Bioorganic & Medicinal Chemistry 18 (2010) 5724–5731

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

1-Azaaurones derived from the naturally occurring aurones as potential antimalarial drugs

Florence Souard [a,*], Sabrina Okombi [a], Chantal Beney [a], Séverine Chevalley [b], Alexis Valentin [b,†], Ahcène Boumendjel [b,†]

[a] Université de Grenoble I/CNRS, UMR 5063, Département de Pharmacochimie Moléculaire, 470, rue de la Chimie, BP 53, Grenoble Cedex 9, France
[b] Université de Toulouse/IRD, UMR 152 LPSNPR, 118 route de Narbonne, F-31062 Toulouse Cedex 9, France

ARTICLE INFO

Article history:
Received 25 January 2010
Revised 17 May 2010
Accepted 4 June 2010
Available online 9 June 2010

Keywords:
Aurones
Azaaurones
Plasmodium falciparum
Malaria

ABSTRACT

We report the synthesis and in vitro antiplasmodial activity of 35 compounds, designed as analogues of the naturally occurring aurones. Several of these analogues showed submicromolar antimalarial activity against a chloroquine-resistant strain of *Plasmodium falciparum* (FcB1-Columbia strain) cultured on human erythrocytes. Substitution of the intracyclic oxygen in aurones by a nitrogen atom and systematic variation of the substituent at the B-ring revealed promising leads showing good activity on the CQ-resistant strain. In particular, 4,6-dimethoxy-4′-ethylazaaurone **22** showed antiplasmodial potency without noticeable toxicity. The easy synthesis of this family of compounds and the relevant antiplasmodial activity are in favor of promising candidates for further development.

1. Introduction

Malaria kills over 1.5 million people a year and it is responsible for human misery in tropical countries. Malaria is caused by a protozoal parasite of the genus *Plasmodium* and remains world wide problem and there is an urgent need to identifying new class of antimalarials.[1]

Traditional medicine and natural compounds have been and still a valuable source of drugs for treatment of malaria.[2–4] In this regard, the quinoleic alkaloid, quinine (**Q**), the active principle of *Cinchona* bark is the most known naturally occurring compound used to treat malaria. Optimization of quinine has led to the discovery of chloroquine (**CQ**) which was used as a major antimalarial drug. More recently, artemisinin has been identified as the active ingredient in decoctions or beverages of *Artemisia annua*.[5] Artemisinin and its hemi-synthetic derivatives, artemether, and artesunate share an endoperoxide moiety that is essential for antimalarial activity.[6,7]

The major hurdle in malaria treatment is the spread of resistance to natural or hemi-synthetic antiplasmodial drugs.[8] Among the various mechanisms identified, those based on drug transport proteins of the ATP-binding cassette (ABC) family appear to play an important role by pumping drugs out of their target sites.[9,10]

Today's meta-analysis provides compelling evidence that drug-resistant malarial parasites are continuing their inexorable global march.[6] Due to the wide spread of malaria and owing to the resistance of the parasite to major drugs, it is urgent to pursue identification for more active and inexpensive drugs to fight malaria.[11–14]

Aurones, (2-benzylidenebenzofuran-3(2*H*)-ones) are secondary metabolite belonging to the flavonoids family (Fig. 1). Aurones are structural isomers of flavones,[15] and compared to other flavonoids subclasses, somehow, aurones still by large less studied. The natural origin of aurones, theirs inhibitory effects on (1) erythrocytic stages of *Plasmodium falciparum* strains in vitro[15–18] and (2) efflux pumps involved in the resistance of the parasites[19–21] has prompted us to investigate them as antimalarial agents targeting resistant strains.

The aim of the present study is to investigate aurones analogs in order to gather more structural elements required for the antimalarial activity. The structure of targeted molecules were chosen according to: (a) from our previous studies, we showed that the

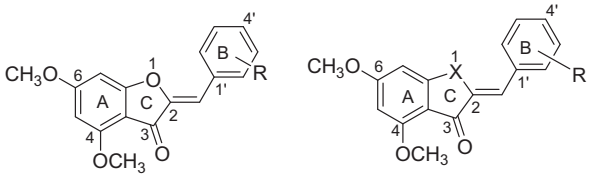

Figure 1. Structures of aurones derivatives investigated.

* Corresponding author.
E-mail address: florence.souard@ujf-grenoble.fr (F. Souard).
† Equal senior investigators.

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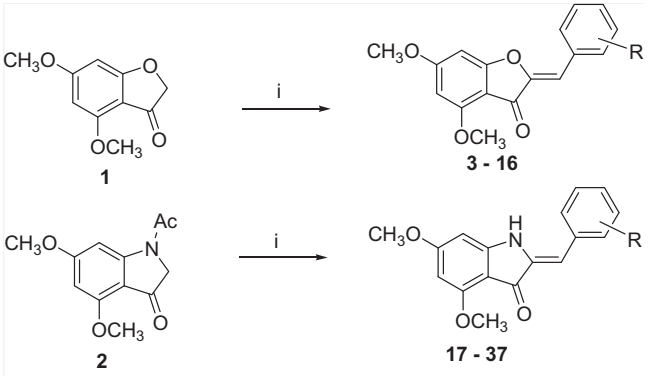
doi:10.1016/j.bmc.2010.06.008

presence of two methoxy groups at positions 4 and 6 of the A-ring was essential for the activity of aurones against the efflux pumps;[17] (b) isosteric replacement of the intracyclic oxygen by N–H; (c) introducing diverse substituents at the B-ring. The general structures of investigated compounds are shown in Figure 1.

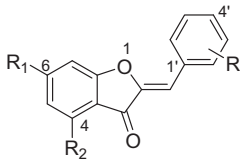
2. Chemistry

We have previously reported a general and convenient synthesis of intermediates **1** and **2** (Scheme 1).[21–23] The condensation of benzofuranone **1** and dihydroindolyl-3-one **2** with benzaldehyde derivatives in the presence of KOH in a mixture of MeOH/H_2O led to aurones and azaaurones. It should be highlighted that both series of compounds were obtained exclusively as *Z*-isomers. The configuration of the exocyclic double bond is clearly evidenced by the chemical shift in the ^{1}H NMR spectrum of the ethylenic proton. The 4,6-dihydroxyaurones were obtained by demethylation of 4,6-dimethoxyaurones with BBr_3 in CH_2Cl_2 as previously reported.[21] During the condensation step of compound **2** with benzaldehydes, the acetyl group was removed. Aurones and azaaurones obtained according to Scheme 1 are summarized in Tables 1 and 2.



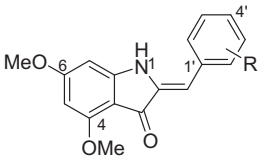
Scheme 1. Synthesis of aurones and azaaurones. Reagents and conditions: (i) KOH (50% in H_2O), MeOH, 70 °C.

Table 1
Structures and activity of aurones



Compd	R	R_1	R_2	Yield	IC_{50} (μM)
3	H	OH	OH	40	94.5
4	H	OMe	OMe	62	60.3
5	4′-Me	OH	OH	98	63.4
6	2′-Et	OMe	OMe	85	21
7	2′-Et	OH	OH	82	113.5
8	4′-Et	OH	H	60	28
9	4′-*t*Bu	OMe	OMe	78	13.3
10	4′-Bu	OMe	OMe	70	11.8
11	4′-Br	OMe	OMe	80	49.8
12	4′-F	OMe	OMe	77	86.7
13	4′-OH	OH	H	92	130
14	4′-OMe	OMe	OMe	85	11
15	4′-Ph	OMe	OMe	97	234
16	4′-Py	OMe	OMe	43	85
CQ					0.19

Table 2
Structures and activity of azaaurones



Compd	R	Yield	IC_{50} (μM)
17	4′-Br	81	49.8
18	4′-Cl	80	17
19	2′-Cl	97	9.9
20	2′,5′-Cl	58	8.4
21	2′-Cl, 6′-F	44	9
22	4′-Et	73	**1**
23	2′-Et	74	12.8
24	2′,6′-Me	25	9.1
25	2′,4′-Me	45	3.6
26	2′,4′,5′-Me	10	5.6
27	2′,3′,5′,6′-Me	18	8.9
28	4′-*i*-Pr	53	4.4
29	4′-*t*-Bu	51	7.2
30	4′-Bu	59	4.1
31	4′-CCH	82	13.4
32	2′,4′-OMe	46	5
33	2′,4′,6′-OMe	60	1.9
34	3′,4′,5′-OMe	42	1.9
35	4′-SMe	60	6.7
36	4′-Morpholino	11	8.9
37	4′-N(Me)$_2$	10	3.7
CQ			0.19

3. Results

Overall, 14 aurones and 21 azaaurones were obtained and tested in vitro for antiplasmodial activity (Tables 1 and 2). Antiplasmodial activity was first evaluated in vitro, using *P. falciparum*, the causative agent of fatal malaria. The IC_{50} (concentration giving 50% of parasite growth inhibition) was determined and compared to chloroquine, used as control drug. For all compounds, cytotoxicity was assessed by MTT assay on human cells and showed no noticeable toxicity (data not shown).

As shown in Tables 1 and 2, half of the investigated compounds were active with an IC_{50} below 10 μM, and seven of them with an IC_{50} below 5 μM. The best candidate was the 4,6-dimethoxy-4′-ethylazaaurone **22** with an IC_{50} of 1 μM. Owing to their known interactions with efflux pumps, the more active compounds (**22**, **28**, **33**, and **34**) were evaluated for their potentialization effect on **CQ** efficiency (Fig. 2). When tested on FcM29 strain (high resistance to **CQ**), a synergistic effect was induced by azaaurones **22**, **28**, and **33**. When tested on F32 strain (CQ-sensitive, *Pfcrt* wild type and PfMDR with no known mutations correlated with resistance), the combination showed only an additive effect (Fig. 2).

The most active derivative (**22**) was evaluated for in vivo efficacy. First, toxicity studies were performed on mice before evaluation of the antiplasmodial activity. Compound **22** was apparently well tolerated by animals, and after 4 days of treatment at 20 mg/kg/day, there were no signs of acute toxicity. In vivo antimalarial activity evaluation was then performed following a 4-day-suppressive test on CD female mice infected by *Plasmodium vinckei petteri*.[24]

Choloroquine **CQ** was tested at two concentrations whereas **22** was tested at three concentrations (1, 10, and 20 mg/Kg/day). The DE_{50} for the title compound was estimated to be higher than 20 mg/ml. The survival data are shown in Table 3 and Figure 3.

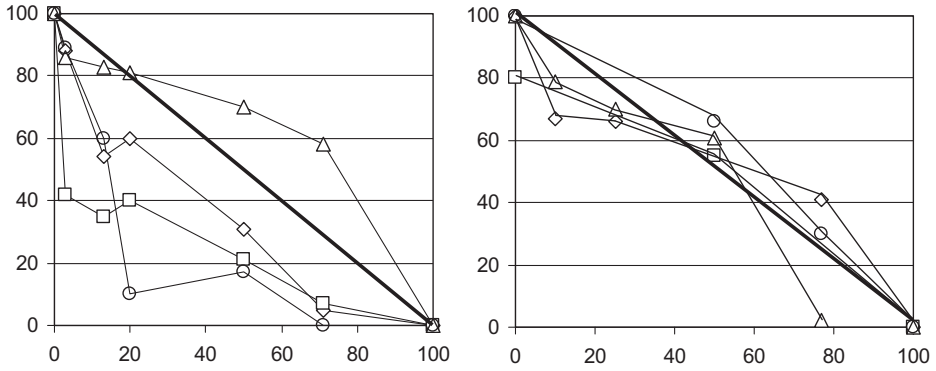


Figure 2. Combination assays with drugs and chloroquine. Left, FcM29 (CQ-resistant strain), right, F32 strain (CQ-sensitive). Except for **34**, the synergistic effect was only found back for FcM29. Data represent the combination of chloroquine (*X*-axis: percentage of the IC_{50} of **CQ**), *Y*-axis: percentage of the IC_{50} of the molecule tested (◇ molecule **22**, □ molecule **28**, ○ molecule **33**, △ molecule **34**).

Table 3
In vivo antiplasmodial activity of (**22**)

	C	CQ		22		
Dose (mg/kg/day)	0	1	10	1	10	20
Parasitaemia (D5, %)	25	38.9	0	24.2	20.8	25
Parasitaemia (SD, %)	8.5	18.4	0	7.1	6.5	16.8
Inhibition (%)	0	0	100	3	17	0
DE_{50} (mg/ml)		5		>20		

C: control, **CQ**: chloroquine, SD: standard deviation.

No differences were observed between the three doses of **22** and **CQ** at 1 mg/kg/day. Compared to **CQ** at 10 mg/kg/day, it appeared that compound **22** had only a little effect on the survival rate (Fig. 3). This low activity was seriously hampered by the low solubility of **22**. Its formulation in more soluble systems would be helpful for better bio-availability.

4. Structure–activity relationship and discussion

Our previous investigations have allowed the assessment of aurones potential in inhibiting ABC proteins, a family of proteins involved in the MDR (multidrug resistance) phenotype.[25–28] The role of protozoal ATP-binding cassette (ABC) transporters in the resistance of *P. falciparum* to antimalarial drugs has been highlighted.[29]

In the aurones series, the structure–activity relationship (Table 1) confirmed that dimethoxylation at 4,6-carbons was highly beneficial (compound **3** vs **4** and **7** vs **6**). This substitution pattern was previously found determinant for inhibiting efflux pumps implied in drug resistance. At the B-ring, the presence of a hydrophobic group was highly favorable (compound **4** vs **8**, **9**, **10**). The chain homologation from an ethyl to a butyl group led to a substantial increase in activity. Further chain elongation led to less active derivatives, probably due to their poor solubility and/or to the size of the chain. The presence of a hydrophobic halogen atom at the 4′-position was quite beneficial as illustrated by comparing derivatives **4** and **11**. These preliminary SAR results permitted us to investigate aza analogs of aurones. The substitution of the intracyclic oxygen atom with its isosteric equivalent, N–H group, globally led to more active analogs. In azaaurones, the positive effect of a hydrophobic group linked to the B-ring as well as its position was confirmed. An ethyl group at the C-4′ position was found to be the optimal substituent. When the ethyl group was placed at the C-2′ position, the compound becomes much less active (**22** vs **23**). When the ethyl group was replaced by its oxidized derivative, an acetylenyl group, the activity was substantially decreased (**22** vs **31**), pointing again to the size and/or lipophilic effects. The methoxylation pattern at the B-ring was highly favorable as shown by the low IC_{50} of derivatives **32**, **33**, and **34**. The presence of a dimethylamino moiety at the 4′-position led to potent compounds **37**. The choice of the later group was motivated by its presence in the chloroquine skeleton. Taken together, the biological results found among aurones and azaaurones point to the importance of the hydrophobicity. Concerning the study of synergy

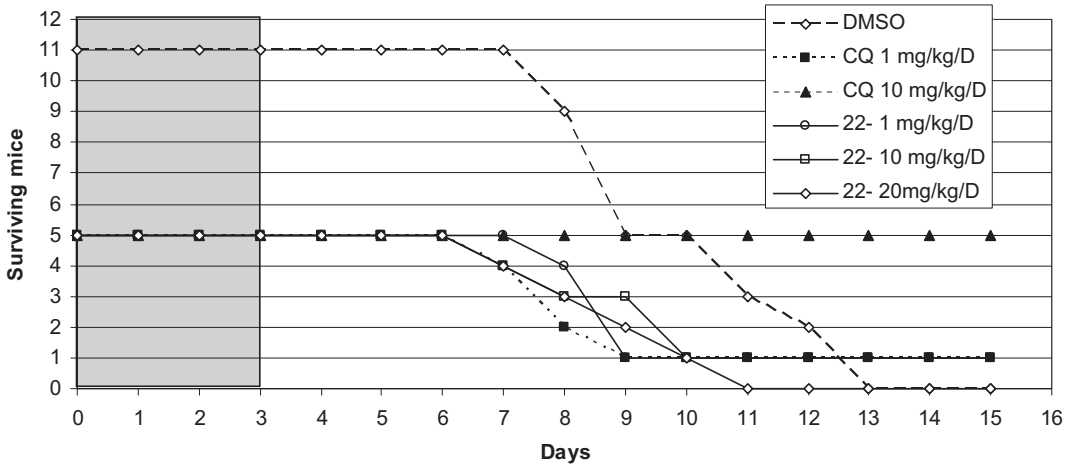


Figure 3. Representative curves of in vivo assay.

between selected azaaurones and **CQ**, it seemed that, more the substituent group was hydrophobic at the 4′-position better was the synergy (**28** vs **22**).

In conclusion, throughout the present study, we report the preliminary results regarding the structural requirements for the antiplasmodial activity of aurones. We have found that the replacement of the intracyclic oxygen by a N–H was revealed to be highly advantageous for the activity. We also optimized (at least in part) the nature of substituents to be present at the A and B-rings. The present results bring essential elements which will be used to go more straightforward for the synthesis of more active azaaurones. This investigation will be facilitated by the easy synthesis of azaaurones through the optimized method reported in this study.

5. Experimental

5.1. Chemistry

5.1.1. Materials and methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz for ^{1}H, 50 MHz for ^{13}C) and Bruker AC-400 instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C). Chemical shifts (δ) are reported in ppm relative to Me_4Si as an internal standard. EI and DCI mass spectra were recorded on a Fisons Trio 1000 instrument. Elemental analyses were performed by the Analytical Department of CNRS, Vernaison, France. IR spectra were recorded on Perkin–Elmer (spectrum one) FT-IR spectrometer. Thin-layer chromatography (TLC) was carried out using Merck Silica Gel F-254 plates (thickness 0.25 mm). Flash chromatography was carried out using Merck Silica Gel 60, 200–400 mesh. All solvents were distilled prior to use. Chemicals and reagents were obtained either from Aldrich or Acros companies and used as obtained.

5.1.2. (*Z*)-2-Benzylidene-4,6-dihydroxybenzofuran-3(2*H*)-one (3)

Yield: 40%; yellow powder; mp 252 °C; ^{1}H NMR (400 MHz, CD_3COCD_3) δ: 7.87 (m, 2H, H2′, H6′); 7.42 (m, 2H, H3′, H5′); 7.35 (m, 1H, H4′); 6.57 (s, 1H, =CH–); 6.30 (d, 1H, J = 1.6 Hz, H5); 6.09 (d, 1H, J = 1.6 Hz, H7); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 181.36, 168.74, 148.50, 132.59, 130.96, 129.31, 128.91, 109.66, 109.04, 91.87, 89.86; IR (NaCl, CH_2Cl_2): 3400 (OH), 1687 (CO), 1546 (C=C) cm^{-1}; HRMS calcd $C_{15}H_{10}O_4$: 252.05; found 253.05 [M−H]$^+$, Anal. ($C_{15}H_{10}O_4$) C, 70.85; H, 4.74.

5.1.3. (*Z*)-2-Benzylidene-4,6-dimethoxybenzofuran-3(2*H*)-one (4)

Yield: 62%; yellow powder; mp 154–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87–7.35 (m, 5H, H2′- H6′), 6.76 (s, 1H, =CH–), 6.39 (d, 1H, J = 1.5 Hz, H7), 6.14 (d, 1H, J = 1.5 Hz, H5), 3.95 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 180.73, 169.10, 169.01, 159.45, 147.88, 132.60, 131.12, 129.33, 129.75, 110.79, 105.24, 94.07, 89.26, 56.24, 56.14; IR (NaCl, CH_2Cl_2): 1694 (CO), 1590 (C=C) cm^{-1}; HRMS calcd $C_{17}H_{14}O_4$: 282.09; found 283.08 [M−H]$^+$, Anal. ($C_{17}H_{14}O_4$) C, 71.27; H, 5.06.

5.1.4. (*Z*)-4,6-Dihydroxy-2-(4-methylbenzylidene)benzofuran-3(2*H*)-one (5)

Yield: 98%; yellow powder; mp 250–252 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, 2H, J = 8 Hz, H2′, H6′), 7.18 (d, 2H, J = 8 Hz, H3′, H5′), 6.71 (s, 1H, =CH–), 6.33 (d, 1H, J = 1.6 Hz, H7), 6.08 (d, 1H, J = 2 Hz, H5), 1.50 (s, 3H, CH_3); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 180.69, 167.61, 167.31, 157.94, 147.46, 139.65, 130.97, 129.86, 129.56, 109.31, 103.26, 97.75, 91.19, 20.59; IR (NaCl, CH_2Cl_2): 3400 (OH), 1615 (CO), 1452 (C=C) cm^{-1}; HRMS calcd $C_{16}H_{12}O_4$: 268.0735; found 268.0721 [M−H]$^+$, Anal. ($C_{16}H_{12}O_4$) C, 71.59; H, 4.72.

5.1.5. (*Z*)-4,6-Dihydroxy-2-(2-ethylbenzylidene)benzofuran-3(2*H*)-one (6)

Yield: 85%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10 (m, 1H, H6′), 7.25 (m, 3H, H3′-H5′), 6.79 (s, 1H, =CH–), 6.26 (d, 1H, J = 2 Hz, H7), 6.09 (d, 1H, J = 2 Hz, H5), 3.90 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 2.80 (q, 2H, J = 7.6 Hz, CH_2), 1.18 (t, 3H, J = 7.6 Hz, CH_3); IR (NaCl, CH_2Cl_2): 1695 (CO), 1614 (C=C) cm^{-1}; HRMS calcd for $C_{19}H_{18}O_4$: 310.353; found: 311.24 [M−H]$^+$.

5.1.6. (*Z*)-4,6-Dihydroxy-2-(4-ethylbenzylidene)benzofuran-3(2*H*)-one (7)

Yield: 82%; yellow powder; mp 208 °C; ^{1}H NMR (400 MHz, CD_3COCD_3) δ: 7.80 (d, 2H, J = 8.4 Hz, H2′, H6′), 7.27 (d, 2H, J = 8.4 Hz, H3′, H5′), 6.56 (s, 1H, =CH–), 6.29 (d, 1H, J = 1.6 Hz, H7), 6.09 (d, 1H, J = 1.6 Hz, H5), 2.62 (q, 2H, J = 7.6 Hz, CH_2), 1.20 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 180.67, 167.89, 167.64, 158.12, 148.27, 144.53, 130.85, 130.11, 129.44, 129.15, 126.39, 105.57, 103.26, 97.84, 91.29, 59.64, 15.38); IR (NaCl, CH_2Cl_2): 3400 (OH), 1634 (CO), 1456 (C=C) cm^{-1}; MS (DCI) m/z 283 [M−H]$^+$.

5.1.7. (*Z*)-2-(4-Ethylbenzylidene)-6-hydroxybenzofuran-3(2*H*)-one (8)

Yield: 60%; yellow powder; mp 228 °C; ^{1}H NMR (400 MHz, CD_3COCD_3) δ: 7.83 (d, 2H, J = 8 Hz, H2′, H6′), 7.56 (d, 1H, J = 8.4 Hz, H4), 7.28 (d, 2H, J = 8 Hz, H3′, H5′), 6.78 (d, 1H, J = 2 Hz, H7), 6.72 (dd, 1H, J_1 = 2 Hz, J_2 = 8.4 Hz, H5), 6.65 (s, 1H, =CH–), 2.63 (q, 2H, J = 7.8 Hz, CH_2), 1.19 (t, J = 7.8 Hz, CH_3); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 181.77, 168.36, 165.94, 147.49, 146.24, 131.37, 130.11, 128.42, 125.73, 113.97, 112.74, 110.45, 95.47, 29.06, 14.97; IR (NaCl, CH_2Cl_2): 3400 (OH), 1694 (CO), 1615 (C=C) cm^{-1}; HRMS calcd for $C_{17}H_{14}O_3$: 266.09429; found: 266.0919 [M−H]$^+$. Anal. ($C_{17}H_{14}O_3$) C 76.51, H 5.30.

5.1.8. (*Z*)-2-(4-*tert*-Butylbenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (9)

Yield: 78%; yellow powder; mp 210–211 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (d, 2H, J = 8.4 Hz, H2′, H6′), 7.39 (d, 2H, J = 8.4 Hz, H3′, H5′), 6.72 (s, 1H, =CH–), 6.32 (d, 1H, J = 1.6 Hz, H5), 6.07 (d, 1H, J = 1.6 Hz, H7), 3.89 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 1.27 (s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 180.91, 169.18, 169.06, 159.59, 153.03, 147.80, 131.15, 129.96, 125.98, 111.10, 105.56, 94.15, 89.41, 56.39, 56.28, 35.08, 31.36; IR (NaCl, CH_2Cl_2): 1694 (CO), 1615 (C=C) cm^{-1}; HRMS calcd for $C_{21}H_{22}O_4$: 338.407; found: 339.19 [M−H]$^+$.

5.1.9. (*Z*)-2-(4-Butylbenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (10)

Yield: 70%; white powder, mp 150 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, 2H, J = 8.4 Hz, H2′, H6′), 7.26 (d, 2H, J = 8.4 Hz, H3′, H5′), 6.56 (s, 1H, =CH–), 6.31 (d, 1H, J = 2 Hz, H7), 6.11 (d, 1H, J = 2 Hz, H5), 3.89 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 2.59 (t, 2H, J = 7.2 Hz, CH_2), 1.58 (m, 2H, CH_2), 1.29 (m, 2H, CH_2), 0.88 (t, 3H, J = 7.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 180.94, 169.21, 169.07, 159.62, 147.70, 144.99, 131.36, 130.20, 129.15, 111.32, 105.60, 94.20, 89.43, 56.43, 56.32, 35.86, 33.59, 22.54, 14.14; IR (NaCl, CH_2Cl_2): 1695 (CO), 1615 (C=C) cm^{-1}; HRMS calcd for $C_{21}H_{22}O_4$: 338.407; found: 329.25 [M−H]$^+$.

5.1.10. (*Z*)-2-(4-Bromobenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (11)

Yield: 80%; yellow powder; mp 171–172 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.72 (d, 2H, J = 1.5 Hz, H2′, H6′), 7.54 (d, 2H, J = 1.5 Hz, H3′, H5′), 6.68 (s, 1H, =CH–), 6.38 (d, 1H, J = 1.7 Hz, H7), 6.14 (d, 1H, J = 1.7 Hz, H5), 3.96 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 180.49, 169.14, 169.06, 159.58, 148.11,

132.35, 131.94, 131.56, 123.59, 109.27, 105.11, 94.12, 89.34, 56.26, 56.13 IR (NaCl, CH_2Cl_2): 1697 (CO), 1594 (C=C) cm^{-1}; HRMS calcd $C_{17}H_{13}BrO_4$: 359.9997; found 361.7930 $[M-H]^+$.

5.1.11. (*Z*)-2-(4-Fluorobenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (12)

Yield: 77%; yellow powder; mp 173–174 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 7.85 (dd, 2H, J_1 = 8.7 Hz, J_2 = 5.5 Hz, H arom), 7.16 (t, 2H, J = 8.7 Hz, H arom), 6.73 (s, 1H, =CH–), 6.38 (d, 1H, J = 1.7 Hz, H7), 6.14 (d, 1H, J = 1.7 Hz, H5), 3.96 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ: 180.7 (CO), 169.1 (C-8), 169.0 (d, J = 250 Hz, C-4), 159.69, 147.66, 133.14, 129.09, 116.13, 109.79, 105.09, 94.27, 89.43, 56.31, 56.26; IR (NaCl, CH_2Cl_2): 1644 (CO) cm^{-1}; HRMS calcd $C_{17}H_{13}FO_4$: 300.0798; found 300.8880 $[M-H]^+$.

5.1.12. (*Z*)-2-(4-Hydroxybenzylidene)-6-hydroxybenzofuran-3(2*H*)-one (13)

Yield: 92%; yellow powder; mp 286–288 °C; ^1H NMR (400 MHz, DMSO-d_6): δ: 10.1 (br s, 1H, OH), 7.73 (d, 2H, J = 8.8 Hz, H2′, H6′), 7.52 (d, 1H, J = 8.8 Hz, H4), 6.81 (d, 2H, J = 8.8 Hz, H3′, H5′), 6.71 (d, 1H, J = 1.6 Hz, H7), 6.65 (s, 1H, =CH–), 6.63 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.8 Hz, H5); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 181.70, 167.97, 166.64, 159.70, 146.1, 133.72, 126.18, 123.49, 116.53, 113.61, 113.34, 111.84, 98.98, 56.2; MS (DCI: NH_3 + isobutane) m/z 255 $[M-H]^+$; Anal. ($C_{15}H_{10}O_4$) C, H.

5.1.13. (*Z*)-2-(4-Methoxybenzylidene)-6-hydroxybenzofuran-3(2*H*)-one (14)

Yield: 85%, yellow powder, mp 167–168 °C, ^1H NMR (200 MHz, DMSO-d_6): δ: 7.73 (d, 2H, J = 8.6 Hz, H3′, H3′, H5′), 6.87 (d, 2H, J = 8.6 Hz, H2′, H6′), 6.62 (s, 1H, =CH–), 6.29 (d, 1H, J = 1.7 Hz, H7), 6.05 (d, 1H, J = 1.7 Hz, H5), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 178.94, 168.74, 168.04, 160.36, 158.85, 146.13, 132.80, 124.67, 114.58, 109.86, 104.22, 94.33, 89.84, 56.52, 56.14, 55.38; IR (NaCl, CH_2Cl_2): 1638 (CO) cm^{-1}; HRMS calcd for $C_{18}H_{16}O_5$: 312.325; found: 313.22 $[M-H]^+$.

5.1.14. (*Z*)-2-(4-Phenylbenzylidene)-6-hydroxybenzofuran-3(2*H*)-one (15)

Yield: 97%, yellow powder, mp 193–195 °C, ^1H NMR (400 MHz, $CDCl_3$): δ: 7.92 (d, 2H, J = 8.7 Hz, H3′, H5′), 7.59 (m, 4H, H3′, H5′, H2″, H6″), 7.44 (t, 2H, J = 7.5 Hz, H3″, H5″), 7.35 (t, 1H, J = 7.5 Hz, H4″), 6.77 (s, 1H, =CH–), 6.39 (s, 1H, H7), 6.13 (s, 1H, H5), 3.94 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 180.73, 169.11, 159.56, 148.10, 142.01, 140.40, 131.75, 131.71, 129.04, 127.92, 127.51, 127.18, 110.56, 105.40, 94.21, 89.43, 56.35, 56.27; IR (NaCl, CH_2Cl_2): 1616 (CO) cm^{-1}; HRMS calcd $C_{23}H_{18}O_4$: 358.29; found 359.32 $[M-H]^+$.

5.1.15. (*Z*)-4,6-Dimethoxy-2-(pyridin-4-ylmethylene)benzofuran-3(2*H*)-one (16)

Yield: 43%, white powder, mp 186–188 °C, ^1H NMR (400 MHz, $CDCl_3$): δ: 8.42 (d, 2H, J = 5.2 Hz, H3′, H5′), 7.03 (d, 2H, J = 5.5 Hz, H2′, H6′), 6.63 (s, 1H, =CH–), 6.14 (d, 1H, J = 1.46 Hz, H7), 6.01 (d, 1H, J = 1.46 Hz, H5), 3.88 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.57, 168.63, 160.43, 156.71, 149.30, 135.92, 132.58, 130.59, 129.61, 127.30, 110.23, 105.09, 91.25, 88.50, 56.01, 55.94; IR (NaCl, CH_2Cl_2): 1634 (CO) cm^{-1}; HRMS calcd $C_{16}H_{13}NO_4$: 283.28; found 284.29 $[M-H]^+$.

5.1.16. (*Z*)-2-(4-Bromobenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (17)

Yield: 80%; yellow powder; mp 171–172 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.72 (d, 2H, J = 1.5 Hz, H arom), 7.54 (d, 2H, J = 8.6 Hz, H arom), 6.68 (s, 1H, =CH–), 6.38 (d, 1H, J = 1.7 Hz, H7), 6.14 (d, 1H, J = 1.7 Hz, H5), 3.96 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 180.44, 169.16, 169.01, 159.50, 148.15, 132.35, 131.90, 131.52, 123.54, 109.28, 105.15, 94.15, 89.34, 56.24, 56.19; IR (NaCl, CH_2Cl_2): 3315 (NH), 1680 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{17}H_{13}O_4Br$: 360.7935; found 361.7930 $[M-H]^+$.

5.1.17. (*Z*)-2-(4-Chlorobenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (18)

Yield: 80%; yellow powder, mp 146–148 °C, ^1H NMR (200 MHz, $CDCl_3$): δ: 7.4 (dd, 2H, J_1 = 8.5 Hz, J_2 = 0.4 Hz, H3′, H5′), 7.3 (dd, 2H, J_1 = 8.5 Hz, J_2 = 0.4 Hz, H2′, H6′), 6.6 (s, 1H, =CH–), 6.1 (d, 1H, J = 1.6 Hz, H7), 5.85 (d, 1H, J = 1.6 Hz, H5), 3.85 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 182.33, 168.89, 160.61, 156.64, 136.64, 133.87, 133.68, 130.54, 129.44, 108.36, 105.07, 91.56, 88.69, 56.06, 56.03, 27.09; IR (NaCl, CH_2Cl_2): 3400 (NH), 1624 (CO); HRMS calcd $C_{17}H_{14}O_3NCl$: 315.751; found 316.12 $[M-H]^+$.

5.1.18. (*Z*)-2-(2-Chlorobenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (19)

Yield: 97%, brown powder, mp 194–195, ^1H NMR (200 MHz, CD_3COCD_3): δ: 7.75 (m, 1H, H3′), 7.33 (m, 3H, H4′, H5′, H6′), 6.75 (s, 1H, =CH–), 6.21 (d, J = 1.6 Hz, 1H, H7), 6.03 (d, J = 1.7 Hz, 1H, H5), 3.67 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3); ^{13}C NMR (CD_3COCD_3, 50 MHz) δ: 182.00, 169.62, 161.30, 158.19, 138.42, 134.84, 134.11, 130.79, 130.65, 129.71, 128.23, 104.72, 102.76, 91.74, 89.70, 56.22, 56.05; IR (NaCl, CH_2Cl_2): 3400 (NH), 1630 (CO) cm^{-1}; HRMS calcd $C_{17}H_{14}ClNO_3$: 316.0502; found 316 $[M-H]^+$.

5.1.19. (*Z*)-2-(2,4-Dichlorobenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (20)

Yield: 58%, yellow powder, mp 235–236 °C, ^1H NMR (200 MHz, CD_3COCD_3): δ: 7.45 (m, 3H, H3′,5′,6′), 6.38 (s, 1H, =CH–), 6.08 (d, J = 1.8 Hz, 1H, H7), 6.0 (d, J = 1.9 Hz, 1H, H5), 3.88 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3); ^{13}C NMR (CD_3COCD_3, 50 MHz) δ: 181.54, 170.05, 161.44, 157.77, 139.84, 135.75, 133.56, 130.62, 129.25, 128.12, 104.33, 101.27, 91.33, 89.04, 56.20, 56.01; IR (NaCl, CH_2Cl_2): 3400 (NH), 1682 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{17}H_{13}Cl_2NO_3$: 350.20; found 350 $[M-H]^+$.

5.1.20. (*Z*)-2-(2-Chloro-6-fluorobenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (21)

Yield: 44%, yellow powder, mp 200–201 °C, ^1H NMR (200 MHz, CD_3COCD_3): δ: 7.4 (m, 2H, H3′, H4′), 7.2 (m, 1H, H4′), 6.6 (s, 1H, =CH–), 6.1 (d, J = 1.9 Hz, 1H, H7), 5.85 (d, J = 1.9 Hz, 1H, H5), 3.68 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3); ^{13}C NMR (CD_3COCD_3, 50 MHz) δ: 181.43, 169.94, 161.44, 130.77, 130.67, 126.59, 126.56, 115.74, 115.52, 96.82, 91.55, 89.40, 56.27, 56.11; IR (NaCl, CH_2Cl_2): 3400 (NH), 1630 (CO) cm^{-1}; HRMS calcd $C_{17}H_{13}ClFNO_3$: 333.74; found 334 $[M-H]^+$.

5.1.21. (*Z*)-2-(4-Ethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (22)

Yield: 70%; yellow powder; mp 215–217 °C ^1H NMR (200 MHz, $CDCl_3$) δ: 7.45 (dd, 2H, J_1 = 8.2 Hz, J_2 = 0.4 Hz, H2′, H6′), 7.25 (dd, 2H, J_1 = 8.2 Hz, J_2 = 0.4 Hz, H3′, H5′), 7.1 (br s, 1H), 6.7 (s, 1H), 6.1 (d, 1H, J = 1.8 Hz, H7), 5.9 (d, J = 1.8 Hz, 1H, H5), 3.9 (s, 3H), 3.8 (s, 3H), 2.7 (q, 2H, J = 6 Hz, $-CH_2$), 1.3 (t, J = 6 Hz, 3H, CH_3); ^{13}C NMR (CD_3COCD_3, 50 MHz) δ: 182.89, 169.88, 161.74, 158.71, 145.59, 137.21, 134.12, 130.91, 129.89, 108.70, 105.62, 92.23, 90.13, 56.77, 56.60, 16.56; IR (NaCl, CH_2Cl_2): 3247 (NH), 1672 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{19}H_{19}NO_3$: 309.359; found 3310.43 $[M-H]^+$.

5.1.22. (*Z*)-2-(2-Ethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (23)

Yield: 74%; yellow powder; mp 197–198 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51–7.49 (m, H6), 7.25 (br s, 3H, H3′, H4′, H5′), 6.90 (s,1H, =CH–), 6.78 (br s, 1H, NH), 6.00 (s, 1H, H7), 5.92 (s, 1H, H5), 3.94 (s, 3H, OCH_3), 2.79–2.73 (q, J = 7.28 Hz, 2H, CH_2), 1.22–1.18 (t, J = 7.52 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 183.58, 170.14, 162.06, 158.04, 145.81, 138.58, 134.67, 130.76, 129.95, 129.83, 127.77, 109.11, 106.66, 92.53, 89.58, 57.38, 57.34, 28.22, 16.04; IR (NaCl, CH_2Cl_2): 3269 (NH), 1675 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{19}H_{19}O_3N$: 309.37; found 310.21 $[M-H]^+$.

5.1.23. (*Z*)-2-(2,6-Dimethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (24)

Yield: 25%; yellow powder; mp 217–218 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.31–7.03 (m, J = 7.62 Hz, 4H, H3′, H4′, H5′, NH), 6.84 (s, 1H, =CH–), 6.03 (s, 1H, H7), 5.92 (s, 1H, H5), 3.93 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 2.37 (s, 3H, CH_3), 2.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 183.49, 170.05, 162.09, 157.90, 138.21, 137.24, 136.81, 135.27, 132.35, 130.49, 130.05, 109.52, 106.79, 92.53, 89.56, 57.47, 57.35, 22.69, 21.19; IR (NaCl, CH_2Cl_2): 3400 (NH), 1592 (CO) cm^{-1}; HRMS calcd $C_{19}H_{19}O_3N$: 309.37; found 310.23 $[M-H]^+$.

5.1.24. (*Z*)-2-(2,4-Dimethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (25)

Yield: 45%, brown powder; mp 235 °C; ^{1}H NMR (200 MHz, CD_3COCD_3): δ: 7.5 (m, 1H, H2′), 7.06 (m, 2H, H4′, H6′), 6.6 (s, 1H, =CH–), 6.2 (d, J = 1.8 Hz, 1H, H7), 5.99 (d, J = 1.78 Hz, 1H, H5), 3.67 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ: 182.07, 169.26, 161.19, 158.18, 138.42, 138.36, 137.22, 132.19, 131.81, 129.26, 127.79, 105.56, 105.11, 91.47, 89.39, 56.18, 56.02, 21.28, 20.13.; IR (NaCl, CH_2Cl_2): 3201 (NH), 1668 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{19}H_{19}O_3N$: 309.37; found 310.23 $[M-H]^+$.

5.1.25. (*Z*)-2-(2,4,5-Trimethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (26)

Yield: 10%; yellow powder; mp 229 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27 (s, 1H, H6′), 7.03 (s, 1H, H3′) 6.84 (br s, 2H, NH, =CH–), 6.04–6.03 (d, J = 1.61 Hz, 1H, H7), 5.91 (d, J = 1.58 Hz, 1H, H5), 3.92 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.25 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 183.56, 169.95, 162.03, 157.89, 138.46, 137.83, 132.29, 135.87, 13.86, 132.73, 130.62, 109.71, 106.84, 92.56, 89.55, 57.41–57.34, 21.13; IR (NaCl, CH_2Cl_2): 3400 (NH), 1671 (CO), 1592 (C=C) cm^{-1}; HRMS calcd $C_{20}H_{21}O_3N$: 323.40; found 324.19 $[M-H]^+$.

5.1.26. (*Z*)-2-(2,3,5,6-Tetramethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (27)

Yield: 18%; yellow powder; mp 260 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.97 (s, 1H, H4′), 6.85 (s, 1H, =CH–), 5.89 (d, J = 1.66 Hz, 1H, H7), 5.87 (d, J = 1.69 Hz, 1H, H5), 3.93 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.25 (s, 6H, 2 CH_3), 2.15 (s, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.79, 170.18, 162.10, 157.57, 139.32, 135.63, 134.55, 134.02, 132.65, 111.82, 92.24, 89.08, 57.43, 57.29, 21.64, 18.33; IR (NaCl, CH_2Cl_2): 3338 (NH), 1682 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{21}H_{23}O_3N$: 337.41; found 338.23 $[M-H]^+$.

5.1.27. (*Z*)-2-(4-Isopropylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (28)

Yield: 53%; yellow powder; mp 252 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47 (d, 2H, J = 4 Hz, H2′, H6′), 7.31 (d, 1H, J = 4 Hz, H3′, H5′), 6,86 (br s, 1H, NH), 6.77 (s, 1H, =CH–), 6.07 (d, 1H, J = 1,6 Hz, H5), 5.94 (d, 1H, J = 2 Hz, H7), 3.94 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.01 (m, 1H, $CH(CH_3)_2$), 1.28 (d, 6H, J = 6,8 Hz, $(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 179.94, 166.27, 158.10, 154.23, 147.01, 133.50, 130.23, 127.22, 124.97, 107.99, 102.78, 88.95, 86.07, 53.88, 53.62, 31.69, 21.58, 21.48; IR (NaCl, CH_2Cl_2): 3294 (NH), 1677 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{20}H_{21}O_3N$: 323.5857; found 323.6541 $[M-H]^+$.

5.1.28. (*Z*)-2-(4-*tert*-Buthylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (29)

Yield: 51%; brown powder; mp 196–197 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.46 (br s, 2H, H2′, H6′), 7.25 (br s, 2H, H3′, H5′), 6.84 (br s,1H, NH), 6.77 (s, 1H, =CH–), 6.06 (d, J = 1.69 Hz, 1H, H7), 5.94 (d, J = 1.67 Hz, 1H, H5), 3.94 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 1.37 (br s, 9H, -$C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 184.07, 170.04, 161.92, 158.12, 152.94, 137.50, 133.69, 130.70, 127.57, 111.34, 106.59, 92.67, 89.88, 57.32, 36.30, 32.73; IR (NaCl, CH_2Cl_2): 3416 (NH), 1678 (CO), 1613 (C=C) cm^{-1}; HRMS calcd $C_{21}H_{23}O_3N$: 337.42; found 336.22 $[M-H]^+$.

5.1.29. (*Z*)-2-(4-Buthylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (30)

Yield: 59%; yellow powder; mp 90–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44 (d, J = 8.2 Hz, 2H, H2′, H6′), 7.27 (d, J = 8.2 Hz, 2H, H3′, H5′), 6.8 (br s, 1H, NH), 6.75 (s, 1H, =CH–), 6.07 (d, J = 1.69 Hz, 1H, H7), 5.93 (d, J = 1.72 Hz, 1H, H5), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 2.63 (t, J = 7.5 Hz, 2H, CH_2-Ph), 1.63 (br s, 4H, CH_2–CH_2), 0.94 (t, J = 7.5 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.02, 161.91, 158.5, 144.86, 137.37, 133.89, 130.85, 111.56, 106.62, 92.69, 89.85, 57.33, 37.06, 35.01, 90.3, 23.91, 15.51; IR (NaCl, CH_2Cl_2): 3402 (NH), 1677 (CO), 1614 (C=C) cm^{-1}; HRMS calcd $C_{21}H_{23}O_3N$: 337.42; found 338.22 $[M-H]^+$.

5.1.30. (*Z*)-2-(4-Ethynylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (31)

Yield: 82%; yellow powder; mp 135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46 (d, 2H, J = 8.4 Hz, H3′, H5′), 7.39 (d, 2H, J = 8.4 Hz, H2′, H6′), 6.80 (br s, 1H, NH), 6.62 (s, 1H, =CH–), 6.01 (d, 1H, J = 2 Hz, H7), 5.88 (d, 1H, J = 1,6 Hz, H5), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.11 (s, 1H, CH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.19, 168.75, 140.47, 156.50, 136.50, 133.73, 133.54, 130.40, 129.31, 108.21, 104.93, 91.52, 88.55, 77.27, 55.92, 55.88, 26.95; IR (NaCl, CH_2Cl_2): 3284 (NH), 1677 (CO), 1612 (C=C) cm^{-1}; HRMS calcd $C_{19}H_{15}O_3N$: 305.327; found 306.241 $[M-H]^+$.

5.1.31. (*Z*)-2-(2,4-Dimethoxybenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (32)

Yield: 46%, orange powder, 214–216 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42–7.39 (d, 1H, J = 8.42, H6′), 6.82 (s, 1H, H3′), 6.56 (d, 1H, J = 8.37 Hz, H5′), 6.51 (s, 1H, =CH–), 6.01 (s, 1H, H7), 5.89 (s, H, H5), 3.92 (s, 6H, 2 OCH_3), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 183.56, 169.95, 162.03, 157.89, 138.46, 137.83, 132.29, 135.87, 13.86, 132.73, 130.62, 109.71, 106.84, 92.56, 89.55, 57.41, 57.34, 21.13; IR (NaCl, CH_2Cl_2): 3400 (NH), 1650 (CO), 1591 (C=C) cm^{-1}; HRMS calcd $C_{19}H_{19}O_5N$: 341,369; found 342.42 $[M-H]^+$.

5.1.32. (*Z*)-2-(2,4,6-Trimethoxybenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (33)

Yield: 60%; yellow powder; mp 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.29 (br s, 1H, NH), 6.98 (s, 1H, =CH–), 6.19 (br s, 2H, H3′, H5′), 5.97 (d, 1H, J = 1.51 Hz, H7), 5.86 (d, 1H, J = 1.47 Hz, 1H, H5), 3.91 (s, 3H, OCH_3), 3.89 (s, 6H, 2 OCH_3), 3.85 (s, 6H, 2 OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.34, 168.20, 162.09, 160.48, 159.32, 155.44, 135.60, 106.88, 105.31, 102.19, 92.04, 90.52, 87.86, 56.61, 56.00, 55.83, 55.64; IR (NaCl, CH_2Cl_2): 3390 (NH), 1671 (CO),

1621 (C=C) cm^{-1}; HRMS calcd $C_{20}H_{21}O_6N$: 371.396; found 372.06 $[M-H]^+$.

5.1.33. (*Z*)-2-(3,4,5-Trimethoxybenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (34)

Yield: 42%, brown powder, mp 200 – 201 °C; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.61 (br s, 1H, NH), 6.95 (s, 1H, =CH–), 6.54 (s, 2H, H2′, H6′), 6.01 (s, 1H, H7), 5.86 (s, 1H, H5), 3.91 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 183.74, 169.85, 161.83, 157.19, 153.22, 151.96, 145.46, 136.72, 117.82, 116.32, 108.11, 106.51, 100.63, 92.20, 89.50, 59.36, 58.13, 57.67, 57.35, 57.29; IR (NaCl, CH_2Cl_2): 3400 (NH), 1603 (C=C) cm^{-1}; HRMS calcd $C_{20}H_{21}O_6N$: 371.396; found 372.10 $[M-H]^+$.

5.1.34. (*Z*)-2-(4-Thiomethylbenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (35)

Yield: 60%, yellow powder, mp 228–230 °C; ^1H NMR (200 MHz, $CDCl_3$) δ: 7.4 (dd, 2H, J_1 = 8.5 Hz, J_2 = 0.4 Hz, H2′, H6′), 7.3 (dd, 2H, J_1 = 8.5 Hz, J_2 = 0.4 Hz, H3′, H5′), 6.8 (s, 1H, =CH–), 6.7 (s, 1H, NH), 6.1 (d, 1H, J = 1.6 Hz, H7), 5.9 (d, 1H, J = 1.6 Hz, H5), 3.9 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 2.5 (s, 3H, SCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.50, 168.73, 160.46, 156.65, 139.30, 135.97, 131.62, 130.59, 129.88, 126.56, 125.05, 109.80, 105.10, 91.40, 88.62, 77.45, 55.98, 15.50; IR (NaCl, CH_2Cl_2): 3400 (NH), 1625 (CO) cm^{-1}; HRMS calcd $C_{18}H_{17}O_3NS$: 327.399; found 328.23 $[M-H]^+$.

5.1.35. (*Z*)-2-(4-Morpholinobenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (36)

Yield: 11%; yellow powder; mp 257 °C; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.46–7.44 (d, 2H, J = 8.7 Hz, H2′, H6′), 6.93 (d, 2H, J = 8.6 Hz, H3′, H5′), 6.82 (br s, 1H, NH), 6.74 (s, 1H, =CH–), 6.08 (d, 1H, J = 1.37 Hz, H7), 5.93 (d, 1H, J = 1.37 Hz, H5), 3.93 (s, 4H, 2 OCH_2), 3.89 (br s, 6H, 2 OCH_3), 3.24 (m, 4H, J = 4.46 Hz, 2 CH_2N); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 183.83, 169.71, 161.88, 157.66, 152.27, 136.30, 132.25, 127.63, 116.89, 112.05, 92.70, 89.82, 68.27, 57.32, 49.91; IR (NaCl, CH_2Cl_2): 3417 (NH), 1662 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{20}H_{20}O_4N_2$: 366.16; found 367.18 $[M-H]^+$.

5.1.36. (*Z*)-2-(4-(Dimethylamino)benzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one (37)

Yield: 10%; red powder; mp 170 °C; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, 2H, J = 8.62 Hz, H2′, H6′), 6.33 (br s, 1H, NH), 6.78 (s, 1H, =CH–), 6.75 (d, 2H, J = 1.42 Hz, H3′, H5′), 6.08 (d, J = 1.51 Hz, 1H, H7), 5.94 (d, J = 1.72 Hz, 1H, H5), 3.93 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.03 (s, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.44, 161.80, 157.42, 151.68, 135.38, 132.50, 113.97, 113.36, 112.57, 107.25, 92.62, 89.70, 57.41, 57.29, 41.77, 41.67; IR (NaCl, CH_2Cl_2): 3400 (Harom), 1629 (CO), 1615 (C=C) cm^{-1}; HRMS calcd $C_{19}H_{20}O_3N_2$: 324.39; found 325.19 $[M-H]^+$.

5.2. Biology

5.2.1. Antiplasmodial activity

The latter was evaluated in vitro, using *P. falciparum*. Blood stages (FcB1-Columbia, FcM29 and F32) were cultured in human erythrocytes maintained in RPMI 1640 supplemented with 5% human serum according to the method described by Trager and Jensen[30] with modifications described by Valentin et al.[31] The cultures were synchronized every 48 h by 5% D-sorbitol lysis (Merck, Darmstadt, Germany) in order to discard old stage parasite. In vitro antimalarial activity testing was performed by following $[^3H]$-hypoxanthine (Perkin–Elmer, France) incorporation.[32] The IC_{50} values were graphically determined from inhibition versus concentration curves as described elsewhere.[33] Combination effect of the drugs was analyzed by combining drugs at sub-inhibitory concentration (Fractional IC_{50} FIC) on either FcM29, (CQ-resistant strain) or F32-Tanzania (CQ-sensitive strain) as described by Garavito et al.[34] Isobolograms representing the combination activities were plotted with an *X*-axis representing the fractional IC_{50} of **CQ** and the *Y*-axis representing the fractional IC_{50} of the tested drug.

5.2.2. In vivo antiplasmodial activity

In vivo effect was evaluated following a 4-day-suppressive assay performed on CD female mice, using *P. vinckei petteri*. Mice (mean body weight: 20 ± 2 g) were infected with 10^6 infected red blood cells in RPMI at day 0. Groups of five mice were treated intraperitoneally from day 0–3 with doses (1, 10, 20 mg/kg/day) with the most efficient drug, compound **22**. On day 4, Giemsa stained smears were made for each mouse after tail blood sampling. Parasitaemia was estimated by visual counting of at least 5000 erythrocytes. Controls were mice treated either with RPMI alone or with chloroquine (1 and 10 mg/kg). The inhibition percentage was calculated with the following formula: (control parasitaemia − parasitaemia with drugs)/(control parasitaemia) × 100.[35] Mice were maintained under institutional animal guidelines. Mice were then followed for 15 days after inoculation in order to evaluate survival time of treated animals.

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