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Probing the Azaaurone Scaffold against the Hepatic and Erythrocytic Stages of Malaria Parasites

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The potential of azaaurones as dual-stage antimalarial agents was investigated by assessing the effect of a small library of azaaurones on the inhibition of liver and intraerythrocytic life-cycle stages of the malaria parasite. The whole series was screened against the blood stage of a chloroquine-resistant *Plasmodium falciparum* strain and the liver stage of *P. berghei*, yielding compounds with dual-stage activity and sub-micromolar potency against erythrocytic parasites. Studies with genetically modified parasites, using a phenotypic assay based on the *P. falciparum* Dd2-*Sc*DHODH line, which expresses yeast dihydroorotate dehydrogenase (DHODH), showed that one of the azaaurone derivatives has the potential to inhibit the parasite mitochondrial electron-transport chain. The global urgency in finding new therapies for malaria, especially against the underexplored liver stage, associated with chemical tractability of azaaurones, warrants further development of this chemotype. Overall, these results emphasize the azaaurone chemotype as a promising scaffold for dual-stage antimalarials.

Introduction

Malaria remains a major global public health threat, and is responsible for high mortality and morbidity burdens in endemic countries. The most severe form of the disease is caused by *Plasmodium falciparum*, which accounts for most fatal cases, particularly in young children and pregnant women,[1] but *P. vivax* also contributes significantly to overall morbidity.[2] Recently reported resistance to artemisinin derivatives, which are critical components of artemisinin-based combination therapies (ACTs), the current mainstay of malaria therapy, highlights the urgent need for novel drugs acting on new or underexploited parasite targets.[3]

Discovery of novel antimalarials has been focused on targeting asexual parasites that invade host erythrocytes and cause clinical symptoms.[4] However, progress toward malaria elimination will be facilitated by intervention at multiple developmental stages of the parasite, including the asymptomatic liver stage that precedes the invasion of host erythrocytes. Targeting liver stage parasites offers advantages, as it can lead to causal prophylaxis and consequently arrest of parasitic transmission. *P. vivax* infections can generate dormant hypnozoites that persist in the liver for long periods of time and, when reactivated, lead to relapsing malaria, a threat to the goal of eliminating malaria. Accordingly, targeting the liver stage of the *Plasmodium* life-cycle is a high priority in antimalarial drug discovery.[4,5] However, despite recent advances in liver stage phenotypic screening methods, the number of scaffolds active against exoerythrocytic forms (EEFs) is still very limited.

Aurones (2-benzylidenebenzofuran-3(2*H*)-ones, **1**, Scheme 1) are secondary metabolites structurally related to flavones.[6] We[7] and others[8] have reported that aurones are active

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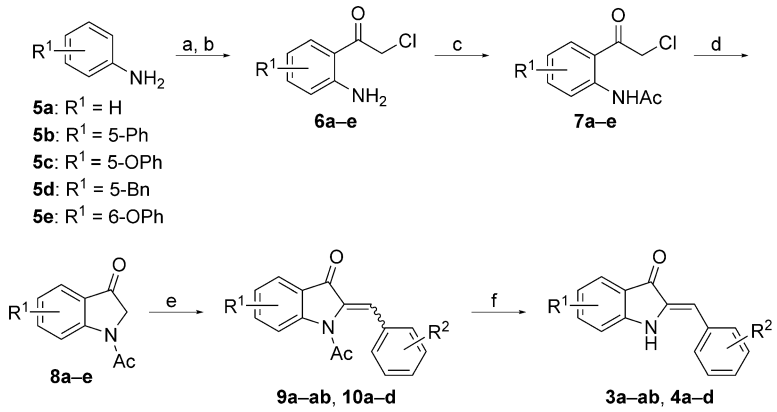


Scheme 1. Synthesis of azaaurones. *Reagents and conditions*: a) BCl_3 (1 M in CH_2Cl_2), chloroacetonitrile, $ZnCl_2$, dry 1,2-DCE, reflux; b) HCl (1 M), reflux; c) AcOH, Ac_2O; d) NaH, dry DMF; e) PhR^2CHO, piperidine (cat.), toluene, reflux; f) KOH (50% in H_2O), MeOH, RT. See Table 1 for R^1 and R^2 groups.

against intraerythrocytic *P. falciparum* in the low micromolar range and non-cytotoxic against mammalian cells. In contrast, bioisosteric azaaurones (2-benzylideneindolin-3-ones, for example, **2**, Figure 1) remain largely underexploited as antimalarial agents. Souard et al. reported that azaaurones inhibit *P. falciparum* growth in the low micromolar range.[8b] These azaaurones were inspired by naturally occurring aurones, with all analogues containing methoxy groups at the C4 and C6 positions of the A-ring. To better understand the structure-activity relationships, we designed a small library of azaaurone derivatives **3** and **4** (Table 1) with a diverse substitution pattern in rings A and B and physicochemical properties. Herein we report azaaurones as dual-stage antimalarials—that is, capable of inhibiting both erythrocytic and liver stage parasites—and describe the studies to gain insight into their mechanism of action.

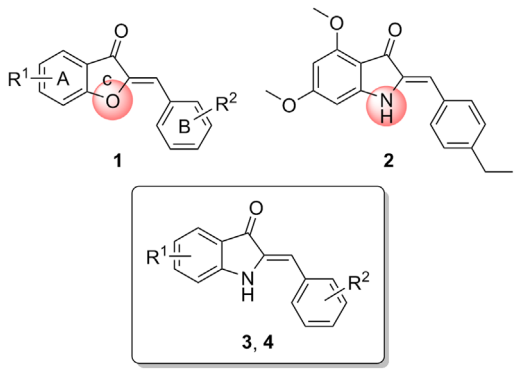


Figure 1. Structures of aurone **1**, azaaurone **2**, and azaaurones from this study, **3** (R^1 = H) and **4** (R^2 = H).

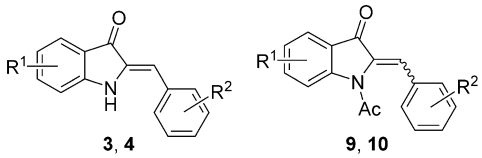
Results and Discussion

The library comprising azaaurone derivatives (Table 1) was prepared using the general synthetic approach depicted in Scheme 1, adapted from Wager and Miller.[9] First, appropriately substituted anilines **5** were reacted with chloroacetonitrile in the presence of BCl_3 and $ZnCl_2$, to form the 2-chloroacetyl derivatives **6**, which were then acetylated at the aniline nitrogen atom to give intermediates **7**.[10] Base-promoted cyclization of **7** afforded the corresponding 1-acetylindolin-3-one (3-oxindole) **8**. Piperidine-catalyzed condensation of 3-oxindoles **8**[11] with the appropriate benzaldehyde followed by the alkaline hydrolysis of the resulting *N*-acetylazaaurones **9** and **10** afforded the target azaaurones **3** and **4** in good yield. The aldehydes required to synthesize azaaurones **3d–g** were obtained from 4-fluorobenzonitrile and the appropriate phenol,[7] while those required to prepare compounds **3i–3ab** were synthesized through a standard Suzuki–Miyaura cross-coupling reaction starting from 4- or 3-bromobenzaldehyde and the appropriate boronic acids.[12]

Precursors **9** and **10** were obtained as inseparable mixtures of *Z* and *E* isomers, as revealed by the presence of two signals for the acetyl as well as for the olefinic protons in the ^{1}H NMR spectra (Supporting Information). Typically, the *Z*/*E* ratio varied from 1:0.1 to 1:0.4. In contrast, the final azaaurones were isolated as single isomers. The stereochemistry of the carbon–carbon double bond in derivatives **3** and **4** was assigned as *Z*-configuration based on both ^{1}H and ^{13}C chemical shifts for the exocyclic (β) carbon atom. These values are consistent with those previously reported by Souard et al.[8b] for (*Z*)-azaaurones. Similar to aurones, it is expected that the *Z* isomer is predominant, as this is regarded as the thermodynamically more stable form.[13] Furthermore, the crystallographic structure of compound **3j** allowed us to confirm that azaaurones are obtained as *Z* isomers (Figure 2).

Azaaurones **3** and **4** and their *N*-acetyl precursors **9** and **10** were first screened for antiplasmodial activity against the chloroquine-resistant *P. falciparum* W2 strain. The most promising compounds were also assayed against human embryonic kidney (HEK) 293T cells for potential cytotoxicity. In general, compounds **3** and **4** revealed moderate to high potency against intraerythrocytic *P. falciparum*, with EC_{50} values within the sub-micromolar range (Table 1). With selectivity indices relative to HEK293T cells typically >200, azaaurones can thus be considered potent and selective antiplasmodial agents. These

Table 1. Structures, activity against the chloroquine (CQ)-resistant *P. falciparum* W2 strain, and cytotoxicity toward HEK293T cells for azaaurones **3** and **4**, and the corresponding *N*-acetylazaaurone precursors **9** and **10**.



3, **4** **9**, **10**

Compd	R^1	R^2	EC_{50} [μM][a] *Pf*(W2)	HEK293T	Compd	R^1	R^2	EC_{50} [μM][a] *Pf*(W2)	HEK293T
3a	H	H	1.76 ± 0.11	> 100	**9a**	H	H	> 10	ND
3b	H	4-Br	0.56 ± 0.02	> 100	**9b**	H	4-Br	5.99 ± 0.02	ND
3c	H	4-NMe_2	2.90 ± 0.28	> 100	**9c**	H	4-NMe_2	> 10	ND
3d	H	4-OPh	0.36 ± 0.01	> 100	**9d**	H	4-OPh	5.02 ± 0.23	ND
3e	H	4-(OC_6H_4-4′-Me)	0.32 ± 0.04	> 100	**9e**	H	4-(OC_6H_4-4′-Me)	5.13 ± 0.34	ND
3f	H	4-(OC_6H_4-4′-Cl)	0.51 ± 0.01	> 100	**9f**	H	4-(OC_6H_4-4′-Cl)	> 10	ND
3g	H	4-(OC_6H_4-4′-CF_3)	0.59 ± 0.02	> 100	**9h**	H	4-OBn	> 10	ND
3h	H	4-OBn	1.62 ± 0.07	> 100	**9i**	H	4-Ph	8.42 ± 0.02	ND
3i	H	4-Ph	0.31 ± 0.04	> 100	**9j**	H	4-(C_6H_4-4′-F)	4.59 ± 0.35	7
3j	H	4-(C_6H_4-4′-F)	0.27 ± 0.01	> 100	**9k**	H	4-(C_6H_4-4′-Me)	3.78 ± 0.31	9
3k	H	4-(C_6H_4-4′-Me)	0.53 ± 0.01	> 100	**9l**	H	4-(C_6H_4-4′-Cl)	5.64 ± 0.17	ND
3l	H	4-(C_6H_4-4′-Cl)	0.83 ± 0.08	> 100	**9m**	H	4-(C_6H_4-4′-CF_3)	5.06 ± 0.06	ND
3m	H	4-(C_6H_4-4′-CF_3)	0.73 ± 0.06	> 100	**9n**	H	4-(C_6H_4-4′-OCF_3)	3.21 ± 0.01	8
3n	H	4-(C_6H_4-4′-OCF_3)	1.08 ± 0.06	> 100	**9o**	H	4-Bn	> 10	ND
3o	H	4-Bn	0.52 ± 0.02	> 100	**9p**	H	4-(3′-quinolyl)	2.03 ± 0.08	19
3p	H	4-(3′-quinolyl)	0.44 ± 0.01	22	**9q**	H	4-(3′-thiophenyl)	9.83 ± 0.24	ND
3q	H	4-(3′-thiophenyl)	0.48 ± 0.01	> 100	**9r**	H	4-(1′-methyl-1*H*-pyrazolyl)	9.43 ± 0.81	ND
3r	H	4-(1′-methyl-1*H*-pyrazolyl)	1.35 ± 0.16	> 100	**9s**	H	3-Ph	9.78 ± 0.07	ND
3s	H	3-Ph	0.45 ± 0.02	> 100	**9t**	H	3-(C_6H_4-4′-F)	9.38 ± 0.88	ND
3t	H	3-(C_6H_4-4′-F)	0.26 ± 0.01	> 100	**9u**	H	3-(C_6H_4-4′-Me)	> 10	4
3u	H	3-(C_6H_4-4′-Me)	0.20 ± 0.02	> 100	**9v**	H	3-(C_6H_4-4′-Cl)	3.65 ± 0.36	ND
3v	H	3-(C_6H_4-4′-Cl)	0.20 ± 0.01	20	**9w**	H	3-(C_6H_4-4′-CF_3)	6.12 ± 0.32	ND
3w	H	3-(C_6H_4-4′-CF_3)	0.11 ± 0.01	> 100	**9x**	H	3-(C_6H_4-4′-OCF_3)	5.08 ± 0.01	ND
3x	H	3-(C_6H_4-4′-OCF_3)	0.99 ± 0.15	> 100	**9y**	H	3-Bn	> 10	ND
3y	H	3-Bn	0.76 ± 0.04	> 100	**9z**	H	3-(3′-quinolyl)	3.92 ± 0.51	14
3z	H	3-(3′-quinolyl)	0.26 ± 0.01	> 100	**9aa**	H	3-(3′-thiophenyl)	8.74 ± 1.60	ND
3aa	H	3-(3′-thiophenyl)	0.47 ± 0.05	> 100	**9ab**	H	3-(1′-methyl-1*H*-pyrazolyl)	> 10	ND
3ab	H	3-(1′-methyl-1*H*-pyrazolyl)	0.34 ± 0.03	> 100	**10a**	5-Ph	H	3.75 ± 0.10	12
4a	5-Ph	H	0.35 ± 0.07	> 100	**10b**	5-OPh	H	> 10	ND
4b	5-OPh	H	0.25 ± 0.06	> 100	**10c**	5-Bn	H	6.77 ± 0.78	ND
4c	5-Bn	H	0.17 ± 0.01	> 100	**10d**	6-OPh	H	> 10	ND
4d	6-OPh	H	0.13 ± 0.01	> 100	CQ			0.14	> 100

[a] Values are the mean ± SD of three independent experiments; ND: not determined.

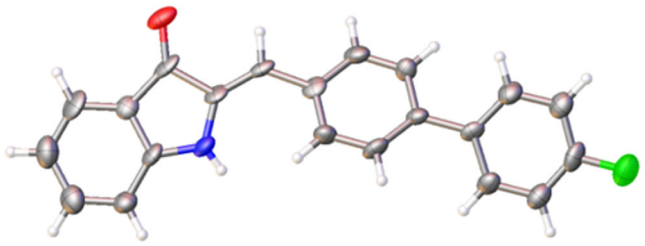


Figure 2. Crystal structure of compound **3j** determined by single-crystal X-ray diffraction data; ellipsoids are set at 50% probability.

results contrast sharply with those obtained for the *N*-acetyl counterparts **9** and **10**, which were shown to be poorly active, with IC_{50} values typically > 5 μM, and cytotoxic (Table 1). This result suggests that the azaaurone NH group is essential to attain good levels of antiplasmodial activity. Interestingly, this is also consistent with the poor-to-moderate activity also displayed by their isosteric aurone counterparts.[8b]

Inspection of data presented in Table 1 reveals that appending an aromatic moiety to ring B leads to improvement in antiplasmodial activity when compared with the parent compound **3a**. For example, the 4- (**3i**) and 3-phenyl (**3s**) derivatives were about six- and four-fold more active than the unsubstituted compound **3a**, respectively. The most potent compounds in the **3** series, **3u**–**3w**, contain the second aromatic moiety appended to the *meta* position of ring B. The azaaurone derivative containing the quinolin-3-yl substituent at the *meta* position, **3z**, was also more potent than its *para*-substituted counterpart, **3p**. Incorporating an oxygen or methylene linker between the two aromatic moieties did not significantly affect activity, as compound **3d**, containing a 4-phenoxy group, and its isosteric 4-benzyl analogue, **3o**, were equipotent to their 4-phenyl counterpart, **3i**. In contrast, the 4-benzyloxy derivative,

3h, was nearly five times less potent than its 4-phenoxy counterpart, **3d**, suggesting that additional flexibility is detrimental.

Compounds **4a–d**, containing an additional aromatic substituent in ring A, displayed EC_{50} values ranging from 0.13 to 0.35 μM, indicating that derivatization at this side of the azaaurone scaffold also leads to improved antiplasmodial activity. The 5-phenoxy (**4b**) and 5-benzyl (**4c**) derivatives were more potent than the 5-phenyl analogue (**4a**), suggesting that an oxygen linker is beneficial to activity. Azaaurone **4d**, containing a 6-phenoxy substituent, was the most potent compound in this series, with a selectivity index of >770 and with no detectable aggregation up to 10 μM (Supporting Information), which could have led to measuring artifacts and false positive results. In contrast, the *N*-acetyl counterparts were at least 10 times less potent in inhibiting *P. falciparum* growth (e.g., **4c**,**d** vs. **10c**,**d**).

Next, azaaurones **3** and **4** were evaluated for their ability to inhibit the development of liver stage parasites. Compounds were first assayed at three different concentrations (1, 5, and 10 μM) using an in vitro infection model that employs a human hepatoma cell line (Huh7) infected with firefly-luciferase-expressing *P. berghei*. Results were compared with those for primaquine (Supporting Information). From the 32 azaaurones studied, 15 led to at least 50% inhibition of the infection at 1 μM relative to controls, while almost all suppressed infection at 10 μM without significantly affecting Huh7 cell proliferation. With these promising results in hand, we then determined the EC_{50} values against the liver stage of *P. berghei* infection of Huh7 cells for the most potent compounds. Inspection of Table 2 shows that azaaurones **3n** and **3x**, both containing a 4-OCF_3 group at the aromatic group appended to ring B, present sub-micromolar EC_{50} values for the liver stage inhibitory activity.

Table 2. EC_{50} values of selected azaaurones, primaquine (PQ), and atovaquone (ATV) for inhibiting the infection of human hepatoma (Huh7) cells by *P. berghei*.

Compd	EC_{50} [μM][a]	Compd	EC_{50} [μM][a]
3h	2.62 ± 1.22	**3s**	2.49 ± 0.21
3i	3.05 ± 0.14	**3t**	1.54 ± 0.29
3j	2.07 ± 0.15	**3w**	0.81 ± 0.20
3k	1.63 ± 0.52	**3x**	0.53 ± 0.26
3n	0.75 ± 0.19	**3y**	1.80 ± 0.53
3o	1.03 ± 0.13	PQ	9.50 ± 2.30
3p	3.00 ± 0.65	ATV	0.0011 ± 0.003

[a] Values are the mean ± SD of three independent experiments.

Azaaurones are structurally related to quinolin-4(1*H*)-ones, and several compounds of this later class have shown antiplasmodial activity, via inhibition of cytochrome bc_1, a key component of the mitochondrial electron-transport chain (mtETC).[14] Atovaquone, the only cytochrome bc_1 inhibitor in clinical use, is active against both erythrocytic and hepatic forms of malaria parasites.[15] Hence, we investigated whether the activity of azaaurones was a consequence of bc_1 complex inhibition by screening several of the most potent compounds in series **3** and **4** using a phenotypic assay based on the *P. falciparum* Dd2-*Sc*DHODH line, which expresses yeast dihydroorotate dehydrogenase (DHODH).[15,16] This parasite, which uses fumarate instead of ubiquinone as the final electron acceptor, has been shown previously to be resistant to mtETC inhibitors.[17] The results presented in Table 3 indicate that most of the tested compounds display similar antiplasmodial activity against both Dd2-*Sc*DHODH and parental Dd2 strains, suggesting that inhibition of the bc_1 complex is not the primary antimalarial mode of action for these azaaurones. The exception was compound **3j**, which showed an inhibitory profile similar to that of atovaquone (Figure 3).

Table 3. Growth inhibition of parental (Dd2) and transgenic (Dd2-*Sc*DHODH) *P. falciparum* strains with azaaurones.

Compd	EC_{50} [μM][a]	
	Dd2	Dd2-*Sc*DHODH
3e	1.46 ± 0.25	1.62 ± 0.36
3j	1.60 ± 0.22	>1000
3t	0.414 ± 0.08	0.343 ± 0.02
3v	1.24 ± 0.14	1.25 ± 0.13
3w	0.800 ± 0.09	0.786 ± 0.07
3z	0.573 ± 0.05	0.493 ± 0.07
4a	1.66 ± 0.40	2.70 ± 0.28
4b	2.62 ± 0.22	4.34 ± 1.22
4c	1.33 ± 0.11	0.906 ± 0.08
4d	1.04 ± 0.10	0.940 ± 0.06
atovaquone	0.000298	>1000
artesunate	0.00287	0.00299

[a] Values are the mean ± SD of three independent experiments.

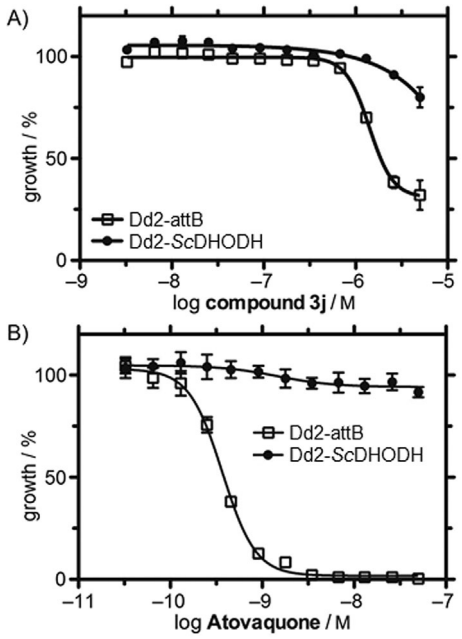


Figure 3. Dose–effect curves for A) azaaurone **3j** and B) atovaquone against Dd2 and Dd2-*Sc*DHODH parasite strains. Wild-type Dd2 (□) and Dd2-*Sc*DHODH (●) parasite strains were grown in the presence of various drug concentrations for 48 h. Growth was assessed by [^{3}H]hypoxanthine incorporation. Growth in the absence of drug was set at 100%. Data are the mean ± SD of triplicate experiments performed at each condition.

Azaaurones contain a Michael acceptor moiety, a structural motif commonly found in cysteine protease inhibitors[18] that have been reported to block the development of cultured erythrocytic parasites and to cure murine malaria.[19] Falcipains are the best characterized cysteine proteases of *P. falciparum*, with falcipain-2 playing a crucial role in hydrolyzing host hemoglobin into amino acids essential to parasite growth during erythrocytic stages of development.[20] We therefore screened azaaurones **3** and **4** for falcipain-2 inhibition; however none of the compounds displayed noticeable activity up to 50 μM (data not shown), ruling out this enzyme as drug target.

To evaluate the potential of azaaurones to undergo Michael addition with thiols we monitored the reaction of compound **3q** with the model thiol, β-mercaptoethanol by ^{1}H NMR. Analysis of the NMR spectra revealed that consumption of **3q** was triggered by addition of triethylamine (1 mol equiv) to the reaction mixture, resulting in the formation of an adduct in a 85:15 ratio to the starting azaaurone. This adduct slowly reverted to the starting compound **3q**, reaching a 60:40 ratio in three days of incubation, suggesting that azaaurones react in reversible manner with cysteine residues, which may explain, at least partially, the lack of inhibition of falcipain-2. These results are in line with those observed for other scaffolds containing an exocyclic Michael acceptor system such as alkylidene rhodanines, which have been reported to react reversibly with cysteine residues in catalytic and allosteric binding sites.[21]

Conclusions

We have identified, for the first time, azaaurones as a new class of antimalarials active against the erythrocytic and liver stages of the malaria parasite. Several compounds displayed EC_{50} values against the liver and asexual blood stages within the same order of magnitude. Although one azaaurone has shown to inhibit the parasite's mtETC, the primary mechanism of action of azaaurones does not appear to involve inhibition of the bc_1 complex. These results suggest that azaaurones may modulate other targets such as kinases. There is a striking resemblance of azaaurones to oxindole inhibitors (e.g., sunitinib) that are active against *Plasmodium*[22] and used clinically for other purposes. The same substitution pattern as in the azaaurones is also found in indirubin kinase inhibitors, which are also active against *P. falciparum*.[23]

The global urgency in finding new therapies for malaria, especially against the underexplored liver stage, associated with the dual activity and chemical tractability of azaaurones, warrants further development of this chemotype. In spite of the concern of potential nonspecific target modulation resulting from the reactivity of the exocyclic Michael acceptors,[24,25] several examples of successful progression of the analogous alkylidene rhodamine-like scaffolds into lead compounds have been reported.[26,27] Overall, our study has revealed azaaurone starting points for lead optimization of dual-stage antimalarials.

Experimental Section

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. Reactions were monitored by analytical thin-layer chromatography (TLC), performed on pre-coated Merck silica gel 60 F_{254} plates. 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 NMR chemical shifts (δ) are expressed in parts per million (ppm) referenced to the solvent used, and proton coupling constants (J) are reported in hertz (Hz). The spin multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), and broad (br). The purity of final compounds was determined by elemental analyses conducted on a CE Instruments EA 1110 apparatus and confirmed to be $\geq$95%.

General procedure for the synthesis of azaaurone derivatives 9a–ab and 10a–d: To a solution of the appropriate 1-acetylindolin-3-one derivative (see Supporting Information for the synthesis of **8a–e**) (0.5 mmol) in toluene (5 mL) at room temperature was added the proper aldehyde (1.2 mmol) and piperidine (1 drop). The mixture was held at reflux for 24 h. After reaction completion, the solvent was removed to provide the crude product.

1-acetyl-2-benzylideneindolin-3-one (9a): Purified by flash chromatography (hexane/EtOAc = 95:5). Obtained as a light-yellow oil, yield: 72%. Mixture of isomers Z/E = 1:0.60. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.30 (d, J = 8.3 Hz, 1H), 7.86 (d, J = 7.6 Hz, 2H), 7.70–7.61 (m, 1H), 7.55 (d, J = 7.6 Hz, 2H), 7.45–7.38 (m, 2H), 7.35 (s, 1H), 7.31 (t, J = 7.6 Hz, 1H), 1.96 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.0, 170.5, 150.2, 136.4, 135.0, 133.9, 130.2, 129.9, 129.2, 125.0, 124.2, 123.9, 122.4, 117.9, 25.1 ppm.

1-acetyl-2-(4-bromobenzylidene)indolin-3-one (9b): Purified by flash chromatography (hexane/EtOAc = 80:20). Obtained as yellow solid, yield: 64%, mp: 138–141 °C. Mixture of isomers Z/E = 1:0.70. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.25 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 8.1 Hz, 1H), 7.69–7.64 (m, 1H), 7.60–7.54 (m, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.34–7.29 (m, 1H), 7.27 (s, 1H), 2.03 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 185.8, 170.2, 150.5, 136.6, 135.3, 133.1, 132.6, 131.7, 125.3, 124.4, 124.3, 124.1, 121.0, 118.0, 26.7 ppm.

1-acetyl-2-(4-(dimethylamino)benzylidene)indolin-3-one (9c): Purified by flash chromatography (CH_2Cl_2/MeOH = 99:1). Obtained as yellow solid, yield: 70%, mp: 147–150 °C. Mixture of isomers Z/E = 1:0.20. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.30 (d, J = 8.3 Hz, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.64 (t, J = 8.3 Hz, 1H), 7.48 (d, J = 8.8 Hz, 2H), 7.33–7.28 (m, 2H), 6.70 (d, J = 8.8 Hz, 2H), 3.06 (s, 6H), 2.14 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 185.9, 171.5, 151.4, 149.7, 135.7, 133.1, 131.812, 125.1, 124.9, 124.7, 123.9, 120.5, 117.9, 112.0, 40.2, 25.5 ppm.

1-acetyl-2-(4-phenoxybenzylidene)indolin-3-one (9d): Purified by flash chromatography (hexane/CH_2Cl_2 = 50:50). Obtained as yellow oil, yield: 57%. Mixture of isomers Z/E = 1:0.30. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.33 (d, J = 8.1 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.71 (t, J = 8.1 Hz, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.39–7.35 (m, 3H), 7.29–7.24 (m, 2H), 7.10–7.07 (m, 4H), 2.15 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 184.1, 168.9, 159.1, 154.1, 150.0, 135.8, 134.2, 133.8, 131.5, 129.3, 129.0, 128.1, 126.7, 124.1, 122.3, 121.2, 119.2, 117.6, 26.6 ppm.

1-acetyl-2-(4-(*p*-tolyloxy)benzylidene)indolin-3-one (9e): Purified by flash chromatography (hexane/CH_2Cl_2 = 50:50). Obtained as yellow oil, yield: 53%. Mixture of isomers *Z*/*E* = 1:0.25. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.27 (d, *J* = 8.2 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.71–7.66 (m, 3H), 7.29–7.34 (m, 4H), 7.01–6.98 (m, 4H), 2.18 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 183.9, 167.0, 158.7, 153.8, 148.7, 134.6, 134.3, 132.3, 131.6, 129.9, 128.9, 127.6, 126.1, 123.9, 122.6, 121.6, 120.0, 118.3, 25.9, 21.2 ppm.

1-acetyl-2-(4-(4-chlorophenoxy)benzylidene)indolin-3-one (9f): Purified by flash chromatography (hexane/CH_2Cl_2 = 50:50). Obtained as yellow solid, yield: 61%, mp: 108–111 °C. Mixture of isomers *Z*/*E* = 1:0.70. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.26 (d, *J* = 8.1 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.69–7.62 (m, 1H), 7.55–7.52 (m, 2H), 7.36–7.31 (m, 3H), 7.03–7.00 (m, 5H), 2.07 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.0, 170.5, 158.9, 154.4, 150.3, 136.4, 134.5, 133.5, 132.5, 130.2, 128.7, 127.3, 125.1, 124.3, 124.3, 121.3, 118.6, 117.6, 25.3 ppm.

1-acetyl-2-(4-(4-(trifluoromethyl)phenoxy)benzylidene)indolin-3-one (9g): Purified by flash chromatography (hexane/CH_2Cl_2 = 50:50). Obtained as yellow oil, yield: 53%. Mixture of isomers *Z*/*E* = 1:0.20. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.28 (d, *J* = 8.1 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.71–7.63 (m, 3H), 7.41–7.32 (m, 3H), 7.02–6.98 (m, 5H), 2.18 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 169.9, 163.6, 158.8, 153.7, 149.7, 135.6, 134.0, 133.9, 132.0, 130.4, 128.6, 127.7, 124.9, 123.7, 123.5, 120.4, 118.3, 117.8, 24.8 ppm.

1-acetyl-2-(4-(benzyloxy)benzylidene)indolin-3-one (9h): Purified by flash chromatography (hexane/EtOAc = 95:5). Obtained as yellow solid, yield: 57%, mp: 151–154 °C. Mixture of isomers *Z*/*E* = 1:0.10. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.29 (d, *J* = 8.3 Hz, 1H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.67 (t, *J* = 8.2 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.45–7.28 (m, 7H), 7.04 (d, *J* = 8.5 Hz, 2H), 5.11 (s, 2H), 2.06 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.9, 160.2, 150.2, 136.3, 133.8, 132.5, 128.8, 128.4, 127.7, 126.4, 125.0, 124.4, 124.2, 123.0, 118.0, 115.7, 70.3, 25.3 ppm.

1-acetyl-2-(biphenyl-4-ylmethylene)indolin-3-one (9i): Purified by flash chromatography (hexane/EtOAc = 80:20). Obtained as yellow solid, yield: 94%, mp: 172–175 °C. Mixture of isomers *Z*/*E* = 1:0.10. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.31 (d, *J* = 8.3 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.71–7.60 (m, 6H), 7.48 (*t*, *J* = 6.9 Hz, 2H), 7.41–7.39 (m, 2H), 7.32 (*t*, *J* = 8.2 Hz, 1H), 7.27 (s, 1H), 2.06 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.7, 150.4, 142.7, 139.8, 136.5, 135.0, 132.8, 131.0, 129.1, 128.2, 127.9, 127.2, 125.1, 124.3, 124.1, 122.3, 118.0, 25.4 ppm.

1-acetyl-2-((4′-fluorobiphenyl-4-yl)methylene)indolin-3-one (9j): Purified by flash chromatography (hexane/EtOAc = 80:20). Obtained as yellow solid, yield: 80%, mp: 70–73 °C. Mixture of isomers *Z*/*E* = 1:0.40. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.30 (d, *J* = 8.3 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.69 (t, *J* = 8.3 Hz, 1H), 7.65–7.58 (m, 6H), 7.37 (s, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.18–7.14 (m, 2H), 2.06 ppm (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.6, 163.0 (d, *J* = 268.7 Hz), 150.3, 141.7, 136.5, 135.9, 135.1, 132.9, 131.0, 128.8 (d, *J* = 8.1 Hz), 127.7, 125.2, 124.3, 124.1, 122.1, 118.0, 116.1 (d, *J* = 22.2 Hz), 25.4 ppm.

1-acetyl-2-((4′-methylbiphenyl-4-yl)methylene)indolin-3-one (9k): Purified by flash chromatography (hexane/EtOAc = 90:10). Obtained as yellow solid, yield: 65%, mp: 135–138 °C. Mixture of isomers *Z*/*E* = 1:0.50. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.15 (d, *J* = 8.2 Hz, 1H), 7.98 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.69–7.60 (m, 3H), 7.57–7.52 (m, 3H), 7.34–7.27 (m, 3H), 2.41 (s, 3H), 2.06 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.0, 169.3, 148.3, 143.0, 138.2, 137.9, 137.6, 136.0, 134.9, 132.0, 130.9, 130.2, 129.7, 127.1, 126.5, 124.8, 124.3, 117.5, 26.6, 25.4 ppm.

1-acetyl-2-((4′-chlorobiphenyl-4-yl)methylene)indolin-3-one (9l): Purified by flash chromatography (hexane/EtOAc = 80:20). Obtained as yellow solid, yield: 75%, mp: 107–110 °C. Mixture of isomers *Z*/*E* = 1:0.35. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.29 (d, *J* = 8.2 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.71–7.55 (m, 7H), 7.45–7.42 (m, 2H), 7.37 (s, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 2.06 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.0, 170.6, 150.3, 141.4, 138.3, 136.6, 135.1, 134.4, 133.2, 131.1, 129.3, 128.4, 127.7, 125.2, 124.4, 124.1, 122.0, 118.0, 25.4 ppm.

1-acetyl-2-((4′-(trifluoromethyl)biphenyl-4-yl)methylene)indolin-3-one (9m): Purified by flash chromatography (hexane/EtOAc = 95:5). Obtained as yellow solid, yield: 78%, mp: 124–127 °C. Mixture of isomers *Z*/*E* = 1:0.80. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.09 (d, *J* = 8.2 Hz, 1H), 7.98 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.76–7.64 (m, 8H), 7.34–7.27 (m, 1H), 2.06 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.5, 162.751, 148.4, 141.2, 136.2, 135.4, 134.0 (q, *J* = 32.1 Hz), 132.3, 131.9, 129.5, 127.5, 126.9, 126.0, 124.8 (q, *J* = 271.2 Hz), 124.7, 124.1, 121.7, 117.4, 25.4 ppm.

1-acetyl-2-((4′-(trifluoromethoxy)biphenyl-4-yl)methylene)indolin-3-one (9n): Purified by flash chromatography (hexane/EtOAc = 95:5). Obtained as yellow solid, yield: 71%, mp: 121–124 °C. Mixture of isomers *Z*/*E* = 1:0.60. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.29 (d, *J* = 8.2 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.72–7.62 (m, 7H), 7.38 (s, 1H), 7.34–7.31 (m, 3H), 2.06 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.4, 166.5, 150.1, 141.2, 138.6, 136.6, 133.1, 131.6, 131.1 (q, *J* = 253.4 Hz), 129.7, 128.6, 127.8, 126.7, 124.4, 123.9, 121.9, 121.5, 118.0, 25.6 ppm.

1-acetyl-2-(4-benzylbenzylidene)indolin-3-one (9o): Purified by flash chromatography (hexane/EtOAc = 92:8). Obtained as yellow oil, yield: 73%. Mixture of isomers *Z*/*E* = 1:0.40. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.30 (d, *J* = 8.2 Hz, 1H), 7.87–7.84 (m, 1H), 7.69–7.64 (m, 1H), 7.50–7.47 (m, 2H), 7.33–7.18 (m, 9H), 4.02 (s, 2H), 2.00 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.7, 150.4, 143.7, 140.1, 136.4, 134.8, 131.8, 130.6, 129.9, 129.1, 128.8, 126.5, 125.0, 124.2, 124.1, 122.6, 118.0, 41.9, 25.2 ppm.

1-acetyl-2-(4-(quinolin-3-yl)benzylidene)indolin-3-one (9p): Purified by flash chromatography (hexane/EtOAc = 70:30). Obtained as yellow solid, yield: 69%, mp: 168–171 °C. Mixture of isomers *Z*/*E* = 1:0.70. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 9.25 (s, 1H), 8.39 (s, 1H), 8.29 (d, *J* = 8.3 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 7.93–7.88 (m, 2H), 7.84–7.60 (m, 7H), 7.40 (s, 1H), 7.33 (*t*, *J* = 7.5 Hz, 1H), 2.07 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.2, 170.5, 150.4, 149.5, 147.7, 139.3, 137.8, 136.6, 136.2, 135.4, 133.9, 133.7, 131.3, 129.4, 128.1, 128.0, 126.9, 125.2, 124.8, 124.4, 123.6, 122.7, 118.0, 25.4 ppm.

1-acetyl-2-(4-(thiophen-3-yl)benzylidene)indolin-3-one (9q): Purified by flash chromatography (hexane/EtOAc = 70:30). Obtained as yellow solid, yield: 72%, mp: 126–129 °C. Mixture of isomers *Z*/*E* = 1:0.25. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.30 (d, *J* = 8.2 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.70–7.56 (m, 6H), 7.45 (s, 2H), 7.36 (s, 1H), 7.31 (t, *J* = 8.1 Hz), 2.05 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.2, 170.8, 150.4, 137.3,

136.5, 135.0, 134.4, 132.6, 131.1, 127.1, 126.9, 126.1, 125.1, 124.3, 124.2, 122.3, 118.0, 25.3 ppm.

1-acetyl-2-(4-(1-methyl-1*H*-pyrazol-4-yl)benzylidene)indolin-3-one (9r): Purified by flash chromatography (CH_2Cl_2/MeOH = 99:1). Obtained as yellow solid, yield: 77%, mp: 161–164 °C. Mixture of isomers *Z*/*E* = 1:0.20. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.29 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 8.1 Hz, 1H), 7.81 (s, 1H), 7.69–7.65 (m, 2H), 7.54–7.52 (m, 4H), 7.33–7.29 (m, 2H), 3.96 (s, 3H), 2.05 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.8, 150.3, 137.1, 136.4, 134.7, 134.5, 131.7, 131.2, 127.5, 126.0, 125.1, 124.3, 124.2, 122.5, 122.3, 118.0, 39.4, 25.3 ppm.

1-acetyl-2-(biphenyl-3-ylmethylene)indolin-3-one (9s): Purified by flash chromatography (hexane/EtOAc = 80:20). Obtained as yellow solid, yield: 65%, mp: 100–103 °C. Mixture of isomers *Z*/*E* = 1:0.30. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.31 (d, *J* = 8.3 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.76 (s, 1H), 7.71–7.67 (m, 1H), 7.63–7.52 (m, 5H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.41–7.37 (m, 2H), 7.32 (t, *J* = 8.2 Hz, 1H), 2.03 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.6, 150.4, 142.4, 140.0, 136.6, 135.3, 134.5, 129.8, 129.1, 129.0, 128.9, 128.8, 128.0, 127.2, 125.1, 124.4, 124.0, 122.3, 117.9, 25.4 ppm.

1-acetyl-2-((4′-fluorobiphenyl-3-yl)methylene)indolin-3-one (9t): Purified by flash chromatography (hexane/EtOAc = 80:20). Obtained as yellow solid, yield: 69%, mp: 165–168 °C. Mixture of isomers *Z*/*E* = 1:0.15. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.30 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.71–7.49 (m, 7H), 7.39 (s, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.16 (t, *J* = 8.4 Hz, 2H), 2.02 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.0, 170.6, 164.1 (d, *J* = 269.1 Hz), 161.7, 150.4, 141.4, 136.6, 135.4, 134.7, 129.9, 128.9, 128.8, 128.6 (d, *J* = 7.9 Hz), 125.1, 124.4, 123.9, 122.1, 117.9, 116.2, 116.0 (d, *J* = 22.3 Hz), 25.4 ppm.

1-acetyl-2-((4′-methylbiphenyl-3-yl)methylene)indolin-3-one (9u): Purified by flash chromatography (hexane/EtOAc = 90:10). Obtained as yellow oil, yield: 62%. Mixture of isomers *Z*/*E* = 1:0.40. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.16–8.12 (m, 2H), 7.81–7.79 (m, 2H), 7.69–7.57 (m, 4H), 7.51–7.47 (m, 2H), 7.29–7.26 (m, 3H), 2.41 (s, 3H), 2.02 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 169.3, 148.3, 141.0, 137.9, 137.4, 136.1, 135.2, 132.5, 139.2, 129.8, 129.7, 128.8, 128.5, 127.2, 124.8, 124.7, 124.3, 122.4, 117.5, 26.5, 25.3 ppm.

1-acetyl-2-((4′-chlorobiphenyl-3-yl)methylene)indolin-3-one (9v): Purified by flash chromatography (hexane/EtOAc = 80:20). Obtained as yellow solid, yield: 66%, mp: 180–183 °C. Mixture of isomers *Z*/*E* = 1:0.20. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.29 (d, *J* = 8.3 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.70–7.50 (m, 7H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.39 (s, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 2.02 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.0, 170.5, 150.4, 141.2, 138.5, 136.7, 135.4, 134.7, 134.2, 129.9, 129.3, 129.2, 128.8, 128.6, 128.5, 125.2, 124.4, 123.9, 122.0, 117.9, 25.4 ppm.

1-acetyl-2-((4′-(trifluoromethyl)biphenyl-3-yl)methylene)indolin-3-one (9w): Purified by flash chromatography (hexane/EtOAc = 95:5). Obtained as yellow solid, yield: 75%, mp: 144–145 °C. Mixture of isomers *Z*/*E* = 1:0.80. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.28 (d, *J* = 8.2 Hz, 1H), 8.21 (s, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 7.81–7.51 (m, 8H), 7.40 (s, 1H), 7.32 (t, *J* = 8.1 Hz, 1H), 2.04 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.0, 170.5, 150.4, 148.3, 140.9, 139.5, 136.3, 135.4, 134.9 (q, *J* = 31.9 Hz), 132.9, 130.8, 130.1, 129.7, 128.7, 127.7, 125.9, 124.9 (q, *J* = 272.1 Hz), 124.5, 123.9, 121.7, 117.4, 26.6 ppm.

1-acetyl-2-((4′-(trifluoromethoxy)biphenyl-3-yl)methylene)indolin-3-one (9x): Purified by flash chromatography (hexane/EtOAc = 90:10). Obtained as yellow solid, yield: 72%, mp: 115–117 °C. Mixture of isomers *Z*/*E* = 1:0.45. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.18 (s, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 7.82–7.49 (m, 7H), 7.32–7.27 (m, 4H), 2.06 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 169.3, 151.4, 150.4, 148.4, 138.6, 138.8, 136.2, 132.8, 130.4 (q, *J* = 254.1 Hz), 130.0, 129.8, 129.6, 128.8, 128.7, 124.9, 124.7, 124.5, 121.6, 121.4, 117.4, 26.9 ppm.

1-acetyl-2-(3-benzylbenzylidene)indolin-3-one (9y): Purified by flash chromatography (hexane/EtOAc = 92:8). Obtained as yellow oil, yield: 72%. Mixture of isomers *Z*/*E* = 1:0.40. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.29 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.70–7.62 (m, 2H), 7.41–7.19 (m, 10H), 4.01 (s, 2H), 1.94 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.5, 150.4, 142.5, 140.3, 136.5, 135.1, 134.2, 130.8, 130.7, 129.5, 129.0, 128.8, 129.1, 126.5, 125.0, 124.3, 124.0, 122.5, 118.0, 41.9, 25.2 ppm.

1-acetyl-2-(3-(quinolin-3-yl)benzylidene)indolin-3-one (9z): Purified by flash chromatography (hexane/EtOAc = 70:30). Obtained as yellow solid, yield: 62%, mp: 145–148 °C. Mixture of isomers *Z*/*E* = 1:0.70. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 9.27 (s, 1H), 8.43 (s, 1H), 8.28 (d, *J* = 8.2 Hz, 1H), 8.80 (d, *J* = 8.0 Hz, 1H), 7.94–7.57 (m, 10H), 7.32 (t, *J* = 7.5 Hz, 1H), 2.07 ppm (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.0, 169.4, 150.5, 149.9, 148.3, 139.1, 137.6, 136.2, 135.2, 133.8, 133.2, 130.7, 130.2, 129.7, 129.5, 129.2, 128.9, 128.3, 127.2, 124.9, 124.7, 124.5, 124.0, 122.0, 117.4, 25.4 ppm.

1-acetyl-2-(3-(thiophen-3-yl)benzylidene)indolin-3-one (9aa): Purified by flash chromatography (hexane/EtOAc = 70:30). Obtained as yellow solid, yield: 69%, mp: 150–153 °C. Mixture of isomers *Z*/*E* = 1:0.75. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.31 (d, *J* = 8.2 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.80–7.60 (m, 4H), 7.50–7.38 (m, 5H), 7.31 (t, *J* = 8.1 Hz, 1H), 2.01 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.7, 150.4, 141.2, 137.0, 136.6, 135.4, 134.6, 129.9, 128.8, 128.1, 126.9, 126.2, 125.1, 124.4, 124.0, 122.1, 121.4, 117.8, 25.4 ppm.

1-acetyl-2-(3-(1-methyl-1*H*-pyrazol-4-yl)benzylidene)indolin-3-one (9ab): Purified by flash chromatography (CH_2Cl_2/MeOH = 99:1). Obtained as yellow solid, yield: 68%, mp: 198–201 °C. Mixture of isomers *Z*/*E* = 1:0.60. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.30 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.77 (s, 1H), 7.70–7.63 (m, 3H), 7.53–7.38 (m, 3H), 7.35 (s, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 3.96 (s, 3H), 2.00 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.7, 150.4, 136.9, 136.6, 135.3, 134.6, 133.9, 129.9, 128.0, 127.3, 127.1, 127.0, 125.1, 124.4, 123.9, 122.3, 122.2, 117.8, 39.3, 25.3 ppm.

1-acetyl-2-benzylidene-5-phenylindolin-3-one (10a): Purified by flash chromatography (hexane/EtOAc = 90:10). Obtained as yellow solid, yield: 67%, mp: 131–134 °C. Mixture of isomers *Z*/*E* = 1:0.25. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.36 (d, *J* = 8.1 Hz, 1H), 8.08 (s, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.64–7.56 (m, 4H), 7.49–7.38 (m, 7H), 1.99 ppm (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.6, 149.4, 140.2, 135.1, 134.1, 133.8, 131.8, 129.4, 128.9, 128.6, 128.4, 128.0, 127.7, 127.1, 127.0, 121.1, 117.7, 26.0 ppm.

1-acetyl-2-benzylidene-5-phenoxyindolin-3-one (10b): Purified by flash chromatography (hexane/EtOAc = 90:10). Obtained as yellow oil, yield: 62%. Mixture of isomers *Z*/*E* = 1:0.20. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.30 (d, *J* = 8.1 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.47–7.33 (m, 8H), 7.16 (t, *J* = 7.4 Hz, 1H), 7.04–6.99

(m, 2H), 1.98 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.0, 170.5, 155.1, 146.2, 135.5, 134.0, 131.2, 130.4, 130.2, 129.4, 127.4, 125.2, 124.1, 123.0, 120.3, 119.6, 119.3, 112.7, 25.1 ppm.

1-acetyl-5-benzyl-2-benzylideneindolin-3-one (10c): Purified by flash chromatography (hexane/EtOAc = 90:10). Obtained as yellow oil, yield: 65%. Mixture of isomers *Z*/*E* = 1:0.45. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.20 (d, *J* = 8.1 Hz, 1H), 7.67 (s, 1H), 7.54–7.51 (m, 2H), 7.47–7.36 (m, 3H), 7.32–7.15 (m, 7H), 4.04 (s, 2H), 1.98 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 186.1, 170.5, 149.0, 140.4, 138.6, 137.3, 135.4, 134.0, 131.2, 130.3, 129.3, 129.0, 128.1, 126.6, 124.3, 124.1, 122.5, 118.1, 41.4, 25.2 ppm.

1-acetyl-2-benzylidene-6-phenoxyindolin-3-one (10d): Purified by flash chromatography (hexane/EtOAc = 90:10). Obtained as yellow oil, yield: 61%. Mixture of isomers *Z*/*E* = 1:0.30. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 8.22 (d, *J* = 8.3 Hz, 1H), 7.67–7.61 (m, 5H), 7.53 (t, *J* = 7.6 Hz, 2H), 7.31–7.12 (m, 6H), 2.01 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, major isomer): δ = 185.9, 170.1, 149.1, 145.6, 136.7, 134.0, 130.9, 129.8, 129.7, 128.7, 127.7, 125.5, 123.9, 123.1, 121.2, 119.2, 118.3, 115.7, 26.0 ppm.

General procedure for the synthesis of azaaurone derivatives 3a–ab and 4a–d: To a solution of the proper acetylated azaaurone derivative (0.25 mmol) in MeOH (2.5 mL) at room temperature was added a solution of KOH 50% in water (375 μL). The mixture was stirred for 45 min. After reaction completion, the reaction mixture was neutralized with extracted with EtOAc. The organic layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product.

(*Z*)-2-benzylideneindolin-3-one (3a): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 90%, mp: 189–191 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.50–7.43 (m, 3H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.01–6.98 (m, 2H), 6.87 ppm (br, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.7, 153.3, 136.3, 135.5, 134.9, 129.6, 129.4, 128.7, 125.2, 121.9, 120.8, 112.1, 111.7 ppm.

(*Z*)-2-(4-bromobenzylidene)indolin-3-one (3b): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 91%, mp: 241–243 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.84 (s, 1H), 7.69–764 (m, 4H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.13 (d, *J* = 7.7 Hz), 6.93 (t, *J* = 7.7 Hz, 1H), 6.59 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.3, 154.2, 136.5, 134.8, 133.3, 131.8, 131.6, 124.2, 121.4, 119.8, 112.6, 108.3 ppm.

(*Z*)-2-(4-(dimethylamino)benzylidene)indolin-3-one (3c): Purified by TLC (toluene/EtOH = 80:20). Obtained as red solid, yield: 87%, mp: 250–252 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 7.7 Hz, 1H), 7.49–7.43 (m, 3H), 7.02 (d, *J* = 7.7 Hz, 1H), 6.95 (t, *J* = 7.7 Hz, 1H), 6.90 (s, 1H), 6.77–6.75 ppm (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.2, 153.0, 135.4, 133.1, 131.6, 124.9, 122.5, 120.4, 118.4, 117.1, 114.3, 122.3, 112.2, 40.3 ppm.

(*Z*)-2-(4-phenoxybenzylidene)indolin-3-one (3d): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 93%, mp: 209–211 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.78 (d, *J* = 7.6 Hz, 1H), 7.55 (d, *J* = 8.7 Hz, 2H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.43–7.39 (m, 2H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.10–7.08 (m, 4H), 7.03–7.01 (m, 2H), 6.89 (s, 1H), 6.78 ppm (br, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.1, 153.2, 136.2, 131.3, 130.1, 125.2, 124.2, 120.9, 119.7, 119.1, 112.2, 111.5 ppm.

(*Z*)-2-(4-(p-tolyloxy)benzylidene)indolin-3-one (3e): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 92%, mp: 175–176 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.74 (s, 1H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 7.5 Hz, 1H), 7.05–6.99 (m, 4H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.65 (s, 1H), 2.32 ppm (s, 3H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.1, 157.5, 154.1, 153.5, 136.2, 133.8, 133.3, 131.8, 130.5, 128.9, 124.1, 120.2, 119.6, 119.3, 118.1, 112.6, 109.7, 20.3 ppm.

(*Z*)-2-(4-(4-chlorophenoxy)benzylidene)indolin-3-one (3f): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 89%, mp: 192–194 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (d, *J* = 8.2 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 1H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.15–7.00 (m, 5H), 6.71 ppm (br, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.0, 156.9, 154.0, 152.8, 136.1, 133.5, 133.2, 131.1, 130.6, 129.0, 124.0, 120.3, 119.3, 119.1, 117.9, 111.9, 110.6 ppm.

(*Z*)-2-(4-(4-(trifluoromethyl)phenoxy)benzylidene)indolin-3-one (3g): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 91%, mp: 204–206 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 8.3 Hz, 1H), 7.63–7.57 (m, 3H), 7.54–7.47 (m, 2H), 7.13–7.10 (m, 3H), 7.02–6.97 (m, 2H), 6.98 (s, 1H), 6.79 ppm (br, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.4, 156.1, 153.1, 153.3, 136.2, 135.1 (q, *J* = 32.2 Hz), 130.8, 128.9, 127.3, 125.1, 124.1 (q, *J* = 270.9 Hz), 121.9, 120.9, 120.1, 118.6, 112.0, 110.7 ppm.

(*Z*)-2-(4-(benzyloxy)benzylidene)indolin-3-one (3h): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 89%, mp: 231–233 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 7.42 Hz, 1H), 7.52–7.35 (m, 8H), 7.05 (d, *J* = 8.72 Hz, 2H), 7.01–6.95 (m, 2H), 6.87 (s, 1H), 6.74 (br, 1H), 5.12 ppm (br, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.0, 159.2, 153.1, 136.5, 136.0, 134.4, 131.3, 128.8, 128.3, 127.7, 127.6, 125.1, 122.2, 120.7, 115.8, 112.3, 112.2, 70.3 ppm.

(*Z*)-2-(biphenyl-4-ylmethylene)indolin-3-one (3i): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 94%, mp: 233–235 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, *J* = 7.6 Hz, 1H), 7.70–7.62 (m, 6H), 7.51–7.45 (m, 3H), 7.38 (t, *J* = 7.3 Hz, 1H), 7.04–6.97 (m, 2H), 6.91 ppm (br, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.6, 153.2, 141.3, 140.2, 136.3, 135.5, 133.9, 130.1, 129.1, 128.0, 127.5, 127.1, 125.2, 121.9, 120.9, 112.2, 111.4 ppm.

(*Z*)-2-((4′-fluorobiphenyl-4-yl)methylene)indolin-3-one (3j): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 91%, mp: 265–267 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.90 (s, 1H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.66–7.60 (m, 6H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 2H), 7.06–7.02 (m, 2H), 6.93 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.4, 162.6 (d, *J* = 267.5 Hz), 154.1, 138.8, 136.4, 135.8, 134.5, 133.3, 130.6, 128.7 (d, *J* = 8.2 Hz), 127.1, 124.2, 120.0, 118.9, 115.9 (d, *J* = 22.2 Hz), 112.7, 109.3 ppm.

(*Z*)-2-((4′-methylbiphenyl-4-yl)methylene)indolin-3-one (3k): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 92%, mp: 169–171 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, 1H, *J* = 7.7 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.1 Hz, 2H), 7.56–7.47 (m, 3H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.03–6.97 (m, 2H), 6.91 (s, 1H), 6.88 (br, 1H), 2.41 ppm (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.1, 153.2, 147.9, 137.9, 137.3, 136.2, 135.4, 133.6, 130.1, 129.8, 127.7, 127.0, 125.2, 120.9, 112.1, 11.6, 21.1 ppm.

(*Z*)-2-((4′-chlorobiphenyl-4-yl)methylene)indolin-3-one (3l): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 87%, mp: 259–261 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.92 (s, 1H), 7.85–7.78 (m, 5H), 7.71–7.52 (m, 5H), 7.16 (d, *J* = 8.1 Hz, 1H), 6.94 (t, *J* = 8.1 Hz, 1H), 6.68 ppm (s, 1H); ^{13}C NMR (101 MHz,

[D_6]DMSO): δ = 186.3, 154.1, 138.4, 138.1, 136.4, 134.5, 133.7, 132.7, 130.6, 129.0, 128.4, 127.0, 124.2, 120.0, 119.9, 112.7, 109.2 ppm.

(*Z*)-2-((4′-(trifluoromethyl)biphenyl-4-yl)methylene)indolin-3-one (3 m): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 90%, mp: 289–291 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.95 (s, 1H), 7.99 (d, J = 8.1 Hz, 2H), 7.87–7.84 (m, 6H), 7.60 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 6.94 (t, J = 7.6 Hz, 1H), 6.70 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.4, 154.2, 143.3, 138.0, 136.5, 134.7, 134.4 (q, J = 32.3 Hz), 130.6, 127.5, 127.5, 125.9, 124.2 (q, J = 270.4 Hz), 120.0, 119.9, 112.7, 108.9 ppm.

(*Z*)-2-((4′-(trifluoromethoxy)biphenyl-4-yl)methylene)indolin-3-one (3 n): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 92%, mp: 280–282 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.92 (s, 1H), 7.89–7.79 (m, 7H), 7.61–7.46 (m, 3H), 7.16 (d, J = 7.8 Hz, 1H), 6.94 (t, J = 7.8 Hz, 1H), 6.69 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.3, 154.1, 152.2, 149.1, 148.1, 138.6, 136.4, 134.6, 133.8, 130.6 (q, J = 253.7 Hz), 128.6, 127.3, 126.5, 124.2, 121.6, 120.0, 112.6, 109.1 ppm.

(*Z*)-2-(4-benzylbenzylidene)indolin-3-one (3 o): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 91%, mp: 194–196 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (d, J = 7.6 Hz, 1H), 7.47–7.45 (m, 3H), 7.13–7.19 (m, 7H), 6.99–6.95 (m, 2H), 6.86 (s, 1H), 6.82 (br, 1H), 4.01 ppm (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.6, 153.2, 142.1, 140.6, 136.2, 135.3, 132.7, 129.9, 129.8, 129.1, 128.7, 126.5, 125.2, 121.9, 120.9, 112.1, 111.8, 41.9 ppm.

(*Z*)-2-(4-(quinolin-3-yl)benzylidene)indolin-3-one (3 p): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 97%, mp: 271–273 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.22 (s, 1H), 8.37 (s, 1H), 8.17 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.83–7.71 (m, 6H), 7.62 (t, J = 7.6 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.05–6.98 (m, 3H), 6.92 ppm (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.3, 153.3, 149.5, 138.8, 136.4, 134.8, 133.6, 130.4, 130.0, 129.2, 128.2, 127.5, 125.3, 121.9, 121.1, 112.2, 110.7 ppm.

(*Z*)-2-(4-(thiophen-3-yl)benzylidene)indolin-3-one (3 q): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 93%, mp: 255–257 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.88 (s, 1H), 8.03 (s, 1H), 7.84 (d, J = 8.3 Hz, 2H), 7.78 (d, J = 8.3 Hz, 2H), 7.67 (s, 2H), 7.59 (d, J = 7.6 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 6.93 (t, J = 7.6 Hz, 1H), 6.67 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.2, 154.0, 140.8, 136.3, 134.9, 134.2, 132.9, 130.6, 127.4, 126.5, 126.2, 124.1, 121.9, 120.0, 119.8, 112.6, 109.6 ppm.

(*Z*)-2-(4-(1-methyl-1*H*-pyrazol-4-yl)benzylidene)indolin-3-one (3 r): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 92%, mp: 247–249 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.82 (s, 1H), 8.26 (s, 1H), 7.98 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.67 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 7.5 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.15 (d, J = 7.5 Hz, 1H), 6.92 (t, J = 7.4 Hz, 1H), 6.65 (s, 1H), 3.88 ppm (s, 3H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.7, 153.4, 136.7, 136.1, 135.9, 135.4, 133.5, 130.4, 128.0, 125.8, 125.0, 123.8, 121.8, 119.4, 112.4, 109.8, 38.5 ppm.

(*Z*)-2-(biphenyl-3-ylmethylene)indolin-3-one (3 s): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 93%, mp: 173–175 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.90 (s, 1H), 8.00 (s, 1H), 7.77–7.74 (m, 3H), 7.76–7.49 (m, 6H), 7.40 (t, J = 7.5 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 6.93 (t, J = 7.6 Hz, 1H), 6.74 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.5, 153.2, 141.4, 140.2, 136.2, 135.5, 133.5, 130.1, 129.3, 128.7, 128.48, 128.1, 126.8, 126.6, 123.9, 121.8, 119.6, 112.4, 109.6 ppm.

(*Z*)-2-((4′-fluorobiphenyl-3-yl)methylene)indolin-3-one (3 t): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 95%, mp: 297–299 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.98 (s, 1H), 7.95 (s, 1H), 7.84–7.80 (m, 2H), 7.74 (d, J = 7.6 Hz, 1H), 7.64–7.52 (m, 4H), 7.36–7.32 (m, 2H), 7.16 (d, J = 7.6 Hz, 1H), 6.95 (t, 1H, J = 7.6 Hz), 6.73 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.4, 162.1 (d, J = 268.1 Hz), 154.3, 139.93, 136.5, 136.2, 123.8 (d, J = 22.1 Hz), 134.7, 129.6, 129.1, 128.7, 128.2, 126.8, 124.2, 120.1, 119.9, 115.6 (d, J = 22.1 Hz), 112.7, 109.7 ppm.

(*Z*)-2-((4′-methylbiphenyl-3-yl)methylene)indolin-3-one (3 u): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 94%, mp: 201–202 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.88 (s, 1H), 7.91 (s, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.65–7.52 (m, 6H), 7.30 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 7.6 Hz, 1H), 6.94 (t, J = 7.6 Hz, 1H), 6.72 (s, 1H), 2.35 ppm (s, 3H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.6, 154.4, 141.0, 137.2, 136.9, 136.6, 134.8, 134.8, 129.7, 128.5, 128.2, 127.0, 126.8, 124.3, 120.2, 120.0, 112.8, 110.1, 20.8 ppm.

(*Z*)-2-((4′-chlorobiphenyl-3-yl)methylene)indolin-3-one (3 v): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 90%, mp: 189–191 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.89 (s, 1H), 7.97 (s, 1H), 7.82–7.73 (m, 4H), 7.67–7.52 (m, 5H), 7.16 (d, J = 7.6 Hz, 1H), 6.94 (t, J = 7.6 Hz, 1H), 6.73 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.1, 154.3, 139.6, 138.6, 136.5, 134.9, 134.7, 132.6, 129.6, 129.0, 128.9, 128.8, 128.3, 126.8, 124.2, 119.9, 112.7, 109.6 ppm.

(*Z*)-2-((4′-(trifluoromethyl)biphenyl-3-yl)methylene)indolin-3-one (3 w): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 91%, mp: 228–230 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, J = 7.7 Hz, 1H), 7.69 (s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.55–7.47 (m, 4H), 7.33 (d, J = 8.5 Hz, 2H), 7.02–6.97 (m, 2H), 6.91 (s, 1H), 6.88 ppm (br, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.5, 164.9, 153.1, 149.0, 140.9, 139.2, 136.3, 135.7, 135.4 (q, J = 32.1 Hz), 129.8, 128.6, 128.4, 128.3, 127.3, 125.1 (q, J = 272.1 Hz), 121.7, 121.4, 120.9, 112.0, 111.0 ppm.

(*Z*)-2-((4′-(trifluoromethoxy)biphenyl-3-yl)methylene)indolin-3-one (3 x): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 92%, mp: 223–225 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.92 (s, 1H), 7.93 (s, 1H), 7.84–7.70 (m, 5H), 7.65–7.43 (m, 5H), 6.98 (t, J = 7.6 Hz, 1H), 6.78 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.4, 153.0, 144.8, 141.8, 141.5, 139.3, 135.3, 129.6 (q, J = 252.9 Hz), 128.2, 127.3, 126.9, 126.1, 125.1, 123.1, 121.8, 121.2, 121.0, 120.2, 114.2, 109.6 ppm.

(*Z*)-2-(3-benzylbenzylidene)indolin-3-one (3 y): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange oil, yield: 96%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.73 (d, J = 7.7 Hz, 1H, H_4), 7.47 (t, J = 7.7 Hz, 1H), 7.38–7.31 (m, 4H), 7.27–7.17 (m, 5H), 6.98–6.93 (m, 2H), 6.82 (s, 1H), 6.71 (br, 1H), 4.04 ppm (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 186.6, 153.2, 142.4, 140.7, 136.2, 135.5, 135.1, 130.2, 129.5, 129.3, 129.2, 128.8, 127.3, 126.5, 125.2, 121.9, 120.8, 112.0, 111.7, 41.8 ppm.

(*Z*)-2-(3-(quinolin-3-yl)benzylidene)indolin-3-one (3 z): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 94%, mp: 266–268 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.95 (s, 1H), 9.37 (s, 1H), 8.76 (s, 1H), 8.20 (s, 1H), 8.09 (d, J = 8.1 Hz, 2H), 7.89–7.79 (m, 3H), 7.70–7.61 (m, 3H), 7.55 (t, J = 7.7 Hz, 1H), 7.17 (d, J = 7.7 Hz, 1H), 6.95 (d, J = 7.7 Hz, 1H), 6.78 ppm (s, 1H); ^{13}C NMR (101 MHz, [D_6]DMSO): δ = 186.4, 154.3, 149.7, 146.9, 137.9, 136.5, 135.1, 134.8, 133.3, 132.4, 129.8, 129.7, 129.4, 128.7, 128.5, 127.6, 127.2, 127.1, 124.2, 120.0, 119.9, 112.7, 109.5 ppm.

(*Z*)-2-(3-(thiophen-3-yl)benzylidene)indolin-3-one (3aa): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 93%, mp: 202–204 °C. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.88 (s, 1H), 8.02–8.01 (m, 2H), 7.72–7.68 (m, 4H), 7.60 (d, J = 7.8 Hz, 1H), 7.56–7.50 (m, 2H), 7.16 (d, J = 7.8 Hz, 1H), 6.94 (t, J = 7.8 Hz, 1H), 6.71 ppm (s, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 186.2, 154.0, 140.8, 136.3, 134.9, 134.2, 132.9, 129.3, 128.1, 127.6, 126.9, 126.3, 126.0, 124.0, 121.5, 120.0, 119.7, 112.5, 109.6 ppm.

(*Z*)-2-(3-(1-methyl-1*H*-pyrazol-4-yl)benzylidene)indolin-3-one (5ab): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 94%, mp: 212–213 °C. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.80 (s, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.66 (s, 1H), 7.62 (s, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.43–7.39 (m, 3H), 7.02 (d, J = 7.8 Hz, 1H), 6.97 (t, J = 7.8 Hz, 1H), 6.86 (br, 2H), 3.94 ppm (s, 3H); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 186.8, 153.4, 136.6, 136.3, 135.7, 135.5, 133.5, 129.8, 127.5, 127.4, 126.8, 125.8, 125.2, 122.7, 121.8, 120.8, 112.2, 111.4, 39.3 ppm.

(*Z*)-2-benzylidene-5-phenylindolin-3-one (4a): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 91%, mp: 234–236 °C. ^{1}H NMR (400 MHz, CDCl3): δ = 8.00 (s, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.59–7.57 (m, 4H), 7.49–7.42 (m, 4H), 7.38–7.32 (m, 2H), 7.08 (d, J = 8.1 Hz), 6.91 ppm (br, 2H); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 186.7, 152.5, 140.2, 135.8, 135.4, 134.82 134.3, 129.7, 129.4, 129.0, 128.8, 127.3, 126.8, 123.3, 122.3, 112.4, 112.1 ppm.

(*Z*)-2-benzylidene-5-phenoxyindolin-3-one (4b): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 91%, mp: 212–214 °C. ^{1}H NMR (400 MHz, CDCl3): δ = 7.82 (s, 1H), 7.77–7.68 (m, 4H), 7.48–7.27 (m, 7H), 7.03 (d, J = 7.6 Hz, 1H), 6.85 ppm (br, 2H); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 186.5, 152.0, 141.0, 136.0, 135.3, 134.1, 133.0, 129.0, 128.9, 128.9, 128.3, 128.0, 126.2, 124.1, 122.4, 112.0, 111.0 ppm.

(*Z*)-5-benzyl-2-benzylideneindolin-3-one (4c): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 92%, mp: 203–205 °C. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.61 (s, 1H), 7.57 (d, J = 7.9 Hz, 2H), 7.47 (t, J = 7.9 Hz, 2H), 7.38–7.20 (m, 7H), 6.94 (d, J = 7.6 Hz, 1H), 6.87 (s, 1H), 6.79 (br, 1H), 3.98 ppm (s, 2H); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 186.7, 152.0, 140.7, 137.3, 135.9, 134.9, 134.0, 129.6, 129.3, 129.0, 128.7, 128.6, 126.4, 125.0, 122.1, 112.2, 111.7, 41.3 ppm.

(*Z*)-2-benzylidene-6-phenoxyindolin-3-one (4d): Purified by TLC (toluene/EtOH = 80:20). Obtained as orange solid, yield: 89%, mp: 216–218. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.02 (d, J = 8.3 Hz, 1H), 7.57–7.43 (m, 5H), 7.53 (t, J = 7.6 Hz, 2H), 7.36–7.24 (m, 5H), 6.91 (s, 1H), 6.85 ppm (br, 1H); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 186.5, 150.4, 144.3, 137.0, 134.9, 131.0, 130.0, 129.2, 128.2, 127.3, 125.1, 124.0, 122.9, 121.0, 118.8, 112.0, 110.8 ppm.

Solid-state characterization of compound 3j: Crystals of **3j** suitable for X-ray diffraction study were mounted with Fomblin© in a cryoloop. Data were collected on a Bruker AXS-KAPPA APEX II diffractometer with graphite-monochromated radiation ($Mo_{K\alpha}$, λ = 0.17073 Å) at 150 K. The X-ray generator was operated at 50 kV and 30 mA, and X-ray data collection was monitored by the APEX2[28] program v. 2014.11-0. All data were corrected for Lorentzian, polarization, and absorption effects using SAINT[28] (v. 8.34A) and SADABS[28] (v. 2014/5) programs. SIR97[29] and SHELXS-97[30] were used for structure solution, and SHELXL-97 was used for full matrix least-squares refinement on F^2. These three programs are included in the package of programs WINGX-Version 1.80.05.[31] Non-hydrogen atoms were refined anisotropically. A full-matrix least-squares refinement was used for the non-hydrogen atoms with anisotropic thermal parameters. All the hydrogen atoms were inserted in idealized positions and allowed to refine in the parent carbon or oxygen atom. OLEX2.1.2.6[32] was used for packing diagrams. CCDC 1419162 contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre. Further details are provided in the Supporting Information, Table S2.

Biological assays

Activity against erythrocyte-stage *P. falciparum*: Drug assays were performed as previously described,[33] with modifications for 384-well format. Briefly, synchronized ring-stage parasites were cultured in the presence of 12-point two-fold serial dilutions of test compounds in 40 μL RPMI supplemented with 0.5% AlbuMAX® II at 1.0% hematocrit and an initial parasitemia of 1.0% in black clear-bottom plates (Greiner Bio-one 781090). Following 72 h incubation under standard culture conditions, SYBR Green I dye (Invitrogen S7563) was added to a dilution of 1:5000, and plates were stored at room temperature until fluorescence signal was read on a Spectramax M5 plate reader (Molecular Devices, λ_{ex}: 480 nm, λ_{em}: 530 nM). After background subtraction and normalization, EC_{50} values were calculated using a nonlinear regression curve fit implemented in the GraphPad Prism software package v. 5.00 (GraphPad Software, CA, USA).

Activity against liver-stage *P. berghei*: Huh7 cells, a human hepatoma cell line, were cultured in RPMI 1640 medium supplemented with 10% *v/v* fetal calf serum (FCS), 1% *v/v* non-essential amino acids, 1% *v/v* penicillin/streptomycin, 1% *v/v* glutamine, and 10 mM HEPES, pH 7, and maintained at 37 °C with 5% CO_2. Inhibition of *P. berghei* liver stage infection was determined by measuring the luminescence of Huh7 cell lysates 48 h after infection with a firefly-luciferase-expressing *P. berghei* line, PbGFP-Luccon, as previously described.[34] Briefly, cells (1.2×10^4 per well) were seeded in 96-well plates the day before drug treatment and infection. Tested compounds were prepared in the following way: 10 mM stock solutions were obtained by dissolving accurately weighed compounds in MeOH and dilutions subsequently made with medium to the desired concentration. Medium was replaced by fresh medium containing the appropriate concentration of each compound 1 h prior to infection. Sporozoites (10000 per well), freshly obtained through disruption of salivary glands of infected female *Anopheles stephensi* mosquitoes, were added to the wells 1 h after compound addition. Sporozoite addition was followed by centrifugation at 1700 *g* for 5 min. At 24 h post-infection, medium was again replaced by fresh medium containing the appropriate concentration of each compound. Parasite load was determined 48 h after infection by luminescence measurement using a Firefly Luciferase Assay Kit (Biotium). The effect of the compounds on the viability of Huh7 cells was assessed by the Alamar Blue assay (Invitrogen, UK) using the manufacturer's protocol. Nonlinear regression analysis was employed to fit the normalized results of the dose–response curves, and EC_{50} values, determined using SigmaPlot software v. 12.0 (Systat Software, San Jose, CA, USA), are the mean value averaged from three independent experiments, each performed in triplicate wells.

In vitro cytotoxicity: Cytotoxicity was assessed using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.[35] Briefly, HEK293T (human embryonic kidney epithelial cell line, ATCC CRL-11268) cells were seeded in 96-well tissue culture plates one day before the experiment, in RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 2 mM L-glu-

tamine, 100 U penicillin G (sodium salt) and 100 μg streptomycin sulfate, at a concentration allowing exponential growth during the assay. Compounds were diluted in DMSO, serially diluted in culture medium, and added to cells incubated at 37 °C in humidified 5% CO_2 atmosphere. After 48 h, culture media was removed and replaced with fresh medium containing MTT dye. After 3 h of incubation the complete media was removed and intracellular formazan crystals were solubilized and extracted with DMSO. After 15 min at room temperature absorbance was measured at 570 nm in a microplate reader. The percentage of cell viability was determined for each tested compound as described previously.[35] EC_{50} values were determined from concentration–response curves using nonlinear regression with GraphPad Prism software package v. 5.02 (GraphPad Software, CA, USA).

Abbreviations: ACTs: artemisinin-based combination therapies, EEFs: exoerythrocytic forms, mtETC: mitochondrial electron-transport chain, DHODH: dihydroorotate dehydrogenase.

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Keywords: antiprotozoal agents · azaaurones · erythrocytic stage · liver stage · malaria

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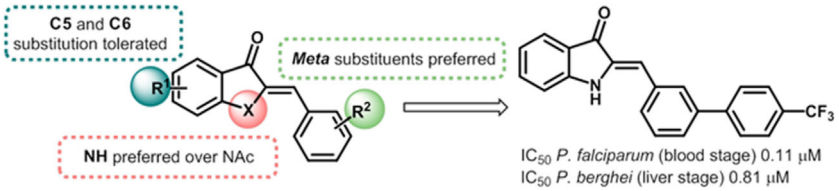
Probing the Azaaurone Scaffold against the Hepatic and Erythrocytic Stages of Malaria Parasites



Once bitten, twice potent: Novel synthetic azaaurone derivatives were synthesized and screened against the blood stage of a chloroquine-resistant *P. falciparum* strain and the liver stage of *P. berghei*. Our report shows several compounds with dual-stage activity and nanomolar potency against erythrocytic parasite, indicating that the azaaurone chemotype is a promising scaffold for dual-stage antimalarial agents.