resistant to

Table 1
Structure, yield, cytotoxic and antiprotozoal activity of 4-arylcoumarins **1–25** and reference drugs.

Product	R_{1-3}	R_4	R_5	R_6	Yield (%)	Cytotoxicity (µM)[a] IC_{50} THP1	Anti-*Ld* activity (µM)[a] IC_{50} Pro	IC_{50} Ama	SI Ama[b]	Anti-*Pf* activity (µM)[a] IC_{50} W2	SI W2[b]
1	H	OMe	OMe	H	82	>176	>176	26.4	>6.7	12	>14,7
2	7-methoxy	OMe	OMe	H	93	64	25.6	> 25	NC[c]	64	1
3	6-methoxy	OMe	OMe	H	99	>160	>160	5.4	>29.6	>160	NC
4	5-methoxy	OMe	OMe	H	88	54	>54	12.8	4.2	>54	NC
5	6,7-dimethoxy	H	OH	H	85	>67	>67	>67	NC	>67	NC
6		OMe	OH	H	74	>137	94.2	30.4	>4.5	22.5	> 6,1
7		OMe	OMe	H	81	>292	>292	1.1	>265	>117	NC
8		OMe	OH	OMe	72	>139	99.3	10	>13.9	22.4	> 6.2
9		OMe	OMe	OMe	68	>268	32	61.8	>4.3	>268	NC
10	5,7-dimethoxy	H	OH	H	86	25.5	>25.5	23.3	1.1	15.4	1.7
11		OMe	OH	H	82	>152	>152	24.1	>6.3	>122	NC
12		OH	OMe	H	74	<15	>15	>15	TOX[d]	>15	TOX
13		OMe	OMe	H	75	>146	>146	9.3	>15.7	61.2	>2.4
14		OAc	OMe	H	81[e]	>135	>135	2.6	>51.9	>135	NC
15		OMe	OH	OMe	91	>139	>139	19.1	>7.2	64	>2.2
16		OMe	OMe	OMe	75	>134	>134	> 25	NC	>134	NC
17	5,6-dimethoxy	OMe	OMe	H	59	88	>88	64.5	1.4	63.7	1.4
18	5,6,7-trimethoxy	H	OH	H	82	>152	>152	74.2	>2.0	28.9	>5.3
19		OH	OH	H	58[e]	45	>45	25.3	1.8	27.9	1.6
20		OMe	OH	H	83	>262	>262	2.4	>109	27.1	>9.7
21		OH	OMe	H	84	<14	>14	13.9	TOX	>14	TOX
22		OMe	OMe	H	78	78	>78	4.3	18.1	25.8	3.0
23		OAc	OMe	H	88[e]	<12.5	>12.5	3.5	TOX	>12.5	TOX
24		OMe	OH	OMe	88	>257	>257	> 50	NC	>103	NC
25		OMe	OMe	OMe	65	>248	>248	>25	NC	72.3	>3.4
Doxorubicin						0.05	–	–	–	–	–
Amphotericin B						14	0.38	0.10	140	–	–
Chloroquine						40	–	–	–	0.70	57.1

[a] Mean of three independent experiments.
[b] Selectivity index was calculated according to the following formula: $SI = IC_{50}$ THP1/IC_{50} Parasite.
[c] NC: not calculated due to insufficient solubility of compound.
[d] TOX: toxic compound (SI < 1).
[e] Overall yield including Suzuki coupling and acetylation or debenzylation.

chloroquine, pyrimethamine and proguanil. Antileishmanial activity was evaluated against extracellular promastigotes and intracellular amastigotes of the reference strain *L. donovani* (MHOM/ IN/80/DD8).

4. Results and discussion

Structure, cytotoxic and antiprotozoal activity of 4-arylcoumarins are reported in Table 1. Compound **23** with a 3-acetoxy-4-methoxyphenyl moiety was highly cytotoxic towards THP1 cells (IC$_{50}$THP1 < 15 μM) as well as compounds **12** and **21** described previously [9]. We reported the importance of the 3-hydroxy-4-methoxyphenyl ring leading to the cytotoxicity of compounds **12** and **21** on human leukemia CEM cells (IC$_{50}$ = 0.083 μM and 0.52 μM respectively) and epithelial mammary HBL100 cells (IC$_{50}$ = 0.087 μM and 0.42 μM respectively) [8,9]. These two molecules disturbed cell survival by depolymerizing the microtubule network leading to a mitototic block and apoptosis induction. Mechanisms of action on cell survival of acetylated derivative **23** were recently studied [10]. The three arylcoumarin analogues were found to be able to promote apoptosis of CLL cells *ex-vivo* through the caspase-dependent mitochondrial pathway. Despite their promising use as antitumoral molecules, these three compounds did not have the antiplasmodial or antileishmanial required selectivity (Table 1).

Among other 4-arylcoumarins tested, only compounds **3**, **7**, **14** and **20** displayed a significant antiprotozoal activity (Table 1, column 9) associated to a weak cytotoxicity towards THP1 cells. These four molecules had only an interesting activity against *L. donovani* amastigotes with an IC$_{50}$Ama of 5.4, 1.1, 2.6 and 2.4 μM respectively, and a selectivity index of at least 29.6, 265, 51.9 and 109 respectively. The selectivity index of compound **7** (SI = 265) is nearly twice than the antileishmanial reference molecule amphotericin B (SI = 140, Table 1).

Despite an important structural similarity between the reported ranges of compounds **1–25**, the difference of activity is quite noteworthy. Considering to un- and monosubstituted coumarin core derivatives **1–4**, 6-methoxylated compound **3** exhibits the highest activity on amastigote form. Importance of presence of 6-OMe group for activity is observed for dimethoxylated analogues also. 6,7-diOMe compound **7** has a very significant activity compared to 5,7-diOMe derivative **13**, with an IC$_{50}$Ama of 1.1 and 9.3 μM and a selectivity index more than 265 and 15.7 respectively. Finally, when the 6-OMe group is introduced on ring A to form the 5,6,7-trimethoxylated analogue **22**, the activity is partially restored (IC$_{50}$Ama amounting to 4 μM). Further structure- activity relationship show that the best substitution pattern on ring B is either a methoxy or acetoxy hydrophobic group on the 3′- and 4′-position.

It is interesting to note major differences between activities of compounds **3**, **7**, **14** and **20** against promastigote and amastigote forms of *L. donovani*. As a consequence of the obligate intracellular development of amastigotes inside the mammalian host macrophages, metabolism is distinct from promastigotes. Such differences will lead us to further investigate the mechanisms of action of these four compounds. Preliminary results suggest that these compounds may modulate the parasite phagocytosis by THP1 cells and there is no evidence to indicate that the active compounds exert their toxic effects through a tubulin mechanism.

5. Conclusion

Among the various 4-arylcoumarins prepared, four compounds exhibit potent antileishmanial activity. In contrast, no antiplasmodial activity was observed. The 3′,4′,6,7-tetramethoxylated

compound **7** is the most selective toward *L. donovani* amastigote forms with an index twice than the reference amphotericin B. These encouraging results justify a further prodrug derivatives development to increase antileishmanial potency of 4-arylcoumarins.

6. Experimental

6.1. Chemistry

Melting points were taken on a Büchi capillary apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker AC 300 (300 MHz) spectrometer using the deuterated solvent as an internal reference. Chemical shifts (δ) are reported in ppm and *J* values are given in hertz. Combustion analyses were performed at the Service d'Analyse Elémentaire of the Saint Jerome Faculty (Marseille, France). Separation by column chromatography was performed using Merck Kieselgel 60. All solvents were purified by standard techniques. Arylboronic acids **26–32** were synthesized according to the reported procedures involving the reaction of the appropriate aryllithium with triisopropylborate [16,18]. 4-Trifluoromethylsulfonyloxy-coumarins **33–40** were prepared as previously reported [16,19].

6.1.1. 6-Methoxy-4-trifluoromethylsulfonyloxycoumarin (35)
Light yellow needles, 93%, mp 76–78 °C. ^{1}H NMR (CDCl$_3$): δ 7.36 (d, *J* = 9.1 Hz, 1H, 8-H), 7.25 (dd, *J* = 9.1, 2.8 Hz, 1H, 7-H), 7.08 (d, *J* = 2.8 Hz, 1H, 5-H), 6.51 (s, 1H, 3-H), 3.88 (s, 3H, OMe).

6.1.2. 5,6-Dimethoxy-4-trifluoromethylsulfonyloxycoumarin (40)
Light yellow crystals, 97%, mp 95–98 °C. ^{1}H NMR (CDCl$_3$): δ 7.24 (d, *J* = 8.6 Hz, 1H, 7-H), 7.12 (d, *J* = 8.6 Hz, 1H, 8-H), 6.31 (s, 1H, 3-H), 3.97 (s, 3H, OMe), 3.91 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 159.5, 157.1, 149.6, 147.5, 144.7, 118.5, 118.3, 112.2, 109.2, 108.4, 61.6, 56.6.

6.1.3. Synthesis of 4-arylcoumarins by Suzuki coupling reaction – General procedure
A mixture of 4-trifluoromethylsulfonyloxycoumarin (1 mmol), arylboronic acid (1.5 eq.), tetrakis (triphenylphosphine) palladium (0) (46 mg, 4 mol%), copper(I) iodide (210 mg, 1.1 eq.) and sodium carbonate (742 mg, 7 eq.) in dry toluene (10 ml) and absolute methanol (3 ml) was refluxed overnight. Then the reaction mixture was diluted with chloroform (40 ml) and filtered through a short pad of Celite. The filtrate was washed with a saturated aqueous solution of sodium bicarbonate (3 × 20 ml) and the combined aqueous layers were extracted with chloroform (3 × 20 ml). The combined organic phases were washed with brine, dried over Na$_2$SO$_4$ and the solvent was distilled under reduced pressure. The residue was purified by column chromatography or directly by crystallization to afford the 4-arylcoumarin derivative.

6.1.3.1. 4-(3′,4′-dimethoxyphenyl)coumarin (1).
Crystallised from ether as fine white needles, 82%, mp 145 °C (lit. [15], 145–146 °C). ^{1}H NMR (CDCl$_3$,): δ 7.60 (d, *J* = 7.9 Hz, 1H, 5-H), 7.56 (dd, *J* = 8.1, 7.2 Hz, 1H, 7-H), 7.42 (d, *J* = 8.1 Hz, 1H, 8-H), 7.25 (dd, *J* = 7.9, 7.2 Hz, 1H, 6-H), 7.06 (dd, *J* = 8.3, 1.3 Hz, 1H, 6′-H), 7.01 (d, *J* = 8.3 Hz, 1H, 5′-H), 6.95 (d, *J* = 1.3 Hz, 1H, 2′-H), 6.38 (s, 1H, 3-H), 3.97 (s, 3H, OMe), 3.92 (s, 3H, OMe).

6.1.3.2. 4-(3′,4′-dimethoxyphenyl)-7-methoxycoumarin (2).
Crystallised from ether as white crystals, 93%, mp 158 °C (lit. [20], 151–153 °C). ^{1}H NMR (CDCl$_3$): δ 7.47 (d, *J* = 8.9 Hz, 1H, 5-H), 7.03 (dd, *J* = 8.3, 1.8 Hz, 1H, 6′-H), 6.98 (d, *J* = 8.3 Hz, 1H, 5′-H), 6.95 (d, *J* = 1.8 Hz, 1H, 2′-H), 6.88 (d, *J* = 2.5 Hz, 1H, 8-H), 6.80 (dd, *J* = 8.9, 2.5 Hz, 1H, 6-H), 6.20 (s, 1H, 3-H), 3.95(s, 3H, OMe), 3.91 (s, 3H, OMe), 3.87 (s, 3H, OMe).

6.1.3.3. 4-(3′,4′-dimethoxyphenyl)-6-methoxycoumarin

(**3**). Crystallised from ether as white crystals, 99%, mp 178 °C (lit. [15], 148–150 °C). ^{1}H NMR (CDCl$_3$): δ 7.35 (d, J = 9.0 Hz, 1H, 8-H), 7.13 (dd, J = 9.0, 2.3 Hz, 1H, 7-H), 7.00–7.06 (m, 3H, 2′-, 5′- and 6′-H), 6.98 (d, J = 2.9, 1H, 5-H), 6.38 (s, 1H, 3-H,), 3.97 (s, 3H, OMe), 3.92 (s, 3H, OMe), 3.75 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 161.1, 155.9, 155.1, 150.3, 149.3, 148.7, 127.8, 121.3, 119.6, 119.1, 118.3, 115.2, 111.6, 111.4, 109.9, 56.1, 56.0, 55.8.

6.1.3.4. 4-(3′,4′-dimethoxyphenyl)-5-methoxycoumarin (**4**).

Crystallised from ether as white crystals, 88%, mp 187 °C. ^{1}H NMR (CDCl$_3$): δ 7.47 (t, J = 8.1 Hz, 1H, 7-H), 7.03 (d, J = 8.1 Hz, 1H, 6-H), 6.89 (d, J = 8.4 Hz, 1H, 6′-H), 6.87 (d, J = 8.4 Hz, 1H, 5′-H), 6.82 (s, 1H, 2′-H), 6.70 (d, J = 8.1 Hz, 1H, 8-H), 6.19 (s, 1H, 3-H), 3.94 (s, 3H, OMe), 3.87(s, 3H, OMe), 3.53 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 160.5, 157.3, 155.5, 155.0, 149.1, 148.0, 132.4, 132.3, 119.7, 116.0, 111.1, 110.2, 110.0, 109.3, 106.7, 56.0, 55.9, 55.6. Anal. Calcd for C$_{18}$H$_{16}$O$_5$ (312.10): C, 69.22; H, 5.16. Found: C, 69.11; H, 5.15%.

6.1.3.5. 4-(4′-hydroxyphenyl)-6,7-dimethoxycoumarin (**5**).

Purified by column chromatography, eluent CH$_2$Cl$_2$–EtOH (49:1), as light yellow crystals, 85%, mp 240 °C (lit. [21], 259–260 °C). ^{1}H NMR (CDCl$_3$): δ 7.38 (d, J = 8.5 Hz, 2H, 2′- and 6′-H), 7.00 (d, J = 8.5 Hz, 2H, 3′- and 5′-H), 6.93 (s, 1H, 5-H), 6.92 (s, 1H, 8-H), 6.22 (s, 1H, 3-H), 5.43 (s, 1H, OH), 3.97 (s, 3H, OMe), 3.78 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 160.4, 158.0, 154.9, 152.2, 149.4, 145.3, 129.1, 126.1, 115.1, 110.7, 110.5, 107.1, 100.2, 55.4, 55.3.

6.1.3.6. 4-(4′-hydroxy-3′-methoxyphenyl)-6,7-dimethoxycoumarin

(**6**). Purified by column chromatography, eluent EtOAc-petroleum spirit (1:1), as white crystals, 74%, mp 164 °C. ^{1}H NMR (CDCl$_3$): δ 6.96–7.06 (m, 4H, 5-, 2′-, 5′- and 6′-H), 6.88 (s, 1H, 8-H), 6.21 (s, 1H, 3-H), 6.11 (s, 1H, OH), 3.94 (s, 3H, OMe), 3.92 (s, 3H, OMe), 3.76 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 161.6, 155.5, 152.8, 150.1, 147.1, 146.9, 146.0, 127.5, 121.7, 114.8, 111.7, 111.4, 110.9, 107.4, 100.2, 56.3, 56.2, 56.1. Anal. Calcd for C$_{18}$H$_{16}$O$_6$ (328.09): C, 65.85; H, 4.91. Found: C, 65.82; H, 4.97%.

6.1.3.7. 4-(3′,4′-dimethoxyphenyl)-6,7-dimethoxycoumarin (**7**).

Crystallised from ether as yellow crystals, 81%, mp 222–223 °C (lit. [22], 220–221 °C). ^{1}H NMR (CDCl$_3$): δ 7.07 (dd, J = 8.3, 1.9 Hz, 1H, 6′-H), 7.02 (d, J = 8.3 Hz, 1H, 5′-H), 6.98 (d, 1H, J = 1.9 Hz, 2′-H), 6.97 (s, 1H, 5-H), 6.92 (s, 1H, 8-H), 6.25 (s, 1H, 3-H), 3.98 (s, 6H, 2× OMe), 3.92 (s, 3H, OMe), 3.79 (s, 3H, OMe).

6.1.3.8. 4-(4′-hydroxy-3′,5′-dimethoxyphenyl)-6,7-dimethoxycoumarin

(**8**). Purified by column chromatography, eluent CH$_2$Cl$_2$, as light pink crystals, 72%, mp 154 °C. ^{1}H NMR (CDCl$_3$): δ 6.98 (s, 1H, 5-H), 6.89 (s, 1H, 8-H), 6.69 (s, 2H, 2′- and 6′-H), 6.23 (s, 1H, 3-H), 5.83 (s, 1H, OH), 3.95 (s, 3H, OMe), 3.92 (s, 6H, 3′- and 5′-OMe), 3.77 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 160.5, 155.6, 152.8, 150.1, 147.3, 146.0, 136.1, 126.7, 111.8, 111.4, 107.3, 105.3, 100.3, 56.5, 56.4, 56.3. Anal. Calcd for C$_{19}$H$_{18}$O$_7$ (358.11): C, 63.68; H, 5.06. Found: C, 63.64; H, 5.10%.

6.1.3.9. 4-(3′,4′,5′-trimethoxyphenyl)-6,7-dimethoxycoumarin

(**9**). Crystallised from ether as white needles, 68%, mp 202 °C. ^{1}H NMR (CDCl$_3$): δ 6.97 (s, 1H, 5-H), 6.91 (s, 1H, 8-H), 6.67 (s, 2H, 2′- and 6′-H), 6.25 (s, 1H, 3-H), 3.97 (s, 3H, OMe), 3.94 (s, 3H, OMe), 3.89 (s, 6H, 3′- and 5′-OMe), 3.78 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 161.4, 155.4, 153.6, 153.0, 150.2, 146.1, 139.2, 131.1, 112.1, 111.3, 107.4, 105.6, 100.4, 61.0, 56.4, 56.4, 56.3. Anal. Calcd for C$_{20}$H$_{20}$O$_7$ (372.12): C, 64.51; H, 5.41. Found: C, 64.28; H, 5.41%.

6.1.3.10. 4-(4′-hydroxyphenyl)-5,7-dimethoxycoumarin (**10**).

Crystallised from ether as fine white needles, 86%, mp 204 °C (lit. [16],

210–213 °C). ^{1}H NMR (CDCl$_3$): δ 7.16 (d, J = 8.5 Hz, 2H, 2′- and 6′-H), 6.86 (d, J = 8.5 Hz, 2H, 3′- and 5′-H), 6.52 (d, J = 2.4 Hz, 1H, 8H), 6.25 (d, J = 2.4 Hz, 1H, 6H), 5.99 (s, 1H, 3-H), 5.51 (s, 1H, OH), 3.87 (s, 3H, OMe), 3.49 (s, 3H, OMe).

6.1.3.11. 4-(4′-hydroxy-3′-methoxyphenyl)-5,7-dimethoxycoumarin

(**11**). Purified by column chromatography, eluent CH$_2$Cl$_2$-EtOH (49:1), and crystallized from ether as white needles, 82%, mp 172 °C (lit. [16], 172–173 °C). ^{1}H NMR (CDCl$_3$): δ 6.92 (d, J = 8.7 Hz, 1H, 5′-H), 6.81 (dd, J = 8.7, 1.9 Hz, 1H, 6′-H), 6.79 (d, J = 1.9 Hz, 1H, 2′-H), 6.52 (d, J = 2.5 Hz, 1H, 8H), 6.26 (d, J = 2.5 Hz, 1H, 6H), 6.02 (s, 1H, 3-H), 5.54 (s, 1H, OH), 3.89 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.50 (s, 3H, OMe).

6.1.3.12. 4-(3′-Hydroxy-4′-méthoxyphényl)-5,7-diméthoxycoumarine

(**12**). Purified by column chromatography, eluent CH$_2$Cl$_2$-EtOH (49:1), and crystallized from ether as white needles, 74%, mp 152 °C (lit. [8], 152 °C). ^{1}H NMR (CDCl$_3$): δ 6.84–6.87 (m, 2H, 2′- et 5′-H), 6.79 (dd, J = 8.1, 1.9 Hz, 1H, 6′-H), 6.51 (d, J = 2.3 Hz, 1H, 8H), 6.24 (d, J = 2.3 Hz, 1H, 6H), 6.00 (s, 1H, 3-H), 5.64 (s, 1H, OH), 3.95 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.50 (s, 3H, OMe).

6.1.3.13. 4-(3′,4′-dimethoxyphenyl)-5,7-dimethoxycoumarin (**13**).

Crystallised from ether as yellow crystals, 75%, mp 170 °C (lit. [23], 170–171 °C). ^{1}H NMR (CDCl$_3$): δ 6.89 (dd, J = 8.3, 1.3 Hz, 1H, 6′-H), 6.86 (d, J = 8.3 Hz, 1H, 5′-H), 6.81 (d, J = 1.3 Hz, 1H, 2′-H), 6.53 (d, J = 2.5 Hz, 1H, 8H), 6.25 (d, J = 2.5 Hz, 1H, 6H), 6.02 (s, 1H, 3-H), 3.94 (s, 3H, OMe), 3.88 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.49 (s, 3H, OMe).

6.1.3.14. 4-(3′-acetoxy-4′-methoxyphenyl)-5,7-dimethoxycoumarin

(**14**). Crystallised from ether as white crystals, 97%, mp 166 °C (CHCl$_3$–Et$_2$O) (lit. [24], 196–198 °C). ^{1}H NMR (CDCl$_3$): δ 7.15 (dd, J = 8.3, 2.2 Hz, 1H, 6′-H), 6.98 (d, J = 2.2 Hz, 1H, 2′-H), 6.96 (d, J = 8.3 Hz, 1H, 5′-H), 6.51 (d, J = 2.4 Hz, 1H, 8H), 6.23 (d, J = 2.4 Hz, 1H, 6H), 6.01 (s, 1H, 3-H), 3.89 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.51 (s, 3H, OMe), 2.32 (s, 3H, Me). ^{13}C NMR (CDCl$_3$): δ 168.9, 163.4, 160.8, 158.2, 157.2, 154.4, 151.1, 138.7, 132.2, 125.5, 122.8, 112.7, 111.2, 103.3, 95.6, 93.5, 56.0, 55.8, 55.5, 20.7.

6.1.3.15. 4-(4′-hydroxy-3′,5′-dimethoxyphenyl)-5,7-dimethoxycoumarin

(**15**). Crystallised from ether as fine white needles, 91%, mp 215 °C (lit. [8], 215 °C). ^{1}H NMR (CDCl$_3$): δ 6.54 (d, J = 2.5 Hz, 1H, 8-H), 6.52 (s, 2H, 2′- and 6′-H), 6.26 (d, J = 2.5 Hz, 1H, 6-H), 6.04 (s, 1H, 3-H), 5.62 (s, 1H, OH), 3.89 (s, 6H, 3′- and 5′-OMe), 3.88 (s, 3H, OMe), 3.51 (s, 3H, OMe).

6.1.3.16. 4-(3′,4′,5′-trimethoxyphenyl)-5,7-dimethoxycoumarin

(**16**). Crystallised from ether as yellow plates, 75%, mp 207 °C (lit. [25], 204–206 °C). ^{1}H NMR (CDCl$_3$): δ 6.54 (d, J = 2.5 Hz, 1H, 8H), 6.49 (s, 2H, 2′- and 6′-H), 6.26 (d, J = 2.5 Hz, 1H, 6H), 6.04 (s, 1H, 3-H), 3.91(s, 3H, OMe), 3.88 (s, 3H, OMe), 3.85 (s, 6H, 3′- and 5′-OMe), 3.50 (s, 3H, OMe).

6.1.3.17. 4-(3′,4′-dimethoxyphenyl)-5,6-dimethoxycoumarin (**17**).

Purified by column chromatography, eluent CH$_2$Cl$_2$–EtOH (49:1), as yellow crystals, 59%, mp 145 °C. ^{1}H NMR (CDCl$_3$): δ 7.16 (d, J = 9.3 Hz, 1H, 7-H), 7.13 (d, J = 9.3 Hz, 1H, 8-H), 6.92–6.87 (m, 3H, 2′-, 5′- and 6′-H), 6.24 (s, 3H, 3-H), 3.94 (s, 3H, OMe), 3.89 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.21 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 160.4, 154.5, 149.7, 149.2, 148.4, 148.0, 146.4, 131.3, 120.1, 117.6, 116.3, 114.2, 112.2, 111.5, 110.2, 61.0, 56.6, 56.0, 55.9. Anal. Calcd for C$_{19}$H$_{18}$O$_6$ (342.11): C, 66.66; H, 5.30. Found: C, 66.51; H, 5.41%.

6.1.3.18. 4-(4′-hydroxyphenyl)-5,6,7-trimethoxy-coumarin (**18**).

Crystallised from ether as light pink plates, 82%, mp 232 °C (lit. [8],

232 °C). ^{1}H NMR (CDCl$_3$): δ 7.24 (d, $J = 8.6$ Hz, 2H, 2′- and 6′-H), 6.88 (d, $J = 8.6$ Hz, 2H, 3′- and 5′-H), 6.73 (s, 1H, 8-H), 6.06 (s, 1H, 3-H), 5.11 (s, 1H, OH), 3.94 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.29 (s, 3H, OMe).

*6.1.3.19. 4-(3′,4′-dihydroxyphenyl)-5,6,7-trimethoxycoumarin (**19**).* Purified by column chromatography, eluent Et$_2$O, as white crystals, 77%, mp 199 °C. ^{1}H NMR (CDCl$_3$): δ 6.92 (d, $J = 8.1$ Hz, 1H, 5′-H), 6.87 (d, $J = 1.9$ Hz, 1H, 2′-H), 6.77 (dd, $J = 8.1$, 1.9 Hz, 1H, 6′-H), 6.71 (s, 1H, 8-H), 6.25 (s, 1H, OH), 6.04 (s, 1H, 3-H), 5.75 (s, 1H, OH), 3.93 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.35 (s, 3H, OMe). ^{13}C NMR (acetone-d6): δ 160.5, 157.9, 156.2, 152.6, 152.2, 146.1, 144.9, 140.4, 131.9, 120.2, 115.9, 115.2, 114.2, 107.9, 97.1, 61.5, 61.1, 56.7. Anal. Calcd for C$_{18}$H$_{16}$O$_7$ (344.09): C, 62.79; H, 4.68. Found: C, 62.89; H, 4.70%.

*6.1.3.20. 4-(4′-hydroxy-3′-methoxyphenyl)-5,6,7-trimethoxycoumarin (**20**).* Purified by column chromatography, eluent CH$_2$Cl$_2$–EtOH (49:1), as white fine needles, 83%, mp 154 °C (lit. [8], 154 °C). ^{1}H NMR (CDCl$_3$): δ 6.96 (d, $J = 8.5$ Hz, 1H, 5′-H), 6.85–6.89 (m, 2H, 2′- and 6′-H), 6.72 (s, 1H, 8-H), 6.08 (s, 1H, 3-H), 5.72 (s, 1H, OH), 3.94 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.32 (s, 3H, OMe).

*6.1.3.21. 4-(3′-Hydroxy-4′-methoxyphenyl)-5,6,7-trimethoxycoumarin (**21**).* Purified by column chromatography, eluent CH$_2$Cl$_2$–EtOH (49:1), and crystallized from ether as fine white needles, 84%, mp 157 °C (lit. [8], 157 °C). ^{1}H NMR (CDCl$_3$): δ 6.92 (d, $J = 2.1$ Hz, 1H, 2′-H), 6.89 (d, $J = 8.2$ Hz, 1H, 5′-H), 6.83 (dd, $J = 8.2$, 2.1 Hz, 1H, 6′-H), 6.71 (s, 1H, 8-H), 6.06 (s, 1H, 3-H), 3.95 (s, 3H, OMe), 3.94 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.36 (s, 3H, OMe).

*6.1.3.22. 4-(3′,4′-dimethoxyphenyl)-5,6,7-trimethoxycoumarin (**22**).* Crystallised from ether as fine white needles, 78%, mp 151 °C. ^{1}H NMR (CDCl$_3$): δ 6.87–6.92 (m, 3H, 2′-, 5′- and 6′-H), 6.72 (s, 1H, 8-H), 6.09 (s, 1H, 3-H), 3.94(s, 6H, 2× OMe), 3.89 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.31 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 160.6, 156.7, 155.1, 151.7, 151.1, 149.0, 147.9, 139.5, 131.5, 119.8, 113.9, 111.3, 110.1, 107.2, 96.2, 61.2, 61.0, 56.2, 55.9, 55.8. Anal. Calcd for C$_{20}$H$_{20}$O$_7$ (372.12): C, 64.51; H, 5.41. Found: C, 64.32; H, 5.42%.

*6.1.3.23. 4-(3′-acetoxy-4′-methoxyphenyl)-5,6,7-trimethoxycoumarin (**23**).* Crystallised from ether as fine white needles, 88%, mp 122 °C (CHCl$_3$–Et$_2$O). ^{1}H NMR (CDCl$_3$): δ 7.19 (1H, dd, J 8.3 and 2.2, 6′-H), 7.06 (1H, d, J 2.2, 2′-H), 7.00 (1H, d, J 8.3, 5′-H), 6.71 (1H, s, 8-H), 6.07 (1H, s, 3-H), 3.94 (3H, s, OMe), 3.89 (3H, s, OMe), 3.80 (3H, s, OMe), 3.34 (3H, s, OMe), 2.32 (3H, s, Me). ^{13}C NMR (CDCl$_3$): δ 168.7, 160.5, 156.9, 154.1, 151.7, 151.1, 139.4, 138.8, 131.3, 126.0, 122.5, 114.2, 111.3, 107.1, 96.2, 61.1, 56.2, 56.0, 20.6. Anal. Calcd for C$_{21}$H$_{20}$O$_8$ (400.12): C, 63.00; H, 5.03. Found: C, 62.98; H, 5.05%.

*6.1.3.24. 4-(4′-hydroxy-3′,5′-dimethoxyphenyl)-5,6,7-trimethoxycoumarin (**24**).* Crystallised from ether as fine white needles, 88%, mp 205 °C (lit. [8], 205 °C). ^{1}H NMR (CDCl$_3$): δ 6.72 (s, 1H, 8-H), 6.58 (s, 2H, 2′- and 6′-H), 6.10 (s, 1H, 3-H), 5.62 (s, 1H, OH), 3.94 (s, 3H, OMe), 3.90 (s, 6H, 3′- and 5′-OMe), 3.81 (s, 3H, OMe), 3.36 (s, 3H, OMe).

*6.1.3.25. 4-(3′,4′,5′-trimethoxyphenyl)-5,6,7-trimethoxycoumarin (**25**).* Crystallised from ether as white needles, 65%, mp 146 °C. ^{1}H NMR (CDCl$_3$): δ 6.70 (s, 1H, 8-H), 6.53 (s, 2H, 2′- and 6′-H), 6.08 (s, 1H, 3-H), 3.92 (s, 3H, OMe), 3.89 (s, 3H, OMe), 3.85 (s, 6H, 3′- and 5′-OMe), 3.78 (s, 3H, OMe), 3.36 (s, 3H, OMe). ^{13}C NMR (CDCl$_3$): δ 160.5, 156.9, 155.1, 152.5, 151.6, 151.0, 139.4, 137.8, 134.5, 113.8, 107.2, 104.8, 96.2, 61.2, 61.0, 60.9, 56.2, 56.1. Anal. Calcd for C$_{21}$H$_{22}$O$_8$ (402.13): C, 62.68; H, 5.51. Found: C, 62.84; H, 5.53%.

6.2. Biology

6.2.1. Reagents

Cell culture medium (RPMI 1640), foetal calf serum (FCS), L-glutamine, non essential amino acids and others medium additives were from Eurobio (Paris, France). All other chemicals were of highest chemical purity and were purchased from Sigma unless mention otherwise. Stock solutions of 4-arylcoumarin derivatives and amphotericin B were prepared in DMSO. Stock solutions of reference drugs (doxorubicin and chloroquine) were prepared in ultrapure H$_2$O.

6.2.2. Cytotoxic assays on human monocytes THP1

Cytotoxicity of 4-arylcoumarins was assessed on human monocytes THP1 (ATCC, Manassas VA, USA) by colorimetric determination of cell viability using the redox indicator Alamar Blue® [26]. Late log-phase THP1 cells were incubated in RPMI 1640 (without phenol red) supplemented with 10% FSC and 1% L-glutamine/penicillin–streptomycin mix (complete RPMI medium), containing 10% Alamar Blue® and a range of compound concentrations incorporated in duplicate (final DMSO concentration less than 0.5%). Appropriate controls treated with or without solvent (DMSO), and various concentrations of doxorubicin, chloroquine and amphotericin B were added to each set of experiments. After a 72 h incubation period at 37 °C with 6% CO$_2$, reduction of Alamar Blue® from blue to red was measured by absorbance monitoring at 570 and 630 nm. IC$_{50}$THP1 was defined as the concentration of compound required to induce a 50% decrease of cell viability, corresponding to a 50% reduction of Alamar Blue® as compared to the control culture. IC$_{50}$ was calculated by non-linear regression analysis processed on dose–response curves, using the Table Curve software 2D v.5.0. IC$_{50}$ values represent the mean value calculated from three independent experiments.

6.2.3. Antiplasmodial activity

Assays used culture-adapted *P. falciparum* reference strain W2. Maintenance in continuous culture was done as described previously [27]. Parasites were cultivated in 75 cm^2-flasks containing RPMI 1640 (20 ml) supplemented with 25 mM HEPES, 25 mM NaHCO$_3$, 10% of A+ human serum and 1 ml of washed A+ erythrocytes (final haematocrit 2.5%). Parasitaemia was maintained daily between 1 and 6%. Dilutions used non-infected A+ erythrocytes. Cultures were incubated at 37 °C, 10% O$_2$, 6% CO$_2$, 84% N$_2$, with 90% humidity. Cultures were monitored daily by microscopic examination of blood smears fixed with methanol and stained with 10% Giemsa stain.

Parasite growth was assessed by flow cytometry using hydro-ethidine (HE, Interchim, Montluçon, France) that is converted by metabolically active parasites into ethidium [28]. After incubation with hydroethidine, parasitized and uninfected erythrocytes were all identified on the basis of fluorescence intensity and size.

Triplicate assays were performed in 96-well plates (Nunc Brand products, Fisher, Paris, France) containing 200 μl of asynchronous parasite cultures at 2% of parasitaemia and 2% haematocrit, and 5 μl of the appropriate extract dissolved in DMSO or ultrapure H$_2$O. Negative control treated by solvents (DMSO or H$_2$O) and positive controls (chloroquine) were added to each set of experiments. After 48 h incubation without medium change, plates were centrifuged and the upper liquids were replaced with 200 μl hydroethidine solution (0.05 mg/ml in phosphate buffered saline (PBS)). Plates were incubated 20 min in the dark at 37 °C and washed three times with PBS. Finally, cells were suspended in 1 ml of PBS to allow determination of the number of cell events (around 300 per s) and parasitaemia by flow cytometry using a FACSort flow cytometer (Beckton Dickinson, Paris, France), equipped with an argon laser (power of 15 mW, and wavelength of 488 nm). Settings were: Forward Scatter (FSC-H), size: Voltage E-1, gain 1, mode Log; Side

Scatter (SSC-H), granulosity: Voltage 250, gain 1, mode Log; Fluorescence 2 (FL2), red fluorescence: Voltage 459, gain 1, mode Log.

The concentration of compounds required to induce a 50% decrease of infected erythrocytes ($IC_{50}W2$) was calculated from three independent experiments as describe above. A specificity index (SI) corresponding to the ratio between toxicity on THP1 and antiplasmodial activity was expressed as follows: SI W2 = $IC_{50}THP1/IC_{50}W2$.

6.2.4. Antileishmanial activity against promastigotes

The effects of 4-arylcoumarins on the growth of *L. donovani* promastigotes were assessed by colorimetric determination of parasite viability using the redox indicator Alamar Blue® [26]. Promastigotes in late log-phase were incubated in complete RPMI medium containing 10% Alamar Blue® and a range of compound concentrations (final DMSO concentration less than 0.5%). Following 48 h incubation at 27 °C, Alamar Blue® reduction from blue to red was assessed by absorbance monitoring at 570 and 630 nm.

$IC_{50}Pro$ was defined as the concentration of compound required to induce a 50% decrease of promastigote viability, corresponding to a 50% reduction of Alamar Blue® as compared to the control culture. IC_{50} was calculated from three independent experiments as described above.

6.2.5. Antileishmanial activity against intracellular amastigotes

Intracellular amastigotes were cultured in human monocyte-derived macrophages as previously described [26]. THP1 differentiation into adherent macrophages was performed by treating exponentially-growing monocytes (10^5 cells/ml) with 1 μM phorbol myristate acetate in complete RPMI medium. After 48 h incubation at 37 °C (6% CO_2) in chamber-slides (Fisher, Paris, France), cells were rinsed with fresh medium and suspended in complete medium containing stationary-phase promastigotes (cells/promastigotes ratio = 1/10). After 24 h incubation at 37 °C (6% CO_2), promastigotes were removed by four successive washes with fresh medium. Adapted dilutions of each compound were added in duplicate chambers and cultures were incubated for 72 h at 37 °C (6% CO_2). Appropriate controls treated with or without solvent (DMSO) or a range of amphotericin B concentrations were added to each set of experiments. After incubation, cells were fixed with methanol and stained with 10% Giemsa stain. The percentage of infected macrophages in each assay was determined microscopically at ×1000 magnification.

$IC_{50}Ama$ was defined as the concentration of compound required to induce a 50% decrease of infected macrophages, and was calculated from three independent experiments as described above. A specificity index (SI Ama) corresponding to the ratio between cytotoxicity and activity against *L. donovani* amastigotes was expressed as follows: SI Ama = $IC_{50}THP1/IC_{50}Ama$.

References

[1] World Health Organization. World Malaria Report (2008) 1–215.
[2] K. Hayton, X.Z. Su, Curr. Genet. 54 (2008) 223–239.
[3] J.P. Daily, J. Clin. Pharmacol. 46 (2006) 1487–1497.
[4] F. Chappuis, S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, J. Alvar, M. Boelaert, Nat. Rev. Microbiol. 5 (2007) S7–S16.
[5] S.L. Croft, S. Sundar, A.H. Fairlamb, Clin. Microbiol. Rev. 19 (2006) 111–126.
[6] M.Q. Klinkert, V. Heussler, Mini. Rev. Med. Chem. 6 (2006) 131–143.
[7] S.L. Croft, D. Snowdon, V. Yardley, J. Antimicrob. Chemother. 38 (1996) 1041–1047.
[8] C. Bailly, C. Bal, P. Barbier, S. Combes, J.-P. Finet, M.P. Hildebrand, V. Peyrot, N. Wattez, J. Med. Chem. 46 (2003) 5437–5444.
[9] C. Rappl, P. Barbier, V. Bourgarel-Rey, C. Gregoire, R. Gilli, M. Carre, S. Combes, J.-P. Finet, V. Peyrot, Biochem. 45 (2006) 9210–9218.
[10] C. Billard, F. Menasria, C. Quiney, A.M. Faussat, J.-P. Finet, S. Combes, J.P. Kolb, Exp. Hematol. 36 (2008) 1625–1633.
[11] I. Köhler, K. Jenett-Siems, F.P. Mockenhaupt, K. Siems, J. Jakupovic, J.C. Gonzalez, M.A. Hernandez, R.A. Ibarra, W.G. Berendsohn, U. Bienzle, E. Eich, Planta Med. 67 (2001) 89–91.
[12] R. Argotte-Ramos, G. Ramirez-Avila, M. del Carmen Rodriguez-Gutierrez, M. Ovilla-Muñoz, H. Lanz-Mendoza, M.H. Rodriguez, M. Gonzalez-Cortazar, L. Alvarez, J. Nat. Prod. 69 (2006) 1442–1444.
[13] M.A. Brenzan, C.V. Nakamura, B.P.D. Filho, T. Ueda-Nakamura, M.C.M. Young, D.A. Garcia Cortez, Parasitol. Res. 101 (2007) 715–722.
[14] M.A. Brenzan, C.V. Nakamura, B.P.D. Filho, T. Ueda-Nakamura, M.C.M. Young, A.G. Correa, A. Oliveira dos Santos, D.A. Garcia Cortez, Biomed. Pharmacother. 62 (2008) 651–658.
[15] G.M. Boland, D.M.X. Donnelly, J.-P. Finet, M.D. Rea, J. Chem. Soc. Perkin Trans. 1 (1996) 2591–2597.
[16] D.M.X. Donnelly, J.-P. Finet, P.J. Guiry, M.D. Rea, Synth. Commun. 29 (1999) 2719–2730.
[17] O.G. Ganina, E. Daras, V. Bourgarel-Rey, V. Peyrot, A.N. Andresyuk, J.-P. Finet, A. Fedorov, A. Yu, I.P. Beletskaya, S. Combes, Bioorg. Med. Chem. 16 (2008) 8806–8812.
[18] H. Ishiurata, S. Sato, M. Kabeya, S. Oda, M. Suda, M. Shibasaki, PCT Int. Appl., WO 0302537, Chem. Abstr. 138 (2003) 89830.
[19] I.P. Beletskaya, O.G. Ganina, A.V. Tsvetkov, A. Fedorov, A. Yu, J.-P. Finet, Synlett 15 (2004) 2797–2799.
[20] H.M. Chawla, R.S. Mittal, Phytochem. 22 (1983) 2625–2626.
[21] V.K. Ahluwalia, N. Rani, Indian J. Chem. Sect. B 15 (1977) 1000.
[22] B.J. Donnelly, D.M.X. Donnelly, A.M. O'Sullivan, J.P. Prendergast, Tetrahedron 25 (1969) 4409–4414.
[23] G. Monache, B. Botta, F. Monache, M. Botta, Phytochem. 24 (1985) 1355–1357.
[24] G. Monache, B. Botta, A.S. Neto, R.A. Lima, Phytochem. 22 (1983) 1657–1658.
[25] G. Bargellini, A. Grippa, Gazz. Chim. Ital. 57 (1927) 141.
[26] C. Di Giorgio, K. Shimi, G. Boyer, G. Delmas, J.P. Galy, Eur. J. Med. Chem. 42 (2007) 1277–1284.
[27] W. Trager, J.B. Jensen, Science 193 (1976) 673–675.
[28] N. Azas, N. Laurencin, F. Delmas, C. Di Giorgio, M. Gasquet, M. Laget, P. Timon-David, Parasitol. Res. 88 (2002) 165–171.