European Journal of Medicinal Chemistry 80 (2014) 523–534

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Probing the aurone scaffold against *Plasmodium falciparum*: Design, synthesis and antimalarial activity

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ARTICLE INFO

Article history:
Received 9 November 2013
Received in revised form
24 April 2014
Accepted 25 April 2014
Available online 28 April 2014

Keywords:
Malaria
Plasmodium falciparum
Aurones
Cross-coupling reactions

ABSTRACT

A library comprising 44 diversely substituted aurones derivatives was synthesized by straightforward aldol condensation reactions of benzofuranones and the appropriately substituted benzaldehydes. Microwave enhanced synthesis using palladium catalyzed protocols was introduced as a powerful strategy for extending the chemical space around the aurone scaffold. Additionally, Mannich-base derivatives, containing a 7-aminomethyl-6-hydroxy substitution pattern at ring A, were also prepared. Screening against the chloroquine resistant *Plasmodium falciparum* W2 strain identified novel aurones with IC_{50} values in the low micromolar range. The most potent compounds contained a basic moiety, with the ability to accumulate in acidic digestive vacuole of the malaria parasite. However, none of those aurones revealed significant activity against hemozoin formation and falcipain-2, two validated targets expressed during the blood stage of *P. falciparum* infection and functional in digestive vacuole of the parasite. Overall, this study highlight (i) the usefulness of aurones as platforms for synthetic procedures using palladium catalyzed protocols to rapidly deliver lead compounds for further optimization and (ii) the potential of novel aurone derivatives as promising antimalarial compounds.

1. Introduction

Malaria remains a critical global health problem, with terrible social and economic consequences in countries where this disease is endemic. The problem is exacerbated by the emergence and spread of parasites that are resistant to well-established antimalarial drugs [1]. For that reason, development of new, effective, nontoxic, and affordable antimalarial drugs is a high priority in medicinal chemistry [2,3]. To achieve this goal, the most important strategies include not only rational drug design but also exploitation of natural products either as a source of antiparasitic agents or as an inspiration to design novel molecular scaffolds [4,5]. Therefore, the evaluation of the antiparasitic activity of plant extracts or compounds isolated from natural sources represents a valuable approach for the design and development of new drugs.

Aurones, 2-benzylidenebenzofuran-3-(2*H*)-ones (Fig. 1), are structural isomers of flavones that contain an exocyclic carbon–carbon double bond bridging the benzofuranone and phenyl rings [6,7]. The therapeutic potential of aurones has been highlighted with recent studies that revealed their anticancer [8–13], antimicrobial [14], antiparasitic [15–18], antiviral [19], and anti-inflammatory [14,20] activities. In addition, aurones can act as modulators of ABC drug transporters [21–25] and present inhibitory activity against acetylcholinesterase [26] and MAO-B [27].

The antimalarial potential of aurones was first reported by Kayser et al. [16]. Moreover, several naturally occurring aurones were synthesized and tested for their ability to inhibit erythrocytic stages of *Plasmodium falciparum*. Some of these compounds exhibited antiplasmodial activity in the micromolar range, and they showed no cytotoxicity against mammalian tumor cell lines. More recently, Souard et al. [18] showed that a series of synthetic aurones

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http://dx.doi.org/10.1016/j.ejmech.2014.04.076
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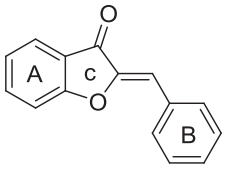
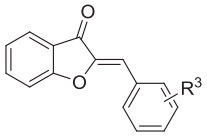


Fig. 1. General structure of aurones.

exhibited IC_{50} values under 5 μM against *P. falciparum* without toxicity against human cells.

Palladium-catalyzed reactions provide an excellent platform for compound library extension due to the wide range of transformations mediated by palladium [28]. Most of the aurone-based libraries already screened against *P. falciparum* were inspired by naturally occurring aurones, typically containing hydroxy, methoxy or acetoxy groups at different positions of this scaffold. Herein we present the synthesis and screening of a library of novel aurones, **1**–**7** (Tables 1–4), designed to probe the chemical space around this scaffold. Many of the target compounds were obtained through Suzuki–Miyaura and Buchwald–Hartwig cross-coupling reactions. Moreover, taking into account recent data suggesting antimalarial activity of aurone-Mannich base derivatives [29], some compounds were also obtained via the introduction of different aliphatic amines in the benzofuranone ring. All compounds were evaluated for their activity against a chloroquine-resistant strain of *P. falciparum* and for their cytotoxicity against human cancer cell lines. To gain insights into the mechanism of action of this class of compounds, additional studies were performed to evaluate the ability of selected aurones to inhibit hemozoin formation and falcipain-2, a cysteine protease from *P. falciparum*, and to address the possibility of synergism when combined with chloroquine.

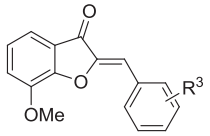
Table 1
Structure, clog*P* values and yields for the synthesis (final step) of aurones **1**–**3**.



Compound	R^3	cLog*P*[a]	Yield %
1a	H	3.39	41
1b	2-Br	4.12	55
1c	3-Br	4.13	54
1d	4-Br	4.12	56
1e	2,4-F	3.72	30
1f	2,4-Cl	4.60	81
1g	3,4,5-OMe	3.01	64
1h	4-NMe_2	3.51	49
2a	4-Ph	5.06	77
2b	4-(C_6H_4-4′-F)	5.21	66
2c	4-(C_6H_4-4′-Cl)	5.65	64
2d	4-(C_6H_4-4′-CHO)	4.70	83
2e	4-Bn	5.24	70
2f	4-(3′-Quinoline)	5.16	87
2g	4-[5′-(Pyridin-2′-NH_2)]	3.69	98
2h	4-(NHPh)	4.83	71
2i	4-(NHBn)	4.62	49
2j	4-(OC_6H_4-4′-Me)	5.40	33
2k	4-(OC_6H_4-4′-Cl)	5.51	51
3a	3-Ph	5.05	71
3b	3-(C_6H_4-4′-F)	5.21	65
3c	3-(C_6H_4-4′-Cl)	5.65	51
3d	3-(C_6H_4-4′-CHO)	4.70	63
3e	3-Bn	5.24	49
3f	3-(3′-Quinoline)	5.16	68
3g	3-[5′-(Pyridin-2′-NH_2)]	3.69	76

[a] clog*P* values calculated using the AlogPS 2.1 software (http://www.vcclab.org/lab/alogps/); reference [56].

Table 2
Structure, clog*P* values and yields for the synthesis (final step) of aurones **4**.



Compound	R^3	cLog*P*[a]	Yield %
4a	H	3.09	62
4b	2,4-F	3.62	95
4c	2,4-Cl	4.55	59
4d	3,4,5-OMe	2.93	99

[a] clog*P* values calculated using the AlogPS 2.1 software (http://www.vcclab.org/lab/alogps/); reference [56].

2. Results and discussion

2.1. Chemistry

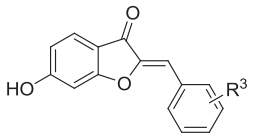
A total of 44 aurone derivatives with a wide range of substituents in ring A and B were synthesized to explore the potential of this scaffold as a platform to design new antimalarial agents (Tables 1–4).

Compounds **1a**–**h**, **2j**–**k** and **4a**–**d** were synthesized from commercially available benzofuran-3(2*H*)-one, **8**, or 7-methoxy-3(2*H*)-benzofuranone, **9**, which were reacted with appropriately substituted benzaldehydes in the presence of neutral alumina [30], as shown in Scheme 1a.

However, this method failed to provide the 6-hydroxy aurones **5a**–**g** in reasonable yields. In contrast, base-catalyzed aldol condensation of 6-hydroxy-3(2*H*)-benzofuranone, **10**, with benzaldehydes afforded aurones **5a**–**d** in good yield (Scheme 1A), while **5e**–**g** were obtained under acidic conditions, in the presence of glacial acetic acid and hydrochloric acid. The aldehydes required for aurones **5f**–**g** were synthesized from 4-fluorobenzonitrile and the appropriate phenol, and via the Weinreb amides **13a,b**, as described in Scheme 1B.

Compounds **2a**–**g**, **3a**–**g** and **6a**–**c** were obtained through a standard Suzuki–Miyaura cross-coupling reaction in the presence

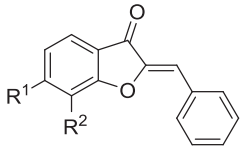
Table 3
Structure, clog*P* values and yields for the synthesis (final step) of aurones **5**.

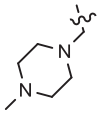
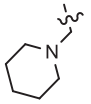
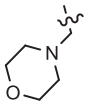
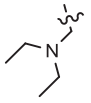


Compound	R^3	cLog*P*[a]	Yield %
5a	H	3.02	99
5b	2,4-F	3.35	98
5c	2,4-Cl	4.25	99
5d	3,4,5-OMe	2.60	98
5e	NMe_2	3.17	71
5f	4-(OC_6H_4-4′-Me)	5.27	51
5g	4-(OC_6H_4-4′-Cl)	5.13	84

[a] clog*P* values calculated using the AlogPS 2.1 software (http://www.vcclab.org/lab/alogps/); reference [56].

Table 4
Structure, clog*P* values and yields for the synthesis (final step) of aurones **6**–**7**.



Compound	R^1	R^2	cLog*P*[a]	Yield %
6a	Ph	H	5.24	78
6b	C_6H_4-4′-F	H	5.05	66
6c	5′-(Pyridin-2′-NH_2)	H	5.20	99
7a	OH		2.55	61
7b	OH		3.73	63
7c	OH		2.47	54
7d	OH		3.55	29

[a] clog*P* values calculated using the AlogPS 2.1 software (http://www.vcclab.org/lab/alogps/); reference [56].

of $Pd(PPh_3)_2Cl_2$ as catalyst, as described by Liu et al. [31] (Scheme 2). Performing the cross-coupling reactions on starting materials **1d** and **1c** afforded aurones **2a**–**g** and **3a**–**g**, respectively, in moderate to very good yields. The synthesis of compounds **6a**–**c** was accomplished through the Suzuki–Miyaura cross-coupling reaction of triflate **15**, obtained from the starting material **10**. Aurones **2h** and **2i** were prepared via the Buchwald–Hartwig cross-coupling reaction following the Fitzmaurice et al. procedure [28]. Starting material **1d** was reacted with the appropriate amine in the presence of $Pd_2(dba)_3$, (*R*)-BINAP, and NaO^tBu in microwave conditions to afford **2h** and **2i** were in moderate yields (Scheme 2).

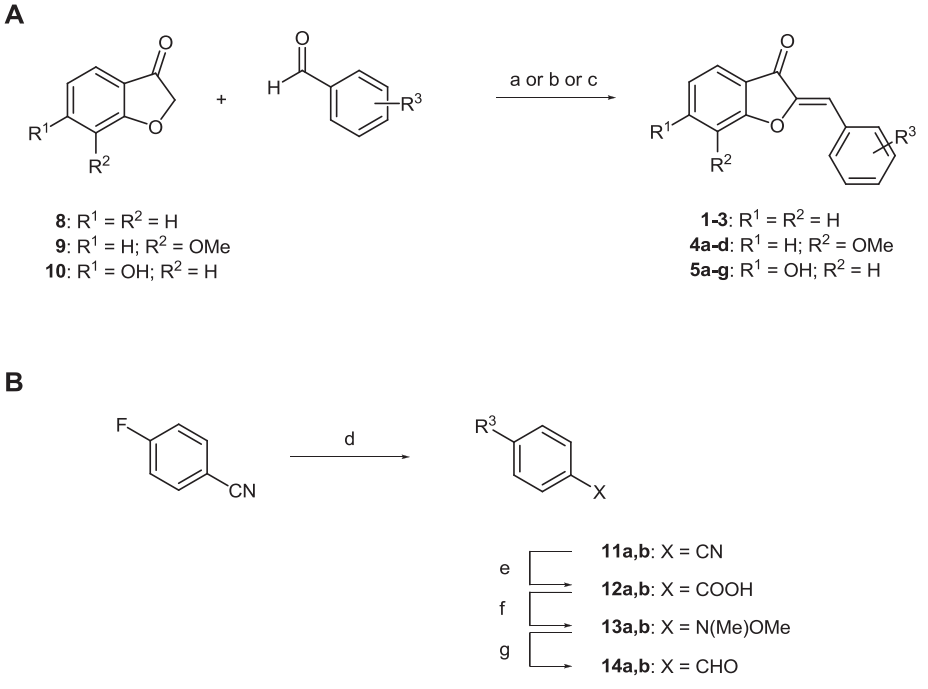
Finally, the Mannich-base derivatives **7a**–**d** were synthesized in moderate yields by reacting the starting material **5a** with the appropriate aliphatic secondary amine in aqueous formaldehyde (Scheme 3).

The stereochemistry of the carbon–carbon double bond in aurones **1**–**7** was assigned as having the (*Z*)-configuration based mainly on ^{13}C chemical shifts for the exocyclic (β) carbon atom. The ^{13}C NMR data obtained for aurones reveal that the chemical shift for the exocyclic carbon range from 108 to 112 ppm, in line with the values reported for (*Z*)-aurones [32,33], the thermodynamically more stable isomer [34]. In contrast, the ^{13}C chemical shifts for the exocyclic carbon in (*E*)-aurones are usually observed at significantly higher frequency (*ca.* 120–130 ppm) [17]. Information from the ^1H NMR spectra was less informative, as the ^1H chemical shifts for the β-hydrogen atoms in most of aurones **1**–**7** ranged from 6.7 to 7.1 ppm, consistent with reported values for (*Z*)-aurones (ca. 6.7 ppm) and (*E*)-aurones (ca. 7.2 ppm) [32–33]. This ambiguity in ^1H chemical shifts values might be ascribed to the electronic properties of substituents at ring B [18].

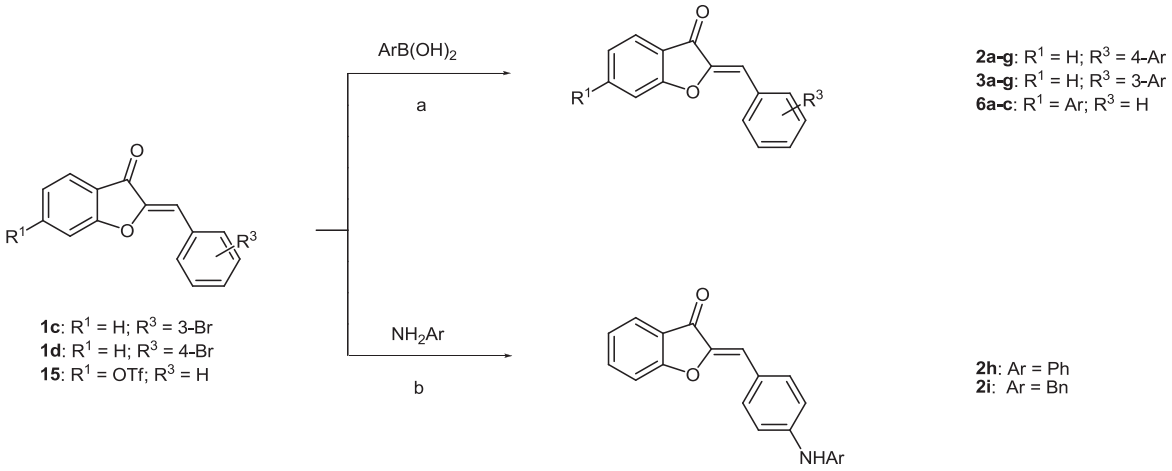
2.2. Biological evaluation

2.2.1. Activity against *P. falciparum*

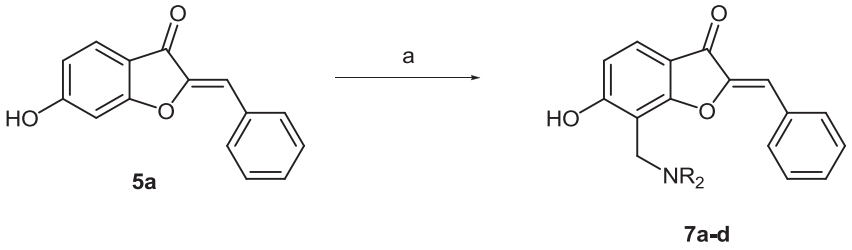
All compounds were assayed for their antiplasmodial activity against the chloroquine-resistant *P. falciparum* W2 strain and for their toxicity against Human Embryonic Kidney 293T cells. The data presented in Table 5 reveals that 21 aurones displayed relevant antiplasmodial activity, with IC_{50} values ranging from 1.2 to 9.9 μM. In addition, aurones **1**–**7** presented negligible cytotoxicity, with



Scheme 1. Reagents and conditions: (a) **8** or **9**, Al_2O_3, MeOH, reflux under N_2, 24 h; (b) **10**, NaOH (1 M), H_2O, RT, 4 h (for **5a** to **5d**); (c) **10**, glacial AcOH, HCl (cat), RT, 4 h (for **5e** to **5g**), (d) substituted phenol, Na_2CO_3, dry DMF, reflux, 24 h; (e) H_2O_2 (30%), KOH, MeOH, EtOH, reflux, 5 h; (f) *N*,*O*-dimethylhydroxylamine, TEA, TBTU, dry DMF, RT, overnight; (g) $LiAlH_4$, dry THF, −5 °C. R^3 is according to Tables 1–3.



Scheme 2. Reactions and conditions: (a) **1c**, **1d**, or **15**, $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (1 M), 1,4-dioxane, 100 °C, 3 h; (b) **1d**, $Pd_2(dba)_3$, (*R*)-BINAP, NaO^{t}Bu, dry toluene, 100 °C, 15 min, MW. R^3 is according to Tables 1–3.



Scheme 3. Reactions and conditions: (a) aliphatic amine, formaldehyde solution, EtOH, reflux, 5 h.

EC_{50} values against cultured human cells ranging from 68 to ≥100 μM. In general, most of the compounds presented selectivity indices (SI = EC_{50}(HEK293T)/IC_{50}(W2)) higher than 10, indicating that aurones are selective and nontoxic antiplasmodial agents.

Inspection of the data in Table 5 shows that aurones containing basic moieties, specifically 3-(2′-amino)pyridine (**2g**, **3g**, **6c**), 3-quinoline (**2f**, **3f**), and Mannich-bases, (**7a**–**d**) generally present improved antiplasmodial activity when compared to their non-basic counterparts. For example, the Mannich-base derivatives, **7a**–**d**, are significantly more active than their parent compound **5a**, an observation in line to that reported for similar Mannich-bases in clinical use such as amodiaquine and pyronaridine [35]. The basic moiety can exert its beneficial effect on activity independently of its localization in the aurone scaffold. For example, compound **6c**, which contains a 2-aminopyridine moiety in ring A, is equipotent to its counterparts **2g** and **3g**, which contain the basic moiety at C-4 and C-3 of ring B, respectively. A similar effect was reported for chalcones, where substitution with a quinoline moiety at ring A or ring B provided compounds with antiplasmodial activity in the low micromolar range [36].

Aurones containing a second aromatic or benzyl moiety at C-3 of ring B were shown to be more potent than their C-4 substituted counterparts (compounds **3a**, **3b** and **3e** versus **2a**, **2b** and **2e**, respectively). Replacement of the benzyl group at ring B by its anilino or phenoxy isosters had a detrimental effect on antiplasmodial activity (**2e** versus **2h** and **2j**,**k**). The same trend in activity was observed when the benzylamine moiety at C-4 of ring B was replaced by its aniline counterpart (**2i** versus **2h**). In general, derivatives containing an hydroxyl or methoxy group at ring A were poorly active, independently of substituents at ring B, with exception of the 7-methoxy derivative **4c**, which presented an IC_{50} value of 4.8 μM.

Overall, these results indicate that introduction of a basic moiety to the aurone scaffold can improve antiplasmodial activity. Aurone **7b** emerged as the most promising compound, with an IC_{50} value of 1.2 μM and an excellent selectivity index SI > 85. Since insight into the mechanism of action is helpful for drug development, we screened aurones against two validated targets expressed during the blood stage of *P. falciparum* infection: hemozoin formation and falcipain-2.

2.2.2. Screening against targets localized in the parasite digestive vacuole

Plasmodium parasites dispose the free, toxic heme that results from digestion of host erythrocyte hemoglobin by crystallizing it into hemozoin crystals. Heme detoxification takes place in the acidic digestive vacuole (DV) of the parasite (pH 5.2), and is believed to be the target of several antimalarial drugs [37]. It was recently reported that Mannich-base derivatives of aurones might interfere with heme polymerization inside the DV of erythrocytic parasites [29]. Since there is strong evidence that pH trapping plays a role in the activity of basic antimalarials such as chloroquine, we assessed the possibility of the quinoline (**2f**, **3f**), 2-aminopyridine (**2g**, **3g**, **6c**) and Mannich-base aurone derivatives (**7a**–**d**) being trapped in the acidic DV, by calculating the vacuolar accumulation ratio (VAR) based on the pH-dependent distribution of these compounds between water and lipid (log D) (Table 6) [37]. We also determined the lipid accumulation ratio (LAR), which measures the expected ratio of compound that would concentrate within the lipid component within the DV [37].

As shown by the VAR values presented in Table 6, basic aurones have the potential to accumulate within the DV, with compound **7a** exhibiting a VAR value comparable to that of chloroquine, reflecting the predominance of its doubly positively charged form at pH 5.2.

Table 5
Antiplasmodial activity (IC_{50}) against the chloroquine-resistant *Plasmodium falciparum* W2 strain, cytotoxicity (EC_{50}) against Human Embryonic Kidney 293T cells and selectivity index (SI = EC_{50}/IC_{50}) for aurones **1**–**7**.

Compd	IC_{50}/μM	EC_{50}/μM	SI	Compd	IC_{50}/μM	EC_{50}/μM	SI	Compd	IC_{50}/μM	EC_{50}/μM	SI
1a	>10	>100	>10	**2h**	8.59	>100	>12	**5a**	>10	>100	>10
1b	>10	>100	>10	**2i**	5.00	>100	>20	**5b**	>10	>100	>10
1c	4.70	>100	>21	**2j**	>10	>100	>10	**5c**	>10	>100	>10
1d	3.21	>100	>31	**2k**	>10	>100	>10	**5d**	>10	>100	>10
1e	>10	>100	>10	**3a**	5.21	>100	>19	**5e**	>10	>100	>10
1f	>10	68	<6.8	**3b**	4.41	>100	>23	**5f**	5.84	95	>16
1g	>10	>100	>10	**3c**	>10	>100	>10	**5g**	9.88	12	>1
1h	7.99	>100	>13	**3d**	>10	>100	>10	**6a**	>10	>100	>10
2a	>10	>100	>10	**3e**	2.31	71	>31	**6b**	>10	>100	>10
2b	>10	>100	>10	**3f**	4.78	>100	>21	**6c**	3.03	>100	>33
2c	>10	>100	>10	**3g**	2.56	79	>31	**7a**	4.01	>100	>25
2d	>10	>100	>10	**4a**	>10	>100	>10	**7b**	1.18	>100	>85
2e	7.34	87	>12	**4b**	>10	>100	>10	**7c**	3.34	>100	>30
2f	6.59	>100	>15	**4c**	4.80	>100	>21	**7d**	3.47	>100	>29
2g	3.70	>100	>27	**4d**	>10	>100	>10	**CQ**	0.14	N.D.	N.D.

With exception of the piperazine Mannich-base **7a**, most of the basic aurones exhibit high LAR values, suggesting the preference of these compounds for a lipophilic environment. Consequently, upon entrance into the digestive food vacuole, the compounds could accumulate within the neutral lipid particles at the sight of hemozoin formation [38,39]. However, no clear correlation emerged between the pIC_{50} values for antiplasmodial activity of compounds **2f**–**g**, **3f**–**g**, **6c**, **7a**–**d**, and their LAR or VAR values. Furthermore, none of these basic-aurones inhibited hemozoin formation at concentrations up to 1 mM, when assayed using a hemozoin-like crystal inhibition assay [40]. Overall, the VAR value obtained for these compounds show their ability to accumulate in the DV, and, as a result, this class of compounds may probably interact with other targets within this compartment (Table 6).

Aurones **1**–**6** were also screened for inhibition of cysteine protease from *P. falciparum*, falcipain-2, which is of particular interest as therapeutic target due to its role in parasite development [41]. Falcipain-2 localizes in the parasite's DV and plays a key role in the hydrolysis of host hemoglobin into amino acids essential to parasite growth [42]. Additionally, falcipain inhibitors that contain a Michael acceptor warhead [43] have been reported to block the development of cultured erythrocytic parasites and to cure mice infected with lethal malaria infections [44]. Although aurones are well known Michael acceptors [6], none of the aurones **1**–**6** inhibited falcipain-2 in concentrations up to 50 μM (data not shown). The only exception was compound **4c**, which showed to be a weak falcipain-2 inhibitor with an IC_{50} value of 18.6 μM. The observation that falcipain-2 is inhibited only in the high μM range, suggests that aurone scaffold is not suitable for enzyme binding.

2.2.3. *In vitro drug combination assay*

Previous studies showed that aurone derivatives inhibit ABC transporters, a family of proteins involved in multidrug resistance [21–25]. Moreover, the importance of this type of transporters was already demonstrated for resistance of malaria parasites to chloroquine [45,46]. Therefore, we evaluated the antimalarial effects of compound **7c** combined with chloroquine by a modified fixed ratio isobologram method [47–50]. Isobologram analysis, based on calculation of the sum of fractional inhibitory concentration values (FICs = IC_{50} of drug in the combination/IC_{50} of drug when tested alone) or combination index (CI) [50] gives an indication of whether the interaction is antagonistic, additive or synergistic. Although there are no defined breakpoints for antimalarial combinations, a recent study defined synergism as $CI < 0.9$, additive effect as $0.90 \leq CI < 1.10$ and antagonism as $CI \geq 1.10$ [51]. The CI value of 1.06 obtained for compound **7c** demonstrated an additive interaction between this compound and chloroquine (Fig. 2). Therefore, synergy of this class of compounds with chloroquine was not demonstrated.

3. Conclusions

Novel aurone derivatives with additional structural complexity and diversity were synthesized and screened against a chloroquine-resistant *P. falciparum* strain. We demonstrated that aurones provide a useful scaffold to generate novel bioactive compounds and that appropriate functionalization of the aurone scaffold yielded compounds with antiplasmodial activity in the low micromolar range and with low cytotoxicity. In particular, aurones containing basic moieties with capacity to protonate under weakly

Table 6
Physicochemical parameters for compounds selected compounds. clog*P* values were calculated using AlogPS 2.1 software from Virtual Computational Chemistry Laboratory [56]. pK_a values were obtained from SPARC software [57], except when indicated. Values of log*D*, VAR and LAR were calculated using the equations available in Ref. [37].

Compd	clog*P*	pK_a^1	pK_a^2	log$D_{7.4}$	log$D_{5.2}$	VAR	LAR	MIC/μM	IC_{50}/μM
2f	5.16	4.49[a]	–	5.16	5.08	1.19	144366	ND	6.59
2g	3.69	6.14[b]	–	3.67	2.70	9.20	4643	NI	3.70
3f	5.16	4.49[a]	–	5.16	5.08	1.19	144,366	ND	4.78
3g	3.69	6.14[b]	–	3.67	2.70	9.20	4643	NI	2.56
6c	5.20	6.14[b]	–	5.18	4.21	9.20	150,233	NI	3.03
7a	2.55	8.50	7.78	1.02	−3.33	22,589	10.6	NI	4.01
7b	3.73	9.08	–	2.04	−0.15	155	110	NI	1.18
7c	2.47	6.46	–	2.42	1.19	17.2	265	NI	3.34
7d	3.55	9.15	–	1.79	−0.40	156	62.0	NI	3.47
CQ	7.72	10.18	8.38	0.92	−3.44	22749	8.25	125	0.14

NI: no inhibition of hemozoin-like crystal formation; ND: not determined.
[a] Calculated from Ref. [58].
[b] Calculated from Ref. [59].

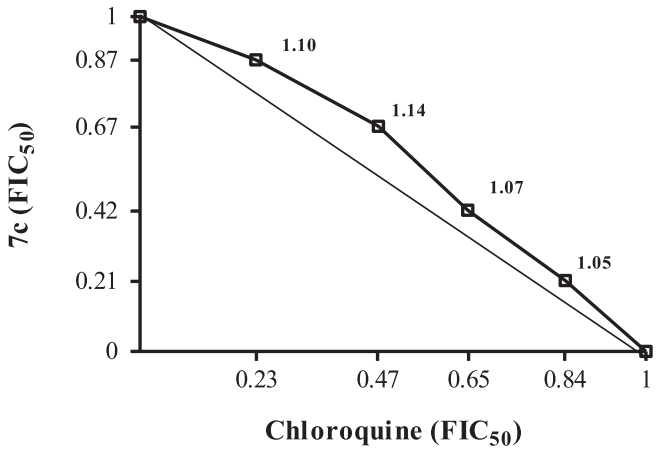


Fig. 2. Isobologram showing the relationship between the FIC_{50} of chloroquine and compound **7c** against *Plasmodium falciparum* Dd2 strain. Numbers on each plotted point correspond to the calculated combination index (CI) value for the utilized combination ratio.

acidic conditions (2-aminopyridine, Mannich-bases) emerged as the most active in this series. Our preliminary results indicate that the primary mechanism of action of these basic aurones does not involve inhibition of hemozoin formation. Furthermore, aurones **1**–**7** are only poorly inhibitors of falcipain-2, which is believed to operate in acidic DV of the parasite. However, the high VAR values obtained for the basic aurones indicate their ability to accumulate in the DV which may be related with their mode of action. Further studies are underway to unravel the antimalarial mechanism of this class of compounds. In conclusion, our results highlight the potential of the aurone scaffold for future antiplasmodial lead optimization.

4. Experimental

4.1. Chemistry

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points were determined using a Kofler camera Bock monoscope M and are uncorrected. Merck Silica Gel 60 F254 plates were used as analytical TLC and flash column chromatography was performed on Merck Silica Gel (200–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 Ultra-Shield (400 MHz). ^{1}H and ^{13}C chemical shifts are expressed in parts per million (ppm, δ) referenced to the solvent used and the proton coupling constants (J) in hertz. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), and broad (br). Low-resolution mass spectra were recorded using a VG Quattro LCMS instruments. HR-ESI-MS were recorded on an ESI-TOF spectrometer (Biotof II Model, Bruker). Elemental analyses were performed using an EA 1110 CE Instruments automatic analyser. The microwave-assisted synthesis was performed in a CEM Corporation Discover® Labmate™.

4.1.1. General procedure for the synthesis of aurones **1a**–**h**, **2j**–**k** and **4a**–**d**

To a solution of benzofuran-3(2*H*)-one (134 mg, 1 mmol) in dry methanol (20 mL) at room temperature was added the appropriate aldehyde (1.2 mmol) and Al_2O_3 (1 mmol). The mixture was refluxed, under N_2, for 48 h. After, the solvent was removed and the solid residue was dissolved in CH_2Cl_2. The organic layer was washed with water, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product.

*4.1.1.1. (Z)-2-Benzylidenebenzofuran-3(2H)-one (**1a**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 80:20) Obtained as yellow solid, yield 41%, mp 113–114 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.97–7.90 (m, 2H), 7.82 (dd, J = 7.6, 0.8 Hz, 1H), 7.66 (ddd, J = 8.5, 7.4, 1.4 Hz, 1H), 7.51–7.38 (m, 3H), 7.34 (d, J = 8.3 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H), 6.91 (s, 1H). ^{13}C NMR (101 MHz, MeOD) δ = 186.22, 167.63, 138.77, 132.71, 132.09, 131.23, 130.04, 129.42, 125.38, 124.98, 122.52, 114.24, 114.15. Anal. Calcd. ($C_{15}H_{10}O_2 \cdot 0.15H_2O$): C, 80.09; H, 4.62%. Found: C, 80.35; H, 4.97%.

*4.1.1.2. (Z)-2-(2-Bromobenzylidene)benzofuran-3(2H)-one (**1b**).* Purified by flash chromatography (Hexane/EtOAc = 95:5). Obtained as yellow solid, yield 55%, mp 169–171 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.32 (dd, J = 7.9, 1.5 Hz, 1H), 7.85–7.79 (m, 3H), 7.59–7.56 (m, 2H), 7.40–7.33 (m, 2H), 7.07 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.15, 166.14, 147.66, 138.55, 133.87, 132.47, 132.09, 131.51, 128.88, 126.06, 125.03, 124.78, 121.02, 113.78, 109.23. Anal. Calcd. ($C_{15}H_9BrO_2$): C, 59.82; H, 3.02%. Found: C, 59.67; H, 3.13%.

*4.1.1.3. (Z)-2-(3-Bromobenzylidene)benzofuran-3(2H)-one (**1c**).* Purified by flash chromatography (Hexane/EtOAc = 95:5). Obtained as yellow solid, yield 54%, mp 123–124 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.18 (s, 1H), 8.03 (d, J = 7.9 Hz, 1H), 7.83–7.81 (m, 2H), 7.66 (d, J = 7.9 Hz, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.48 (t, J = 7.9 Hz, 1H), 7.34 (t, J = 8.2 Hz, 1H), 6.96 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.15, 166.02, 147.31, 138.42, 134.81, 133.88, 133.03, 131.56, 130.59, 124.90, 124.67, 122.68, 121.14, 113.82, 110.80. Anal. Calcd. ($C_{15}H_9BrO_2$): C, 59.82; H, 3.02%. Found: C, 59.81; H, 3.05%.

*4.1.1.4. (Z)-2-(4-Bromobenzylidene)benzofuran-3(2H)-one (**1d**).* Purified by flash chromatography (Hexane/EtOAc = 95:5). Obtained as yellow solid, yield 56%, mp 180–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.91 (d, J = 8.3 Hz, 2H), 7.84–7.80 (m, 2H), 7.72 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.5 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 6.95 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.66, 165.47, 146.59, 137.88, 133.14, 132.10, 131.21, 124.42, 124.16, 123.65, 120.78, 113.28, 110.92. Anal. Calcd. ($C_{15}H_9BrO_2$): C, 59.82; H, 3.02%. Found: C, 59.88; H, 3.04%.

*4.1.1.5. (Z)-2-(2,4-Difluorobenzylidene)benzofuran-3(2H)-one (**1e**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 80:20). Obtained as yellow solid, 30% yield, mp 150–152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.36 (td, J = 8.6, 6.5 Hz, 1H), 7.83 (dd, J = 7.7, 1.3 Hz, 1H), 7.73–7.63 (m, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.29–7.22 (m, 1H), 7.13 (s, 1H), 7.02 (dd, J = 11.9, 5.6 Hz, 1H), 6.95–6.85 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 184.55, 166.20, 147.39, 137.25, 133.16, 125.00, 123.91, 121.63, 113.05, 112.33, 104.40, 103.27. Anal. Calcd. ($C_{15}H_8F_2O_2 \cdot 0.25H_2O$): C, 68.61; H, 3.27%. Found: C, 68.60; H, 3.27%.

*4.1.1.6. (Z)-2-(2,4-Dichlorobenzylidene)benzofuran-3(2H)-one (**1f**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 70:30). Obtained as a yellow solid, 81% yield, mp 213–214 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.31 (d, J = 8.6 Hz, 1H), 7.84–7.82 (m, 1H), 7.70–7.66 (m, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.37 (dd, J = 8.6, 2.1 Hz, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.28 (s, 1H), 7.26–7.24 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 184.57, 166.23, 147.82, 137.38, 136.61, 135.98, 132.98, 130.03, 129.15, 127.69, 125.07, 124.03, 121.51, 113.08, 106.94. Anal. Calcd. ($C_{15}H_8Cl_2O_2 \cdot 0.1$Hex): C, 62.50; H, 3.17%. Found: C, 62.38; H, 3.32%.

*4.1.1.7. (Z)-2-(3,4,5-Trimethoxybenzylidene)benzofuran-3(2H)-one (**1g**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 70:30). Obtained as a yellow solid, 64% yield, mp 200–201 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.82 (d, J = 7.6 Hz, 1H), 7.70–7.63 (m, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.24 (dd, J = 12.5, 4.9 Hz, 1H), 7.19 (s, 2H), 6.83 (s, 1H), 3.95 (s, 6H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 184.70, 166.03, 153.44, 146.53, 140.14, 136.92, 127.82, 124.83, 123.68, 121.83, 113.52, 113.02, 109.01, 61.18, 56.33. Anal. Calcd. ($C_{18}H_{16}O_5$): C, 69.22; H, 5.17%. Found: C, 69.30; H, 5.36%.

*4.1.1.8. (Z)-2-(4-(Dimethylamino)benzylidene)benzofuran-3(2H)-one (**1h**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 50:50). Obtained as orange solid, 49% yield, mp 189–191 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (d, 2H, J = 8.8 Hz), 7.81 (d, 1H, J = 7.5 Hz), 7.61 (t, 1H, J = 8.3 Hz), 7.32 (d, 1H, J = 8.3 Hz), 7.19 (t, 1H, J = 7.5 Hz), 6.93 (s, 1H), 6.75 (d, 2H, J = 8.8 Hz), 3.07 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 184.14, 165.42, 151.47, 145.16, 135.96, 133.78, 124.46, 123.02, 122.59, 120.15, 115.45, 112.90, 112.08, 40.23. Anal. Calcd. ($C_{17}H_{15}NO_2$•0.15H_2O): C, 76.18; H, 5.77, N, 5.28%. Found: C, 76.20; H, 5.60, N, 5.33%.

*4.1.1.9. (Z)-2-(4-(p-Tolyloxy)benzylidene)benzofuran-3(2H)-one (**2j**).* Purified by flash chromatography (Hexane/EtOAc = 80:20). Obtained as yellow solid, 33% yield, mp 116–118 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.02 (d, 2H, J = 8.8 Hz), 7.82–7.79 (m, 2H), 7.55 (d, 1H, J = 8.6 Hz), 7.32 (t, 1H, J = 7.4 Hz), 7.25 (d, 2H, J = 8.4 Hz), 7.07 (d, 2H, J = 8.7 Hz), 7.02 (d, 2H, J = 8.4 Hz), 6.97 (s, 1H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.39, 165.72, 159.49, 153.41, 146.06, 138.00, 134.10, 133.99, 131.07, 126.92, 124.72, 124.39, 121.47, 120.11, 118.32, 113.66, 112.51, 20.80. Anal. Calcd. ($C_{22}H_{16}O_3$·0.15H_2O): C, 79.81; H, 5.28%. Found: C, 79.59; H, 5.01%.

*4.1.1.10. (Z)-2-(4-(4-Chlorophenoxy)benzylidene)benzofuran-3(2H)-one (**2k**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 50:50). Obtained as yellow solid, 51% yield, mp 112–113 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.05 (d, 2H, J = 8.7 Hz), 7.83–7.80 (m, 2H), 7.55 (d, 1H, J = 8.6 Hz), 7.48 (d, 2H, J = 8.8 Hz), 7.33 (t, 1H, J = 7.4 Hz), 7.16–7.13 (m, 4H), 6.98 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 183.95, 165.80, 158.44, 155.01, 146.26, 138.08, 134.06, 130.55, 128.54, 127.76, 124.76, 124.44, 121.57, 121.44, 119.14, 113.68, 112.26. Anal. Calcd. ($C_{21}H_{13}ClO_3$•0.15H_2O): C, 71.76; H, 3.82%. Found: C, 71.37; H, 3.84%.

*4.1.1.11. (Z)-2-Benzylidene-7-methoxybenzofuran-3(2H)-one (**4a**).* Purified by flash chromatography (Hexane/EtOAc = 90:10). Obtained as yellow solid, 62% yield, mp 168–169 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (d, 2H, J = 7.3 Hz), 7.56–7.47 (m, 4H), 7.35 (d, 1H, J = 7.8 Hz), 7.26 (t, 1H, J = 7.8 Hz), 6.99 (s, 1H), 4.01 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.88, 154.91, 146.32, 145.62, 131.89, 131.44, 130.29, 129.17, 129.71, 122.16, 119.64, 115.17, 112.75, 56.35. Anal. Calcd. ($C_{16}H_{12}O_3$): C, 76.18; H, 4.80%. Found: C, 76.32; H, 5.03%.

*4.1.1.12. (Z)-2-(2,4-Difluorobenzylidene)-7-methoxybenzofuran-3(2H)-one (**4b**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 50:50). Obtained as yellow solid, 95% yield, mp 167–168 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.38 (td, J = 8.6, 6.6 Hz, 1H), 7.38 (dd, J = 6.7, 2.1 Hz, 1H), 7.20–7.14 (m, 2H), 7.13 (s, 1H), 7.07–6.99 (m, 1H), 6.92–6.84 (m, 1H), 4.02 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 184.66, 155.83, 147.35, 146.05, 133.38, 124.36, 122.96, 118.81, 116.04, 112.48, 104.31, 103.68, 56.44. Anal. Calcd. ($C_{16}H_{10}F_2O_3$): C, 66.66; H, 3.50%. Found: C, 66.65; H, 3.84%.

*4.1.1.13. (Z)-2-(2,4-Dichlorobenzylidene)-7-methoxybenzofuran-3(2H)-one (**4c**).* Purified through recrystallization from Hexane/CH_2Cl_2. Obtained as yellow solid, 59% yield, mp 289–290 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.35 (d, J = 8.6 Hz, 1H), 7.48 (d, J = 2.0 Hz, 1H), 7.42–7.37 (m, 2H), 7.30 (s, 1H), 7.21–7.14 (m, 2H), 4.02 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 184.73, 155.86, 147.78, 146.04, 136.62, 136.03, 133.17, 129.96, 129.10, 127.88, 124.50, 122.87, 119.02, 116.13, 107.42, 56.47. Anal. Calcd. ($C_{16}H_{10}Cl_2O_3$·H_2O): C, 56.66; H, 3.57%. Found: C, 56.97; H, 3.45%.

*4.1.1.14. (Z)-7-Methoxy-2-(3,4,5-trimethoxybenzylidene)benzofuran-3(2H)-one (**4d**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 80:20). Obtained as yellow solid, 99% yield, mp 167–168 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39 (dd, J = 6.8, 1.9 Hz, 1H), 7.24 (s, 2H), 7.18–7.12 (m, 2H), 6.85 (s, 1H), 4.01 (s, 3H), 3.95 (s, 6H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 184.81, 155.81, 153.41, 146.59, 146.09, 140.08, 127.83, 124.09, 123.22, 118.93, 116.06, 113.95, 109.04, 61.17, 56.69, 56.23. Anal. Calcd. ($C_{19}H_{18}O_6$·H_2O): C, 63.33; H, 5.59%. Found: C, 63.07; H, 5.89%.

*4.1.2. General procedure for the synthesis of aurones **5a** to **5d***

To a solution of 6-hydroxybenzofuran-3(2*H*)-one (134 mg, 1 mmol) in H_2O (15 mL) at room temperature was added the appropriate aldehyde (1.1 mmol) and KOH 1 M (2 mmol). The mixture was stirred, at room temperature, for 4 h. After, some drops of HCl 37% were added to the mixture until the solution reach pH 2. The mixture was extracted with ethyl acetate and combined organic layers were washed with brine, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product.

*4.1.2.1. (Z)-2-Benzylidene-6-hydroxybenzofuran-3(2H)-one (**5a**).* Purified through recrystallization from CH_2Cl_2. Obtained as yellow solid, 99% yield, mp 228–230 °C. ^{1}H NMR (400 MHz, DMSO) δ = 11.26 (s, 1H), 7.95 (d, 2H, J = 7.4 Hz), 7.64 (d, 1H, J = 8.4 Hz), 7.52–7.42 (m, 3H), 6.81–6.80 (m, 2H), 6.73 (d, 1H, J = 8.4 Hz). ^{13}C NMR (101 MHz, DMSO) δ = 181.51, 168.02, 166.67, 147.41, 132.11, 131.10, 129.71, 129.03, 126.07, 113.15, 112.75, 110.38, 98.69. Anal. Calcd. ($C_{15}H_{10}O_3$·0.15H_2O): C, 74.77; H, 4.32%. Found: C, 74.50; H, 4.19%.

*4.1.2.2. (Z)-2-(2,4-Difluorobenzylidene)-6-hydroxybenzofuran-3(2H)-one (**5b**).* Purified through recrystallization from CH_2Cl_2. Obtained as yellow solid, 98% yield, mp 277–279 °C. ^{1}H NMR (400 MHz, DMSO) δ = 8.32–8.22 (m, 1H), 7.65 (d, J = 8.5 Hz, 1H), 7.48–7.38 (m, 1H), 7.32–7.25 (m, 1H), 6.80 (d, J = 1.9 Hz, 1H), 6.73 (dd, J = 8.5, 2.0 Hz, 1H), 6.71 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ = 181.19, 168.19, 167.07, 148.20, 132.69, 126.36, 116.77, 116.69, 113.47, 112.78, 112.47, 104.66, 99.74, 98.85. Anal. Calcd. ($C_{15}H_8F_2O_3$·0.15Hex): C, 66.50; H, 3.55%. Found: C, 66.74; H, 3.86%.

*4.1.2.3. (Z)-2-(2,4-dichlorobenzylidene)-6-hydroxybenzofuran-3(2H)-one (**5c**).* Purified through recrystallization from CH_2Cl_2. Obtained as yellow solid, 99% yield, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO) δ = 8.26 (d, J = 8.6 Hz, 1H), 7.80 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 6.88 (s, 1H), 6.79 (s, 1H), 6.74 (d, J = 8.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ = 181.17, 168.19, 167.14, 148.69, 134.98, 134.61, 132.68, 129.58, 128.75, 128.17, 126.42, 113.50, 112.29, 103.15, 98.84. Anal. Calcd. ($C_{15}H_8Cl_2O_3$·H_2O): C, 55.41; H, 3.10%. Found: C, 55.10; H, 3.23%.

*4.1.2.4. (Z)-6-Hydroxy-2-(3,4,5-trimethoxybenzylidene)benzofuran-3(2H)-one (**5d**).* Purified through recrystallization from CH_2Cl_2. Obtained as yellow solid, 98% yield, mp 264–266 °C. ^{1}H NMR (400 MHz, DMSO) δ = 7.62 (d, J = 8.4 Hz, 1H), 7.32 (s, 2H), 6.83 (s, 1H), 6.76 (s, 1H), 6.72 (d, J = 8.5 Hz, 1H), 3.85 (s, 6H), 3.72 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 181.48, 167.93, 166.68, 153.10, 146.95,

139.18, 127.64, 126.07, 113.25, 112.88, 111.03, 108.84, 98.91, 60.33, 56.10. Anal. Calcd. ($C_{18}H_{16}O_6$): C, 65.84; H, 4.92%. Found: C, 65.62; H, 5.00%.

4.1.3. General procedure for the synthesis of aurones ***5e–g***

To a solution of 6-hydroxybenzofuran-3(2*H*)-one (0.57 mmol) in glacial acetic acid (5.7 mL) at room temperature was added the appropriate aldehyde (0.68 mmol) and HCl (cat, 3 drops). The reaction mixture was stirred for 4 h at room temperature. After, the mixture was dropped in cold water and the precipitate formed was filtered and washed with water.

*4.1.3.1. (Z)-2-(4-(Dimethylamino)benzylidene)-6-hydroxybenzofuran-3(2H)-one (**5e**).* Obtained as orange solid, 71% yield, mp 235–237 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.00 (s, 1H), 7.73 (d, 2H, J = 8.9 Hz), 7.55 (d, 1H, J = 8.4 Hz), 6.70–6.61 (m, 5H), 2.99 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 182.10, 167.38, 165.43, 150.17, 145.56, 132.77, 125.15, 119.69, 114.04, 112.79, 112.27, 111.59, 98.40. Anal. Calcd. ($C_{17}H_{15}NO_3$): C, 72.58; H, 5.39, N, 4.98%. Found: C, 72.19; H, 5.31, N, 5.09%.

*4.1.3.2. (Z)-6-Hydroxy-2-(4-(p-tolyloxy)benzylidene)benzofuran-3(2H)-one (**5f**).* Obtained as yellow solid, 51% yield, mp 248–250 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.22 (br, 1H), 7.96 (d, 2H, J = 8.8 Hz), 7.62 (d, 1H, J = 8.5 Hz), 7.24 (d, 1H, J = 8.1 Hz), 7.06–6.99 (m, 4H), 6.79–6.77 (m, 2H), 6.71 (dd, 1H, J = 8.5, 1.8 Hz). ^{13}C NMR (101 MHz, CDCl3): δ = 181.38, 167.82, 166.53, 158.67, 153.14, 146.71, 133.59, 133.17, 130.64, 126.73, 125.99, 119.64, 117.92, 113.11, 112.92, 110.14, 98.61, 20.38. Anal. Calcd. ($C_{22}H_{14}O_4 \cdot 0.2H_2O$): C, 75.93; H, 5.05%. Found: C, 75.66; H, 5.03%.

*4.1.3.3. (Z)-2-(4-(4-Chlorophenoxy)benzylidene)-6-hydroxybenzofuran-3(2H)-one (**5g**).* Obtained as yellow solid, 84% yield, mp 243–244 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.21 (br, 1H), 7.80 (d, 2H, J = 8.8 Hz), 7.56 (d, 1H, J = 9.1 Hz), 7.27–7.25 (m, 2H), 6.97–6.93 (m, 4H), 6.67–6.63 (m, 3H). ^{13}C NMR (101 MHz, CDCl3): δ = 181.39, 167.86, 166.56, 157.59, 154.71, 146.88, 133.24, 130.11, 128.00, 127.56, 126.02, 121.06, 118.72, 113.12, 112.87, 109.91, 98.62. Anal. Calcd. ($C_{21}H_{13}ClO_4 \bullet 0.15H_2O$): C, 68.63; H, 3.66%. Found: C, 68.57; H, 3.64%.

4.1.4. General procedure for the synthesis of aurones derivatives ***2a–g****,* ***3a–g****, and* ***6a–c*** *via Suzuki coupling*

To a solution of the appropriate aurone (0.23 mmol) in dioxane (2.3 mL) was added $Pd(PPh_3)_2Cl_2$ (0.023 mmol) and Na_2CO_3 1 M (690 μL) followed by the proper boronic acid (0.28 mmol). The resulting mixture was degassed and stirred at 100 °C for 3 h under N_2. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2, filtered under celite and concentrated under pressure to give the crude product.

*4.1.4.1. (Z)-2-(biphenyl-4-ylmethylene)benzofuran-3(2H)-one (**2a**).* Purified by flash chromatography (Hexane/EtOAc = 90:10). Obtained as yellow solid, 77% yield, mp 133–135 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.10 (d, J = 8.0 Hz, 2H), 7.85–7.75 (m, 6H), 7.61 (d, J = 8.4 Hz, 1H), 7.52–7.49 (m, 2H), 7.41 (m, 1H), 7.34 (t, J = 7.3 Hz, 1H), 7.03 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.60, 165.45, 146.42, 141.50, 139.11, 137.73, 132.08, 131.08, 129.10, 128.15, 127.23, 126.82, 124.38, 124.07, 120.94, 113.30, 111.97. Anal. Calcd. ($C_{21}H_{14}O_2 \cdot 0.15H_2O$): C, 83.78; H, 4.80%. Found: C, 83.67; H, 4.89%.

*4.1.4.2. (Z)-2-((4′-Fluorobiphenyl-4-yl)methylene)benzofuran-3(2H)-one (**2b**).* Purified by flash chromatography (Hexane/EtOAc = 92:8). Obtained as yellow solid, 66% yield, mp 174–175 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.10 (d, J = 8.3 Hz, 2H), 7.84–7.80 (m, 6H), 7.61 (d, J = 8.5 Hz, 1H), 7.36–7.31 (m, 3H), 7.03 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.61, 165.45, 162.27, 146.43, 140.41, 137.75, 135.60, 132.08, 131.05, 128.90, 127.18, 124.39, 124.09, 120.94, 115.94, 113.31, 111.90. Anal. Calcd. ($C_{21}H_{13}FO_2 \cdot 0.1H_2O$): C, 79.28; H, 4.19%. Found: C, 79.07; H, 4.33%.

*4.1.4.3. (Z)-2-((4′-chlorobiphenyl-4-yl)methylene)benzofuran-3(2H)-one (**2c**).* Purified by flash chromatography (Hexane/EtOAc = 90:10). Obtained as yellow solid, 64% yield, mp 171–173 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.10 (d, J = 8.3 Hz, 2H), 7.86–7.79 (m, 6H·), 7.61 (d, J = 8.7 Hz, 1H), 7.56 (d, J = 8.5 Hz, 2H), 7.35 (t, J = 7.5 Hz, 1H), 7.03 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.05, 165.91, 146.95, 140.52, 138.35, 138.20, 133.47, 132.53, 131.86, 129.49, 129.01, 127.63, 124.82, 124.54, 121.36, 113.74, 112.22. Anal. Calcd. ($C_{21}H_{13}ClO_2 \cdot 0.15H_2O$): C, 75.18; H, 4.00%. Found: C, 75.16; H, 3.97%.

*4.1.4.4. (Z)-4′-((3-Oxobenzofuran-2(3H)-ylidene)methyl)biphenyl-4-carbaldehyde (**2d**).* Purified by flash chromatography (Hexane/EtOAc = 90:20). Obtained as yellow solid, 83% yield, mp 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.07 (s, 1H), 8.14 (d, J = 8.3 Hz, 2H), 8.04–7.99 (m, 4H), 7.94 (d, J = 8.3 Hz, 2H), 7.83–7.81 (m, 2H), 7.61 (d, J = 8.5 Hz, 1H), 7.35 (t, J = 7.4 Hz, 1H), 7.04 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 192.81, 183.69, 165.52, 146.70, 144.76, 139.95, 137.85, 135.49, 132.22, 132.13, 130.26, 127.75, 127.53, 124.45, 124.17, 120.89, 113.34, 111.61. Anal. Calcd. ($C_{21}H_{14}O_3 \cdot 0.75H_2O$): C, 77.74; H, 4.61%. Found: C, 77.43; H, 4.47%.

*4.1.4.5. (Z)-2-(4-Benzylbenzylidene)benzofuran-3(2H)-one (**2e**).* Purified by flash chromatography (Hexane/EtOAc = 92:8). Obtained as yellow solid, 70% yield, 154–156 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.93 (d, J = 7.9 Hz, 2H), 7.81–7.79 (m, 2H), 7.56 (d, J = 8.6 Hz, 1H), 7.39 (d, J = 7.9 Hz, 2H), 7.34–7.25 (m, 5H), 7.20 (t, J = 7.2 Hz, 1H), 6.93 (s, 1H), 4.01 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.60, 165.43, 146.11, 143.86, 140.71, 137.69, 131.67, 129.75, 129.48, 128.77, 128.54, 126.16, 124.33, 124.01, 120.95, 113.26, 112.33, 41.01. Anal. Calcd. ($C_{22}H_{16}O_2 \cdot 0.15H_2O$): C, 83.86; H, 5.23%. Found: C, 83.87; H, 5.12%.

*4.1.4.6. (Z)-2-(4-(Quinolin-3-yl)benzylidene)benzofuran-3(2H)-one (**2f**).* Purified by flash chromatography (Hexane/EtOAc = 70:30). Obtained as yellow solid, 87% yield, mp 205–207 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.35 (s, 1H), 8.77 (s, 1H), 8.19 (d, J = 8.4 Hz, 2H), 8.09–8.07 (m, 4H), 7.86–7.78 (m, 3H), 7.69–7.62 (m, 2H), 7.35 (t, J = 7.4 Hz, 1H), 7.07 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.08, 165.93, 149.78, 147.47, 147.07, 138.80, 138.24, 133.68, 132.65, 132.24, 130.38, 129.15, 129.02, 128.12, 128.05, 127.65, 127.62, 124.86, 124.58, 121.35, 113.78, 112.15. Anal. Calcd. ($C_{24}H_{15}NO_2 \cdot 0.4H_2O$): C, 80.87; H, 4.48; N, 3.93%. Found: C, 80.67; H, 4.27; N, 4.06%.

*4.1.4.7. (Z)-2-(4-(6-aminopyridin-3-yl)benzylidene)benzofuran-3(2H)-one (**2g**).* Purified by flash chromatography (EtOAc/MeOH = 97:3). Obtained as orange solid, 99% yield, mp 195–197 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.38 (s, 1H), 8.04 (d, J = 8.4 Hz, 2H), 7.84–7.80 (m, 3H), 7.76 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 8.5 Hz, 1H), 7.34 (t, J = 7.4 Hz, 1H), 7.00 (s, 1H), 6.55 (d, J = 8.6 Hz, 1H), 6.24 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.91, 165.75, 160.10, 146.75, 146.52, 140.16, 138.03, 135.74, 132.62, 130.16, 125.95, 124.75, 124.44, 123.02, 121.46, 113.72, 112.83, 108.48. Anal. Calcd. ($C_{20}H_{14}N_2O_2 \cdot 0.4H_2O$): C, 74.70; H, 4.65; N, 8.71%. Found: C, 74.57; H, 4.43; N, 8.79%.

*4.1.4.8. (Z)-2-(biphenyl-3-ylmethylene)benzofuran-3(2H)-one (**3a**).* Purified by flash chromatography (Hexane/EtOAc = 92:8). Obtained as yellow solid, 71% yield, mp 123–125 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.24 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.84–7.80 (m, 2H), 7.77–7.73 (m, 3H), 7.64–7.60 (m, 2H), 7.54–7.50 (m, 2H), 7.42 (t, J = 7.3 Hz, 1H), 7.34 (t, J = 7.4 Hz, 1H), 7.07 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.79, 165.59, 146.57, 140.96, 139.51, 137.84, 132.62, 130.06, 129.99, 129.76, 129.13, 128.47, 127.90, 126.87, 124.43, 124.13, 120.89, 113.41, 112.27. Anal. Calcd. ($C_{21}H_{14}O_2 \cdot 0.1H_2O$): C, 84.03; H, 4.78%. Found: C, 83.94; H, 4.80%.

*4.1.4.9. (Z)-2-((4′-Fluorobiphenyl-3-yl)methylene)benzofuran-3(2H)-one (**3b**).* Purified by flash chromatography (Hexane/EtOAc = 92:8). Obtained as yellow solid, 65% yield, mp 103–105 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.22 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.81–7.78 (m, 5H), 7.64–7.60 (m, 2H), 7.37–7.32 (m, 3H), 7.06 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.78, 165.59, 162.15, 146.58, 139.89, 137.85, 135.95, 132.63, 129.96, 129.78, 128.93, 128.85, 128.38, 124.43, 124.14, 120.88, 116.03–115.82, 113.41, 112.18. Anal. Calcd. ($C_{21}H_{13}FO_2 \cdot 0.1H_2O$): C, 79.28; H, 4.19%. Found: C, 79.23; H, 4.15%.

*4.1.4.10. (Z)-2-((4′-Chlorobiphenyl-3-yl)methylene)benzofuran-3(2H)-one (**3c**).* Purified by flash chromatography (Hexane/EtOAc = 85:15). Obtained as yellow solid, 51% yield, mp 187–189 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.24 (s, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.85–7.76 (m, 5H), 7.65–7.56 (m, 4H), 7.34 (t, 1H, J = 7.5 Hz), 7.06 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.82, 165.62, 146.64, 139.63, 138.33, 137.91, 132.83, 132.73, 130.35, 129.94, 129.89, 129.11, 128.67, 128.38, 124.47, 124.19, 120.88, 113.44, 112.11. Anal. Calcd. ($C_{21}H_{13}ClO_2 \cdot 0.15H_2O$): C, 75.18; H, 4.00%. Found: C, 74.79; H, 4.20%.

*4.1.4.11. (Z)-3′-((3-oxobenzofuran-2(3H)-ylidene)methyl)biphenyl-4-carbaldehyde (**3d**).* Purified by flash chromatography (Hexane/EtOAc = 75:25). Obtained as yellow solid, 63% yield, mp 193–195 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.09 (s, 1H), 8.35 (s, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.06–7.98 (m, 4H), 7.88–7.82 (m, 3H), 7.68 (t, 1H, J = 7.8 Hz), 7.62 (d, 1H, J = 8.5 Hz), 7.35 (t, 1H, J = 7.4 Hz), 7.09 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 192.83, 183.76, 165.59, 146.66, 145.13, 139.54, 137.86, 135.38, 132.81, 130.97, 130.29, 128.73, 127.95, 127.55, 124.43, 124.15, 120.84, 113.42, 111.89. Anal. Calcd. ($C_{22}H_{14}O_3 \cdot 0.25H_2O$): C, 79.86; H, 4.43%. Found: C, 79.48; H, 4.57%.

*4.1.4.12. (Z)-2-(3-Benzylbenzylidene)benzofuran-3(2H)-one (**3e**).* Purified by flash chromatography (Hexane/EtOAc = 92:8). Obtained as yellow oil, 60%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.86–7.79 (m, 4H), 7.52 (t, 1H, J = 8.2 Hz), 7.44 (t, 1H, J = 7.6 Hz), 7.34–7.27 (m, 6H), 7.23–7.22 (m, 1H), 6.90 (s, 1H), 4.02 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.80, 165.54, 146.41, 142.41, 140.94, 137.90, 132.11, 131.77, 130.72, 129.32, 129.26, 128.93, 128.67, 126.26, 124.48, 124.17, 120.94, 113.33, 112.39, 41.00. HRMS calc. ($C_{22}H_{17}O_2$): 313.1223. Found: 313.1226.

*4.1.4.13. (Z)-2-(3-(Quinolin-3-yl)benzylidene)benzofuran-3(2H)-one (**3f**).* Purified by flash chromatography (Hexane/EtOAc = 80:20). Obtained as yellow solid, 68% yield, mp 195–196 °C. ^{1}H NMR (400 MHz, pyridine-d_6): δ = 9.53 (s, 1H), 8.53 (s, 1H), 8.42 (d, 1H, J = 8.4 Hz), 8.37 (s, 1H), 8.19 (d, 1H, J = 7.8 Hz), 8.05 (d, 1H, J = 8.1 Hz), 7.90 (d, 1H, J = 7.7 Hz), 7.85 (d, 1H, J = 7.4 Hz), 7.78 (t, 1H, J = 7.6 Hz), 7.67–7.61 (m, 3H), 7.40 (d, 1H, J = 8.1 Hz), 7.26–7.18 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.80, 166.74, 148.56, 147.97, 139.23, 137.84, 134.13, 133.94, 133.66, 131.43, 131.21, 130.56, 130.29, 130.13, 129.46, 129.11, 128.83, 127.83, 125.05, 124.45, 124.17, 122.24, 113.85, 112.55. Anal. Calcd. ($C_{24}H_{15}NO_2 \cdot 0.3H_2O$): C, 81.24; H, 4.44; N, 3.95%. Found: C, 81.59; H, 4.83; N, 3.92%.

*4.1.4.14. (Z)-2-(3-(6-Aminopyridin-3-yl)benzylidene)benzofuran-3(2H)-one (**3g**).* Purified by flash chromatography (CH_2Cl_2/MeOH = 97:3). Obtained as yellow solid, 76% yield, mp 187–189 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.32 (s, 1H), 8.14 (s, 1H), 7.98 (d, J = 7.7 Hz, 1H), 7.83–7.80 (m, 2H), 7.77 (dd, J = 8.6, 2.5 Hz, 1H), 7.67 (d, J = 7.9 Hz, 1H), 7.63–7.61 (m, 1H), 7.55 (t, J = 7.7 Hz, 1H), 7.34 (t, J = 7.4 Hz, 1H), 7.03 (s, 1H), 6.56 (d, J = 8.6 Hz, 1H), 6.15 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.17, 165.97, 159.89, 145.87, 146.35, 138.19, 135.87, 132.93, 131.99, 130.09, 129.28, 127.50, 124.80, 123.52, 121.31, 113.82, 112.89, 108.49. Anal. Calcd. ($C_{20}H_{14}N_2O_2 \cdot 0.5H_2O$): C, 74.28; H, 4.69; N, 8.67%. Found: C, 74.43; H, 4.55; N, 8.97%.

*4.1.4.15. (Z)-2-Benzylidene-6-phenylbenzofuran-3(2H)-one (**6a**).* Purified by flash chromatography (Hexane/CH_2Cl_2 = 70:30). Obtained as yellow solid, 78% yield, mp 141–143 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.95 (d, 2H, J = 7.3 Hz), 7.87 (d, 1H, J = 8.0 Hz), 7.57 (d, 2H, J = 7.1 Hz), 7.56 (s, 1H), 7.53–7.40 (m, 7H), 6.92 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.14, 166.24, 149.52, 146.92, 138.58, 131.99, 131.44, 130.16, 129.23, 129.20, 129.10, 127.44, 124.79, 122.86, 124.48, 119.81, 112.18, 111.06. Anal. Calcd. ($C_{21}H_{14}O_2 \cdot 0.6H_2O$): C, 81.58; H, 4.97%. Found: C, 81.41; H, 5.28%.

*4.1.4.16. (Z)-2-Benzylidene-6-(4-fluorophenyl)benzofuran-3(2H)-one (**6b**).* Purified by flash chromatography (Hexane/EtOAc = 90:10). Obtained as yellow solid, 66% yield, mp 172–174 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.04 (d, 2H, J = 7.2 Hz), 7.94–7.90 (m, 3H), 7.87 (d, 1H, J = 8.1 Hz), 7.64 (dd, 1H, J = 8.1, 1.3 Hz), 7.55–7.48 (m, 3H), 7.38 (t, 2H, J = 8.8 Hz), 6.98 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.10, 166.22, 164.08–161.63, 148.35, 146.91, 135.03, 131.98, 131.43, 130.17, 129.73–129.64, 129.10, 124.81, 122.78, 119.76, 116.21–115.99, 112.21, 111.04. Anal. Calcd. ($C_{21}H_{13}FO_2 \cdot 0.3H_2O$): C, 78.39; H, 4.27%. Found: C, 78.14; H, 4.22%.

*4.1.4.17. (Z)-6-(6-Aminopyridin-3-yl)-2-benzylidenebenzofuran-3(2H)-one (**6c**).* Purified by flash chromatography (CH_2Cl_2/MeOH = 98:2). Obtained as orange solid, 98% yield, mp 191–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.50 (s, 1H), 8.02 (d, 2H, J = 7.3 Hz), 7.92 (dd, 1H, J = 8.7, 2.5 Hz), 7.82–7.78 (m, 1H), 7.58 (d, 1H, J = 8.1 Hz), 7.53 (t, 2H, J = 7.3 Hz), 7.48–7.45 (m, 1H), 6.92 (s, 1H), 6.56 (d, 1H, J = 8.7 Hz), 6.44 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 182.75, 166.53, 160.35, 147.83, 147.36, 147.12, 135.82, 132.09, 131.32, 130.01, 129.08, 124.76, 121.94, 121.00, 118.51, 111.52, 108.53, 108.02. Anal. Calcd. ($C_{20}H_{14}N_2O_2 \cdot 0.3H_2O$): C, 75.12; H, 4.61; N, 8.76%. Found: C, 74.91; H, 4.55; N, 8.64%.

*4.1.5. General procedure for the synthesis of aurones derivatives **2h–i** via Buchwald coupling*

(*Z*)-2-(4-bromobenzylidene)benzofuran-3(2*H*)-one (**1d**) (0.23 mmol), $Pd_2(dba)_3$ (0.0115 mmol), (*R*)-BINAP (0.075 mmol) and NaO^{t}Bu (0.322 mmol) were dissolved in dry toluene (2.3 mL). The resulting mixture was degassed and the appropriate amine (0.276 mmol) was added. The mixture was stirred at 100 °C for 15 min under MW conditions. After cooling to room temperature, the reaction mixture was diluted with Et_2O, filtered under celite and concentrated under pressure to give the crude product.

*4.1.5.1. (Z)-2-(4-(Phenylamino)benzylidene)benzofuran-3(2H)-one (**2h**).* Purified by flash chromatography (Hexane/EtOAc = 80:20). Obtained as orange solid, 71% yield, mp 191–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.87 (s, 1H), 7.90 (d, 2H, J = 8.7 Hz), 7.79–7.76 (m, 2H), 7.55 (d, 1H), 7.34–7.29 (m, 3H), 7.20 (d, 2H, J = 7.6 Hz),

7.15 (d, 2H, $J = 8.7$ Hz), 6.98 (t, 1H, $J = 7.3$ Hz), 6.91 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 183.21, 165.18, 146.57, 144.95, 141.80, 137.36, 134.02, 129.80, 124.46, 124.08, 122.61, 122.13, 121.92, 119.41, 115.66, 114.30, 113.58. Anal. Calcd. ($C_{21}H_{15}NO_2 \cdot 0.15H_2O$): C, 79.80; H, 4.89; N, 4.43%. Found: C, 79.56; H, 5.12; N, 4.68%.

*4.1.5.2. (Z)-2-(4-(Benzylamino)benzylidene)benzofuran-3(2H)-one (**2i**).* Purified by flash chromatography (Hexane/EtOAc = 80:20) followed by TLC (Hexane/EtOAc = 70:30). Obtained as orange oil, 49% yield. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.82–7.80$ (m, 3H), 7.62 (t, 1H, $J = 7.6$ Hz), 7.39–728 (m, 6H), 7.20 (m, 1H, $J = 7.6$ Hz), 6.91 (s, 1H), 6.70 (d, 2H, $J = 8.5$ Hz), 4.66 (br, H), 4.43 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.22, 165.41, 149.82, 145.12, 138.47, 136.08, 133.93, 128.86, 127.59, 127.44, 124.40, 123.03, 122.37, 121.48, 115.21, 112.90, 112.86, 47.71. HRMS calc. ($C_{22}H_{17}NO_2$): 328.1332. Found: 328.1334.

*4.1.6. Synthesis of (Z)-2-benzylidene-3-oxo-2,3-dihydrobenzofuran-6-yl trifluoromethanesulfonate (**15**)*

Compound **5a** (0.25 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and dry TEA (0.275 mmol) was added to the solution followed by Tf_2O (0.275 mmol). The mixture was stirred for 45 min at room temperature under N_2. After completion, water was added to the reaction mixture and the product was extracted with CH_2Cl_2. The organic layer was washed with water, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure permitting to obtain the pure product. Obtained as yellow solid, 98% yield. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 8.04–7.97$ (m, 4H), 7.53–7.44 (m, 4H), 7.05 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 182.17, 165.67, 154.11, 146.55, 131.69, 131.59, 130.56, 129.13, 126.56, 121.37, 118.23, 117.57, 113.51, 107.89.

*4.1.7. General procedure for the synthesis of Mannich bases **7a–d***

To a solution of (*Z*)-2-benzylidene-6-hydroxybenzofuran-3(2*H*)-one (0.29 mmol) in absolute ethanol (1 mL) was added the appropriate amine (0.32 mmol) followed by formaldehyde solution (0.32 mmol). The mixture was refluxed for 3 h30. After, the solvent was removed and the solid residue was dissolved in CH_2Cl_2 and extracted with HCl 1 M. The aqueous layer was neutralized with $NaHCO_3$ saturated solution and extracted with CH_2Cl_2. The organic layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product.

*4.1.7.1. 2-Benzylidene-6-hydroxy-7-((4-methylpiperazin-1-yl)methyl)benzofuran-3(2H)-one (**7a**).* Purified by flash chromatography (CH_2Cl_2/MeOH = 98:2). Obtained as yellow solid, 61% yield, mp 178–179 °C. Mixture of isomers $Z/E = 1{:}0.15$. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.97$ (d, 2H, $J = 7.4$ Hz), 7.54–7.49 (m, 3H), 7.45–7.41 (m, 1H), 6.77 (s, 1H), 6.65 (d, 1H), 3.90 (s, 2H), 2.50 (br, 8H), 2.18 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 181.24, 167.75, 166.19, 147.67, 132.32, 131.07, 129.57, 129.05, 124.62, 113.64, 111.48, 109.91, 105.77, 54.30, 52.00, 50.83, 45.47. Anal. Calcd. ($C_{21}H_{22}N_2O_3 \cdot 0.3H_2O$): C, 70.88; H, 6.42; N, 7.87%. Found: C, 70.75; H, 6.48; N, 7.66%.

*4.1.7.2. 2-Benzylidene-6-hydroxy-7-(piperidin-1-ylmethyl)benzofuran-3(2H)-one (**7b**).* Purified by flash chromatography (CH_2Cl_2/MeOH = 98:2). Obtained as yellow solid, 63% yield, mp 198–200 °C. Mixture of isomers Z/E = 1:0.4. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.94$ (d, 2H, $J = 7.4$ Hz), 7.51–7.41 (m, 4H), 6.70 (s, 1H), 6.50–6.45 (m, 1H), 4.06 (s, 2H), 2.82–2.76 (m, 4H), 1.63–1.48 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 180.26, 171.55, 166.39, 148.16, 132.50, 130.91, 129.05, 128.28, 124.90, 115.03, 109.47, 109.07, 103.14, 52.69, 51.91, 24.49, 22.69. HRMS calc. ($C_{21}H_{22}NO_3$): 336.1594. Found: 336.1594.

*4.1.7.3. 2-Benzylidene-6-hydroxy-7-(morpholinomethyl)benzofuran-3(2H)-one (**7c**).* Purified by flash chromatography (CH_2Cl_2/MeOH = 99:1). Obtained as yellow solid, 54% yield, 179–181 °C. Mixture of isomers Z/E = 1:0.1. ^{1}H NMR (400 MHz, acetone-d_6): $\delta = 7.99$ (d, 2H, $J = 7.3$ Hz), 7.58–7.44 (m, 4H), 6.74 (s, 1H), 6.67 (d, 1H, $J = 8.4$ Hz), 4.11 (s, 2H), 3.96–3.75 (m, 8H). ^{13}C NMR (101 MHz, acetone-d_6): δ = 167.85, 133.51, 132.06, 129.83, 125.38, 114.11, 111.03, 67.21, 53.71. Anal. Calcd. ($C_{20}H_{19}NO_4 \cdot 0.25H_2O$): C, 70.26; H, 5.76; N, 4.10%. Found: C, 70.17; H, 5.72; N, 4.24%.

*4.1.7.4. (Z)-2-Benzylidene-7-((diethylamino)methyl)-6-hydroxybenzofuran-3(2H)-one (**7d**).* Purified by flash chromatography (CH_2Cl_2/MeOH = 97:3) followed by TLC (CH_2Cl_2/MeOH = 95:5). Obtained as yellow solid, 29% yield, mp 165–167 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.83$ (d, 2H, $J = 7.3$ Hz), 7.60 (d, 1H, $J = 8.5$ Hz), 7.46 (t, 2H, $J = 7.5$ Hz), 7.40–7.38 (m, 1H), 6.78 (s, 1H), 6.62 (d, 1H, $J = 8.5$ Hz), 4.06 (s, 2H), 2.78 (q, 4H, $J = 7.2$ Hz), 1.21 (t, 6H, $J = 7.2$ Hz). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.93, 148.28, 132.88, 131.23, 129.48, 129.01, 125.22, 114.05, 112.94, 111.17, 104.51, 49.54, 47.10, 11.34.

5. Biological assays

5.1. Activity against P. falciparum W2 strain

Compounds were assayed against human red blood cells infected with 1% ring stage W2-strain *P. falciparum* synchronized with 5% sorbitol. These cells were incubated with tested compounds in 96-well plates at 37 °C for 48 h in RPMI-1640 medium supplemented with 25 mM HEPES pH 7.4, 10% heat inactivated human serum (or 0.5% Albumax/2% human serum), and 100 μM hypoxanthine under an atmosphere of 3% O_2, 5% CO_2, 91% N_2. After 48 h, the cells were fixed in 2% formaldehyde in PBS, transferred into PBS with 100 mM NH_4Cl, 0.1% Triton X-100, 1 nM YOYO-1, and then analyzed in a flow cytometer (FACSort, Beckton Dickinson; EX 488 nm, EM 520 nm). Values of IC_{50} were calculated using GraphPad PRISM software.

5.2. Hemozoin-like crystals growth inhibition

Inhibition of hemozoin-like crystals formation by tested compounds was assessed using the previously described *in vitro* method [39,40]. In short, a hemozoin-like crystals stock suspension was sonicated for 3 min and diluted in fresh broth medium to the final concentration of 2 μM (heme equivalents) in the wells of a 96-wells plate. Stock solutions of tested compounds were prepared at 25 mM in DMSO. Stock solutions of chloroquine (positive control) and gentamicin (negative control) were prepared at 100 mM in distilled water; and all were 0.22 μm-filtered previous to being diluted to 0–1000 μM final concentrations in the wells. Plates were incubated at 37 °C in a 5% CO_2 atmosphere for 5 days to observe the presence or absence of crystal growth. All tests were performed in triplicate.

5.3. In vitro drug combination assay

5.3.1. Parasite cultivation

Laboratory-adapted *P. falciparum* Dd2 (chloroquine-resistant) strain was continuously cultured as previously described by Trager and Jensen [52], with minor modifications. Briefly, parasites were cultivated on human erythrocytes suspended in RPMI 1640 medium supplemented with 25 mM HEPES, 6.8 mM hypoxanthine and 10% AlbuMAX II, at pH 7.2. Cultures were maintained at 37 °C under an atmosphere of 5% O_2, 3–5% CO_2, and N_2 and synchronized by double sorbitol treatment prior to the assays [53]. Staging and

parasitaemia were determined by light microscopy of Giemsa-stained thin blood smears.

5.3.2. In vitro antimalarial activity

The antimalarial activity of compound **7c** and chloroquine were determined using the SYBR Green I assay as previously described. Stock solutions of the drugs (10 mM) were prepared in DMSO and serially diluted in complete media. Parasitized erythrocytes at the early ring stage were added to a final 1% parasitaemia and 3% hematocrit to each triplicate well of a 96-well plate, and incubated for 48 h at 37 °C prior to growth assessment with SYBR Green I nucleic acid. Each compound was analyzed at a final concentration range of 0–50 μM (0.2% DMSO), whereas chloroquine was assayed at a concentration range of 0–10 μM. SYBR Green I fluorescence was quantified using a multi-mode microplate reader (Dynex Triad) and analyzed by nonlinear regression using GraphPad Prism 5 demo version.

5.3.3. Combination assay

Analysis of the combination effects of compound **7c** with chloroquine was determined by a modified fixed ratio isobologram method [47–50]. Initially, the 50% inhibitory concentration (IC_{50}) values of the individual test compounds were determined. Subsequently, dose–response experiments at 2-fold dilutions were run for different drug combinations (IC_{50} ratios equals 5:0, 4:1, 3:2, 2:3, 1:4 or 0:5) and their IC_{50}s in the combination determined, as described above. The fractional inhibitory concentration (FIC; FIC_{50} = IC_{50} of drug in the combination/IC_{50} of drug when tested alone) of each drug was calculated and plotted as an isobologram.

5.3.4. In vitro cytotoxicity

The cytotoxicity was assessed using general cell viability endpoint MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) [54,55]. Briefly, the day before experiment cells NIH 3T3 (mouse embryonic fibroblast cell line, ATCC CRL-1658) or HEK 293T (human embryonic kidney epithelial cell line, ATCC CRL-11268) are seeded in 96 well tissue culture plates, in RPMI 1640 culture medium supplemented with 10% Fetal serum bovine, 100 units of penicillin G (sodium salt) and 100 μg of streptomycin sulfate and 2 mM L-glutamine, at a concentration that allow cells to grow exponentially during the time of the assay. Compounds to be tested are diluted in dimethylsulfoxide (DMSO) and then serially diluted in the culture medium. Compounds at different concentrations (until reaching 100 μM) and DMSO are then added to the cells. Cells are incubated at 37 °C in humidified 5% CO_2 atmosphere. After 48 h, cell media containing DMSO (for control cells) or tested compound solution (for test cells) was removed and replaced with fresh medium containing MTT dye. After 3 h of incubation the complete media was removed and the intracellular formazan crystals were solubilized and extracted with DMSO. After 15 min at room temperature the absorbance measured at 570 nm in microplate reader.

The percentage of cell viability was determined for each concentration of tested compound as described previously [54,55]. The concentration of a compound reflecting a 50% inhibition of cell viability (i.e. IC_{50}) was determined from the concentration–response curve. This was done by applying non-linear regression procedure to the concentration response data using GraphPad PRISM software.

Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia (FCT, Portugal) through projects PTDC/SAU-FAR/118459/2010 and Pest-OE/SAL/UI4013/2011. A.S.N. and M.P.C. acknowledge FCT for grants SFRH/BD/41276/2007 and SFRH/BD/61611/2009, respectively. P.J.R. is a Doris Duke Charitable Foundation Distinguished Clinical Scientist.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.076.

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