European Journal of Medicinal Chemistry Reports 14 (2025) 100258

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry Reports

journal homepage: www.editorialmanager.com/ejmcr/default.aspx

Structural optimization of indolizinoindolones to obtain potent new antimalarials with dual stage activity

Paulo A.F. Pacheco [a], Ricardo J.F. Ferreira [a], Diana Fontinha [b], Caroline Conceição Sousa [c], Jenny Legac [d], Valentina Barcherini [a], Philip J. Rosenthal [d], Miguel Prudêncio [b,e], Diogo R.M. Moreira [c], Maria M.M. Santos [a,*]

[a] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof.Gama Pinto, 1649-003, Lisboa, Portugal
[b] GIMM - Gulbenkian Institute for Molecular Medicine, Avenida Prof. Egas Moniz, 1649-035, Lisboa, Portugal
[c] Instituto Gonçalo Moniz, FIOCRUZ, Salvador, CEP 40296-710, BA, Brazil
[d] Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA94143, USA
[e] Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz MB, 1649-028, Lisboa, Portugal

ARTICLE INFO

Keywords:
Antimalarials
Cytotoxicity
Dual-stage
Indolizinoindolones
Plasmodium

ABSTRACT

Malaria continues to represent a major public health concern due to the emergence of resistance to most available drugs. We report the optimization of the indolizinoindolone scaffold to increase activity against erythrocytic stages of *Plasmodium* (*P.*) *falciparum* and against hepatic stages of the rodent parasite *P. berghei*. Twenty-six enantiopure indolizinoindolones were synthesized, with IC_{50} values in the low micromolar and sub-micromolar range against both stages, and no significant cytotoxicity against mammalian cell lines. The most active compound showed nanomolar activity against *P. falciparum* blood stages *in vitro*, low micromolar activity against hepatic *P. berghei* infection *in vitro*, and a 7-fold higher selectivity index than that of chloroquine. This compound was also tested in *P. berghei*-infected mice, inhibiting the development of parasitemia relative to untreated mice. Overall, we identified a new set of lead antimalarial compounds. Further optimization of the pharmacokinetic properties of this scaffold is warranted.

1. Introduction

Malaria is a potentially life-threatening disease that occurs in tropical and subtropical regions of the world [1,2]. Despite continued efforts to reduce or even eliminate the burden of malaria in endemic areas, this disease still represents a major public health concern. Efforts to eradicate malaria were impacted by the COVID-19 pandemic, resulting in an estimated additional 13.4 million cases during this period [3,4]. In fact, in 2022, 249 million new cases were estimated in 85 endemic countries and areas, representing an increase of 5 million cases compared to 2021. Globally, in 2022, 608,000 deaths due to malaria were estimated [4]. Most of these cases and deaths were concentrated in Africa, and the most vulnerable group was children aged under 5 years, who accounted for approximately 76 % of all malaria deaths [4].

Malaria is caused by protozoan parasites belonging to the *Plasmodium* genus [5]. To date, approximately 200 *Plasmodium* species have been identified that can cause malaria in different vertebrate hosts, but 6 species routinely cause disease in humans: *P. malariae*, *P. falciparum*, *P. vivax*, *P. knowlesi, P. ovale curtisi,* and *P. ovale wallikeri* [6]. Among them, *P. falciparum* and *P. vivax* represent the predominant causative agents of malaria worldwide, with *P. falciparum* causing over 90 % of cases of malaria and of severe malaria.

Plasmodium presents a complex life cycle that alternates between a sexual phase (sporogony) in the mosquito vector and an asexual phase (schizogony) that occurs in the vertebrate host [7,8]. Human malaria is initiated with the bite of an infected female *Anopheles* mosquito which delivers sporozoites into the bloodstream. The sporozoites then migrate to the liver, where they invade and infect hepatocytes. Following a period of intrahepatic multiplication and differentiation, newly formed merozoites are released into the bloodstream, where they repeatedly infect red blood cells (RBCs) [7,9]. During this period, sexual forms of the parasite, known as gametocytes, develop and can be ingested by a

* Corresponding author. Medicinal Organic Chemistry Group, iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal.
E-mail address: mariasantos@ff.ulisboa.pt (M.M.M. Santos).

https://doi.org/10.1016/j.ejmcr.2025.100258
Received 30 November 2024; Received in revised form 14 February 2025; Accepted 22 February 2025
Available online 28 February 2025

female mosquito, thus completing the parasite's lifecycle [7,9]. Moreover, *P. vivax* and *P. ovale* parasites can generate hypnozoites, dormant liver forms capable of reactivating and causing disease relapses, even after the successful clearance of blood-stage parasites [10,11].

Human malaria has a wide spectrum of clinical manifestations, ranging from asymptomatic parasitemia, mild malaria with fever, severe malaria with organ system dysfunction, and death [12]. The severity of the disease depends on several factors, such as *Plasmodium* species, immunity from prior infections, and genetic background [13,14]. The development of resistance by *P. falciparum* to most currently available drugs highlights the urgent need for the discovery and development of novel antimalarial agents [15–17]. Ideally, a treatment should present efficacy against the hepatic and intraerythrocytic parasites. Furthermore, to ensure therapeutic efficacy and reduce the risk of selecting drug-resistant strains, new treatments should consist of combinations of pharmaceuticals with distinct mechanisms of action [1].

As part of the worldwide effort to control and eradicate malaria, it is crucial to identify new compounds that are effective against multidrug-resistant *P. falciparum* strains and ideally active against multiple stages of the parasite life cycle, preventing transmission and occurrence of relapse [18–21]. Indole-based compounds have emerged as a promising molecular scaffold for the development of new antiplasmodial agents [22–24]. For example, indole-based natural alkaloids such as dihydrousamabarensine (**1**), voacamine (**2**) and cryptolepine (**3**), have been identified as antiplasmodial compounds (Fig. 1) [23,25]. Moreover, synthetic indole derivatives have also been developed, including the spiroindolone cipargamin (**4**) (formerly known as NITD609 or KAE609) that is currently under phase II clinical evaluation and has sub-nanomolar activity against blood stage *P. falciparum* (Fig. 1) [22,23,26].

Recently, our group reported the antiplasmodial activity of a series of benzoindolizinoindolones synthesized from chiral 1,2-aminoalcohol tryptophanol. The assessed compounds showed promising activity against both asexual intraerythrocytic and liver stages of *Plasmodium* parasites [28]. As part of our effort to identify more potent and selective antiplasmodial agents, we now report the synthesis and evaluation of new indolizinoindolones against both hepatic and intraerythrocytic parasite stages. This synthetic design features novel modifications in the molecular scaffold, including the attachment of *N*-alkyl groups with different sizes and a chemically diversified set of *para*- and *meta*-substituted phenyl groups at C-13b position (Fig. 2).

2. Results and Discussion

2.1. Chemistry

In this work, we seek to investigate the effect of structural modifications, such as, elongation of the alkyl chain attached to the indole nitrogen, variation of substituents at the C-13b phenyl group, and stereochemistry. Previously, we demonstrated that benzoindolizinoindolones **6a** and **6b** could be obtained by $BF_3{\cdot}OEt_2$ catalyzed Pictet-Spengler reaction starting from oxazoloisoindolinone **5**. To investigate the impact of various substituents at the C-9 position and at the *N*-indole on the diastereoselectivity and antimalarial efficacy of indolizinoindolones, we synthesized thirteen *N*-indole protected isoindolinone derivatives (**9a**−**j** and **10a**−**c**) with different substituted phenyl groups at the C-9 position. These compounds were prepared following the synthetic route described in Scheme 1. Initially, oxazoloisoindolinones **7** and **8** were prepared according to previously reported methodology [29] through the stereoselective cyclocondensation of enantiopure (*S*)- and (*R*)-tryptophanol, respectively, with keto-acids, under Dean–Stark conditions. Next,

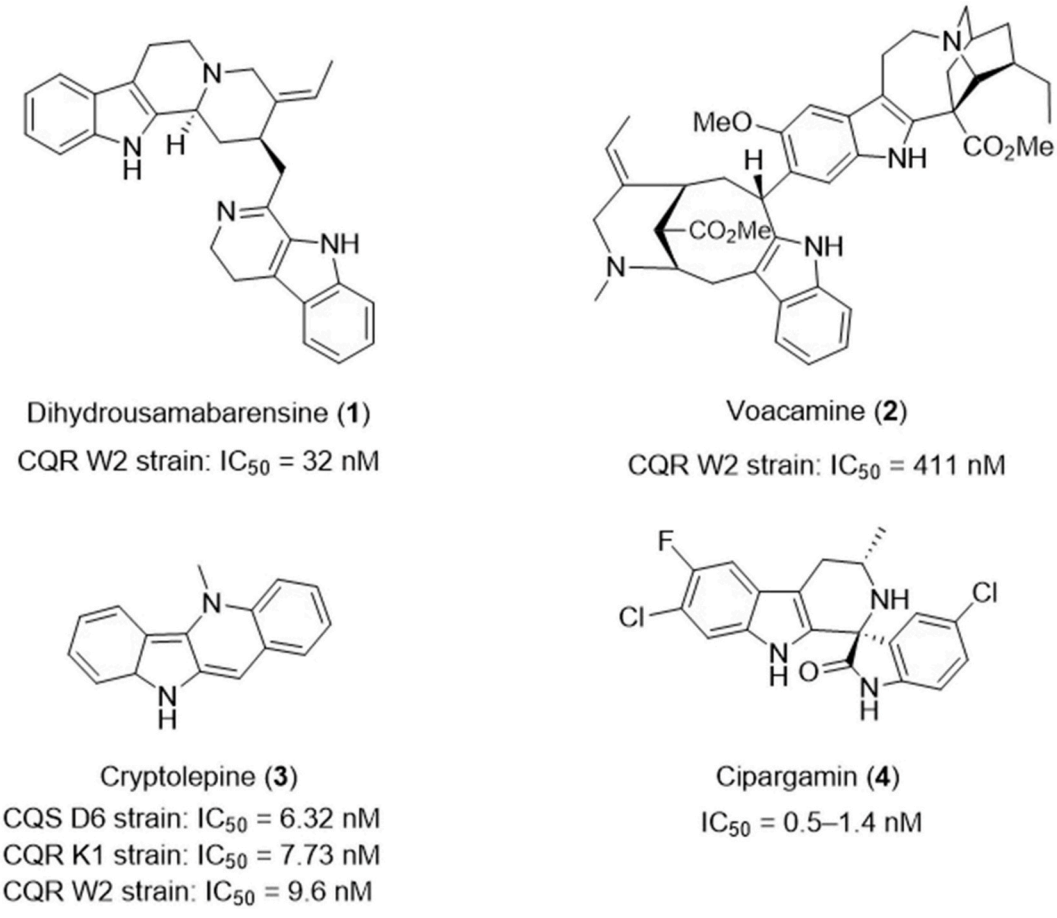


Fig. 1. Indole-based compounds with antimalarial activity. CQR= Chloroquine-resistant; CQS= Chloroquine-sensitive strains [27].

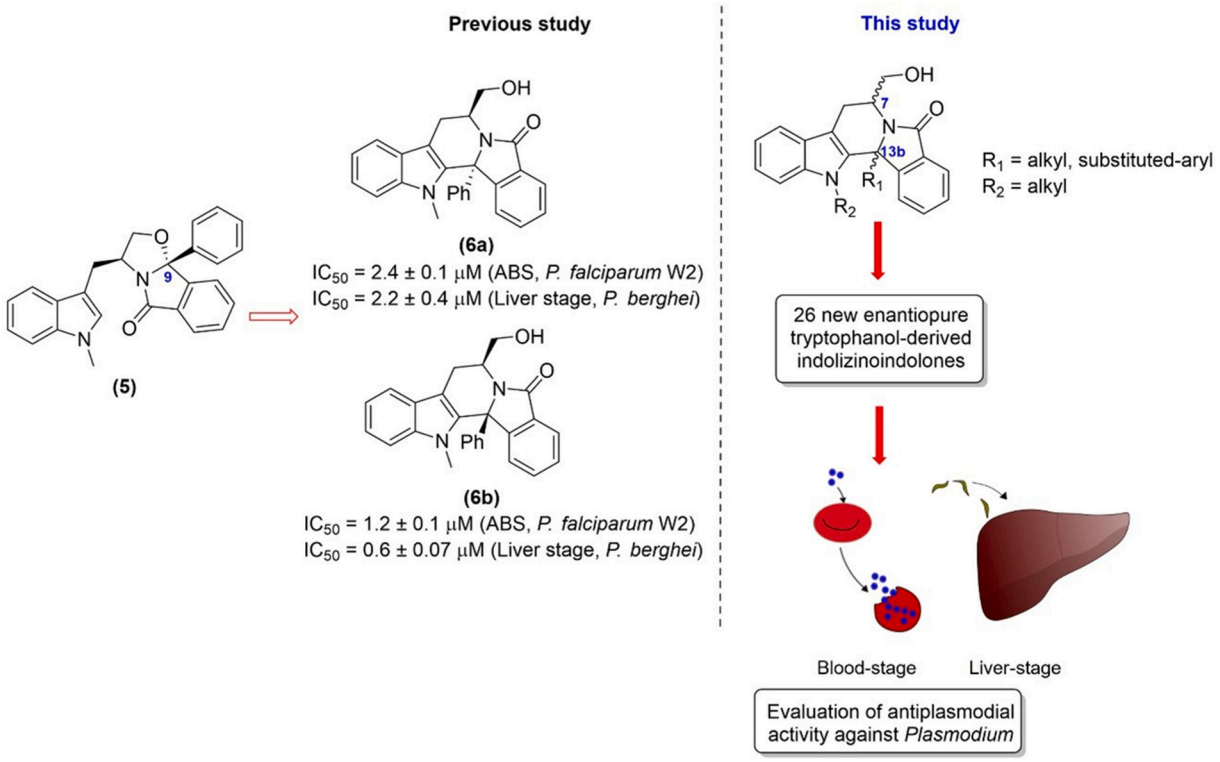


Fig. 2. Structures of the indolizinoindolones previously reported with antimalarial activity and the new compounds developed in this study. ABS = asexual blood stages.

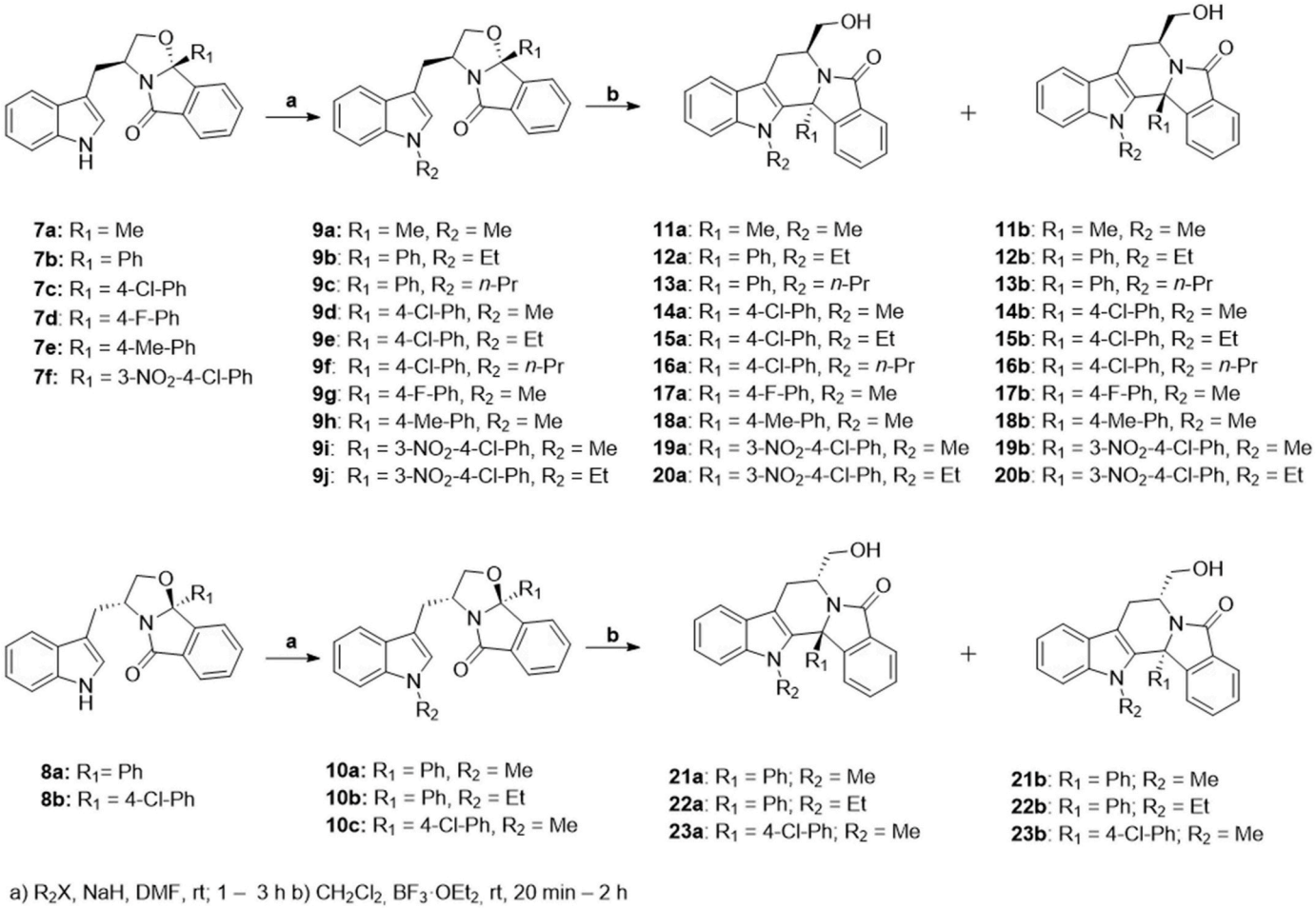
N-alkylation was performed by treatment of tryptophanol-derived isoindolinones with alkyl halides in the presence of sodium hydride, generating the oxazoloisoindolinones derivatives **9a**−**j** and **10a**−**c**. Finally, the target compounds **11**−**23** were obtained after $BF_3{\cdot}OEt_2$--promoted intramolecular α-amidoalkylation.

The absolute stereochemistry of the new stereogenic centers formed after Pictet-Spengler cyclization reaction was determined using ^{13}C NMR spectroscopy and comparing with our previous reports [28]. The chemical shift of C-7 is around 54 ppm for the 7*S*,13b*S* diastereoisomers (and corresponding enantiomers), while is around 51 ppm for the 7*S*, 13bR diastereoisomers (and corresponding enantiomers). The yields and diastereoselectivity ratio (determined by HPLC of the crude of each reaction) are presented in Table 1. Following the same trend observed in our previous work, Lewis acid-induced Pictet–Spengler cyclization of *N*-alkylated oxazoloisoindolinones resulted in two epimers with the 7*S*, 13b*S* (for the (*S*)-tryptophanol derivatives)/7*R*,13bR (for the (*R*)-tryptophanol derivatives) diastereoisomers as preferential products. The diastereoselectivities of these reactions were strongly dependent on the nature of the substituents in the C-13b and NH-indole positions. Considering the presence of the small *N*-methyl group, the replacement of the phenyl by a methyl group at position C-13b resulted in a decrease in diastereoselectivity (entries **1** and **2**). The diastereoselectivity was also influenced by the nature of the substituent on the aromatic ring at position 13b. The presence of *para*-chlorine, *para*-fluorine, or *para*--methyl group (entries **5**, **8** and **9**) favored the formation of the 7*S*,13b*S* epimer, with *para*-methyl strongly accentuating the diastereoselectivity (entry **9**). Interestingly, the presence of a bulkier group, such as a phenyl substituted at the *meta* and *para* positions (entry **10**), reversed this trend towards the formation of the 7*S*,13b*R*-diastereoisomer. When keeping the same substituent at C-13b, it was observed that increase in the size of the alkyl chain in the NH-indole also affected the diastereoselectivity ratio, leading to an increase of the 7*S*,13b*S*-diastereoisomer (entries **3** and **4**; entries **5** to **7**). This effect was reversed for the two examples bearing bulkier substituents on the aromatic ring (entries **10** and **11**).

2.2. Assessment of the compounds' pharmacological properties

The antiplasmodial activities of compounds **11**−**23** were determined against the asexual blood stages (ABS) of *P. falciparum* as well as against the liver stages of the rodent parasite *P. berghei*. For the ABS, screening was performed against the chloroquine (CQ)-resistant W2 strain. CQ was included as a positive control. IC_{50} values were determined, enabling us to compare drug potency and to perform a structure-activity relationship study. The results are summarized in Table 2. Most of the tested compounds inhibited parasite growth, with IC_{50} values ranging from 25 nM to 5.8 μM. In general, (*R*)-tryptophanol derived indolizinoindolones exhibited higher potency against blood-stage parasites when compared to the corresponding enantiomers (**6a** vs **21a**, **6b** vs **21b**, **12a** vs **22a**, **12b** vs **22b**, **14a** vs **23a** and **14b** vs **23b**). We identified 11 derivatives with submicromolar IC_{50} values, 5 of which had low-mid nanomolar potency: **14b** (IC_{50} = 0.071 ± 0.014 μM), **17b** (IC_{50} = 0.033 ± 0.005 μM), **18b** (IC_{50} = 0.043 ± 0.002 μM), **21a** (IC_{50} = 0.052 ± 0.005 μM) and **23a** (IC_{50} = 0.025 ± 0.002 μM). Confirming the same trend observed in our previous work, the stereochemistry of position C-13b proved to be an essential feature regarding the antimalarial activity of the compounds [28]. Among all the products derived from (*S*)-tryptophanol, compounds **b**, with the hydroxy methyl group on the same side as R_1, were more potent than counterparts **a**. Regarding the second library derived from (*R*)-tryptophanol, a reversal of this trend was observed, with greater potency for compounds **a** (except for the pair **21a/b**).

The nature of the substituent R_1 at the C-13b position also affected the antimalarial activity of the tested compounds. For instance, the replacement of a methyl group by a *para*-substituted aromatic ring generally improved the potency of the compounds, as seen for **11a/b** vs **14a/b**, **11a/b** vs **17a/b** and **11a/b** vs **18a/b**. These results support the previously observed trend, showing that analogues with an aromatic group at position C-13b exhibit higher activity than the ones with a methyl group [28]. However, the introduction of a nitro group at the



Scheme 1. Synthesis of *N*-alkylated indolizinoindolone derivatives.

Table 1
Reaction yields and diastereoselectivity ratios.

				η (%)		d.r.
Entry	Compounds	R_1	R_2	7*S*,13b*S*	7*S*,13b*R*	
1	**6**	Ph	Me	58	24	2.5:1 [28]
2	**11**	Me	Me	40	28	1.5:1
3	**12**	Ph	Et	71	29	2:1
4	**13**	Ph	n-Pr	69	21	3:1
5	**14**	4-Cl-Ph	Me	59	28	1.7:1
6	**15**	4-Cl-Ph	Et	66	23	2.3:1
7	**16**	4-Cl-Ph	n-Pr	68	29	4:1
8	**17**	4-F-Ph	Me	51	25	2:1
9	**18**	4-Me-Ph	Me	80	12	6.4:1
10	**19**	3-NO_2-4-Cl-Ph	Me	17	67	1:4
11	**20**	3-NO_2-4-Cl-Ph	Et	16	67	1:2.7
				7*R*,13b*R*	**7*R*,13b*S***	
12	**21**	Ph	Me	50	33	ND
13	**22**	Ph	Et	61	24	ND
14	**23**	4-Cl-Ph	Me	56	23	ND

ND – Not determined; η (%) – yield; d.r. – diastereoselectivity ratio; η (%) and d.r. were determined by HPLC of the crude for each reaction.

ortho-position resulted in weaker antiplasmodial activity (**14a**, IC_{50} = 1.48 μM vs **19a**, IC_{50} = 4.15 μM; **14b**, IC_{50} = 0.071 μM vs **19b**, IC_{50} = 3.2 μM), indicating that an increase in steric effect or electron-withdrawing effect in the aromatic ring may lead to a decrease in activity. Another structural feature that appeared to be significant was the length of the *N*-alkyl group. In our previous work, we observed that N-methylation of the indole nitrogen atom only slightly influenced antimalarial activity in comparison to the unprotected counterpart [28]. However, with an increase in the carbon chain length a decrease in potency was observed. When comparing compounds bearing the same phenyl group at the C-13b position but with an increasing *N*-alkyl group, decreasing activity was observed (**14b**, IC_{50} < **15b**, IC_{50} < **16b**, IC_{50}).

The most active compounds were evaluated for their cytotoxic potential against two cell types: the murine macrophage cell line J774 and human liver cell line HepG2 (Table 3). Except for **15b**, **18a** and **22b**, all compounds exhibited CC_{50} values higher than the reference drug chloroquine. Highlighting the remarkable pharmacological potential of this compound class, compounds **14b**, **18b**, and **23a** demonstrated exceptional selectivity for targeting parasites. In particular, the selectivity index of compound **23a** was 7-fold higher than that of chloroquine.

Due to the growing interest in identifying new drugs active against hepatic *Plasmodium* parasites, which can be targeted for chemoprotection, we further evaluated all compounds against this stage of infection in rodent *P. berghei* parasites, using a well-established luminescence-based *in vitro* assay [30]. Contrary to chloroquine, primaquine is active against the hepatic stage of *Plasmodium* infection and was, therefore, employed as a reference drug in these assays. As observed for the blood stage parasites, most compounds reduced infection of the

Table 2
In vitro screening of antiplasmodial activity for *N*-alkylated indolizinoindolones **11**−**23** against the ABS of *P. falciparum*.

Compounds	R_1	R_2	W2 strain of *P. falciparum* IC_{50} (μM, mean ± S.D.)[a]
(*S*)-tryptophanol-derived compounds			
6a	Ph	Me	2.4 ± 0.1 [28]
6b	Ph	Me	1.2 ± 0.1 [28]
11a	Me	Me	5.8 ± 0.81
11b	Me	Me	2.6 ± 0.08
12a	Ph	Et	3.0 ± 0.12
12b	Ph	Et	0.66 ± 0.08
13a	Ph	n-Pr	2.1 ± 0.18
13b	Ph	n-Pr	0.37 ± 0.05
14a	4-Cl-Ph	Me	1.5 ± 0.40
14b	4-Cl-Ph	Me	0.071 ± 0.014
15a	4-Cl-Ph	Et	1.6 ± 0.27
15b	4-Cl-Ph	Et	0.93 ± 0.13
16a	4-Cl-Ph	n-Pr	2.2 ± 0.76
16b	4-Cl-Ph	n-Pr	1.7 ± 0.40
17a	4-F-Ph	Me	1.1 ± 0.40
17b	4-F-Ph	Me	0.033 ± 0.005
18a	4-Me-Ph	Me	1.8 ± 0.42
18b	4-Me-Ph	Me	0.043 ± 0.002
19a	3-NO_2-4-Cl-Ph	Me	4.2 ± 0.41
19b	3-NO_2-4-Cl-Ph	Me	3.2 ± 0.51
20a	3-NO_2-4-Cl-Ph	Et	2.6 ± 0.41
20b	3-NO_2-4-Cl-Ph	Et	1.7 ± 0.04
(*R*)-tryptophanol-derived compounds			
21a	Ph	Me	0.052 ± 0.005
21b	Ph	Me	0.15 ± 0.04
22a	Ph	Et	1.1 ± 0.40
22b	Ph	Et	0.20 ± 0.08
23a	4-Cl-Ph	Me	0.025 ± 0.002
23b	4-Cl-Ph	Me	0.14 ± 0.02
Chloroquine	–	–	0.15 ± 0.007

[a] Data represent the mean ± SD of 3 independent experiments performed in triplicate.

Table 3
Cytotoxicity for mammalian cells and selectivity index of *N*-alkylated indolizinoindolones.

Compounds	CC_{50} (μM)[a]		% of hemolysis in uRBC[b]	Selectivity index[c]
	J774	HepG2		
12a	>80	ND	ND	>26.7
12b	>80	ND	ND	>121
13a	>80	ND	ND	>38.1
13b	>80	ND	ND	>217.4
14a	>80	ND	ND	>54.0
14b	>80	ND	ND	>1126.8
15a	>80	ND	ND	>50
15b	14.4 (11.2–17.5)	44.3 (39.9–47.8)	7.5 (5.2–8.7)	15.0
17a	~80	ND	ND	~70.2
17b	>80	ND	ND	>186.0
18a	~22.5 (21.9–23.8)	~80	5.0 (2.2–5.9)	~12.3
18b	~56.9	ND	ND	~1323.2
21a	>80	ND	ND	>1538.5
21b	56.9	ND	ND	384.5
22a	>80	ND	ND	>70.2
22b	23.9 (19.0–27.7)	~80	10.3 (9.0–11.5)	119.5
23a	~63.1	ND	ND	~2524.0
23b	>80	ND	ND	>555.5
Chloroquine	51.5 (48.7–56.2)	>80	0	348.0
Gentian violet	8.1 (8.0–9.0)	12.1 (10.7–17.3)	ND	ND

ND – Not determined. [a] Cytotoxic concentration for 50 % (CC_{50}) against the murine macrophages of the J774 cell lineage and the human hepatocellular carcinoma of the HepG2 cell lineage, determined 72 h after incubation with compounds using the CellTiter-Glo readout. Values were calculated the mean (95 % CI) of one single experiment, each concentration in triplicate. [b] Percentage of hemolysis (in comparison to untreated samples) using uninfected human red blood cells (RBC). Compounds were tested at a concentration of 10 μM. Values are the mean (95 % CI) of one single experiment. [c] Determined as CC_{50}/IC_{50} using CC_{50} values from J774 cells.

human hepatoma cell line, Huh7, with negligible cytotoxicity. Further, the 7*S*,13b*S*-diastereoisomers displayed a higher potency against exo-erythrocytic than against intraerythrocytic parasite forms, except for the pair **14a/b** (R_1 = 4-Cl-Ph; R_2 = Me) (Table 4). However, the data also suggests that the steric bulk of the substituent on C-13b had no significant impact on the activity of the compounds against the hepatic stage of *P. berghei*, since the replacement of a small methyl group by *para*-substituted or unsubstituted aromatic rings resulted in only a slight change in potency.

The two most potent and selective antiplasmodial agents against the asexual blood stage of *P. falciparum*, both with low micromolar activity against the liver stage of *P. berghei*, were selected for determination of their efficacy in *P. berghei*-infected mice by a Peter's suppression test. To this end, mice were infected with asexual blood stages parasites and, 24 h after infection, compounds were injected intraperitoneally for three consecutive days. Untreated and chloroquine (CQ)-treated mice were employed as negative and positive controls. Based on previous data on efficacy and pharmacokinetics for indolizinoindolones, as well as for cipargamin [31–33], compounds were administered at 20 and 40 mg/kg. At 20 mg/kg, CQ inhibited development of parasitemia, and at 40 mg/kg, CQ cured the mice (Table 5). Compound **18b** did not show activity in the murine model at the dosages studied. At both dosages employed, compound **23a** inhibited the development of parasitemia relative to untreated mice, with approximately half the efficacy of CQ (Table 5). Relative to untreated mice, compound **23a** inhibited the development of parasitemia by 43 % at 20 mg/kg and by 55 % at 40 mg/kg. However, treatment with **23a** did not increase the median survival time of the mice. One reason for the sub-optimal efficacy obtained could be due to the relatively low to moderate aqueous solubility of these compounds.

3. Conclusions

We report on the synthesis and antiplasmodial activity of 26 new enantiopure indolizinoindolones. We observed that the diastereoselectivity ratio of $BF_3.OEt_2$-induced cyclization was dependent on the nature of the substituents at C-13b and on the size of the alkyl group attached to the indole moiety. Interestingly, a higher diastereoselectivity ratio was obtained when R_1 was a 4-methylphenyl group. However, if the phenyl was substituted with two strong electron-withdrawing groups (e.g. chlorine and nitro) instead of a methyl, the diastereoselectivity ratio reversed. Moreover, the size of the substituent at the NH-indole also impacted the diastereoselectivity ratio. The compounds were assessed for their potential to inhibit asexual hepatic and blood stages of *Plasmodium* infection, as well as for their cytotoxicity against mammalian cell lines and selectivity. We identified eleven derivatives with submicromolar IC_{50} values, 5 of which had low-mid nanomolar potency: **14b** (IC_{50} = 0.071 ± 0.014 μM), **17b** (IC_{50} = 0.033 ± 0.005 μM), **18b** (IC_{50} = 0.043 ± 0.002 μM), **21a** (IC_{50} = 0.052 ± 0.005 μM) and **23a** (IC_{50} = 0.025 ± 0.002 μM) μM. Moreover, two compounds, **11b** and **19b**, were more active against the exo erythrocytic forms of *P. berghei* than our previous hit compound [28]. However, these two compounds exhibited relatively low potency against blood stage parasites. Most compounds showed low cytotoxicity against two

Table 4
Activity of *N*-alkylated indolizinoindolones **11**−**23** against hepatic *P. berghei* infection.

Compounds	*P. berghei* IC_{50} (μM)[†]
6a	2.2 ± 0.4 [28]
6b	0.6 ± 0.07 [28]
11a	ND
11b	0.56 ± 0.17
12a	2.9 ± 1.9
12b	0.63 ± 0.12
13a	2.9 ± 0.055
13b	2.9 ± 1.2
14a	1.01 ± 0.09
14b	4.5 ± 1.8
15a	4.8 ± 2.2
15b	1.2 ± 0.14
16a	3.3 ± 0.55
16b	ND
17a	4.96 ± 2.2
17b	0.83 ± 0.08
18a	5.5 ± 0.88
18b	1.2 ± 1.02
19a	7.9 ± 3.3
19b	0.58 ± 0.47
20a	ND
20b	1.04 ± 0.26
21a	9.5 ± 3.2
21b	ND
22a	0.81 ± 0.20
22b	4.5 ± 0.86
23a	3.4 ± 0.17
23b	2.2 ± 0.29
Primaquine (PQ)	9.5 ± 2.3

ND – Not determined.

Table 5
Efficacy of compounds in suppressing parasitemia in *P. berghei*-infected mice.

Groups	Dose (mg/kg of animal)/I. P.	Parasitemia inhibition (%)[a]	Median of animal survival (days)	Number of cured mice on 30 days (%)
Vehicle	–	–	10, 13	0
18b	20	7.6 ± 6.9	9	0
18b	40	0.1 ± 0.7	10	0
23a	20	42.9 ± 10.8	16	0
23a	40	55.3 ± 9.7	10	0
CQ	20	87 ± 1.59	21*	0
CQ	40	>99	>30*	100

[a] Values are mean and standard deviation determined in comparison to vehicle group using n = 4/group and from one single experiment. *$p < 0.05$ (Log-rank and Mantel-Cox test) versus vehicle. CQ = chloroquine. I.P. = intraperitoneal injection.

mammalian cell lines. Remarkably, compound **23a** presented a selectivity index 7-fold higher than that of chloroquine, and inhibited parasite development in mice. In conclusion, we identified a promising lead antimalarial compound. Further optimization is required to enhance its pharmacodynamic properties.

4. Experimental section

4.1. Chemistry

General: All reagents and solvents were available from commercial suppliers and used without further purification. Chloroquine free base was employed for *in vitro* assays and Chloroquine diphosphate (Sigma-Aldrich) was employed for *in vivo* assays in mice (dosage of salt was adjusted to free base). Compounds, **7a-f** [29], **8a-b** [28,29], **9a-b**, **10a-b** [29] were synthesized according to literature. Analytical thin layer chromatography was performed with silica gel plates (Merck, TLC silica gel 60 F254), and the spots were visualized using UV lamp. Flash column chromatography was performed with Merck Silica Gel 60 (200–400 mesh). Melting points were obtained using a Kofler camera Bock monoscope M. The specific rotation values were determined with P-200 high-accuracy digital polarimeter JASCO. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz/54 mm Ultra-Shield Spectrometer (Wissenbourg, Bas-Rhin, France). ^{1}H and ^{13}C NMR chemical shifts are reported in parts per million (ppm, δ) referenced to the solvent used and the proton coupling constants J in Hertz (Hz). Multiplicities are given as: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet) and m (multiplet). Spectra were assigned using appropriate COSY, APT, HMQC and HMBC sequences. All compounds showed purity ≥95 % by HPLC experiments, performed in a LaChrom HPLC constituted of a Merck Hitachi pump L-7100, Merck Hitachi autosampler L-7250, and a Merck Hitachi UV detector L-7400. Analyses were performed with a LiChrospher®100 RP-8 (5 μm) LiChroCART® 250-4 column at room temperature, using a mobile phase solution constituted of 70 % acetonitrile and 30 % Milli-Q water. Peaks were detected at λ = 254 nm. High resolution mass spectrometry (HRMS) analysis was performed by direct injection on a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer using electrospray ionization (ESI) in positive mode.

General procedure for the reactions of *N*-alkylation: To a stirred solution of the (*R*)- or (*S*)-tryptophanol-derived oxazoloisoindolinone (1.0 equiv.) in anhydrous dimethylformamide under inert atmosphere of nitrogen and ice bath, was added sodium hydride (2.2 equiv. of 80 % dispersion in mineral oil) was added. After stirring for 20 min, alkyl halide (1.5 equiv.) was added, and the reaction was stirred at room temperature for 1–3 h.

(3*S*,9bR)-9b-((4-chlorophenyl)-3-((1-methyl-1*H*-indol-3-yl) methyl)-2,3-dihydrooxazolo [2,3-*a*] isoindol-5(9b*H*)-one (9d): Following the general procedure, starting from compound **7b** (0.140g, 0.338 mmol), sodium hydride 95 % anhydrous reagent (0.0162 g, 0.676 mmol) and methyl iodide (0.042 mL, 0.677 mmol, d = 2.28 g mL^{-1}) were added. The reaction was stirred at room temperature for 30 min. Eluent for flash chromatography *n*-hexane/ethyl acetate 7:3. Recrystallization with the same mixture of solvents. The product was obtained as a white crystalline light solid (0.127 g, 88 %). mp: 69–71 °C. $[\alpha]_D^{22}$ = +150.1 (c = 0.1, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.79 (m, 1H), 7.52–7.46 (m, 5H), 7.34–7.29 (m, 2H), 7.26 (m, 1H), 7.23 (dd, J = 6.6, 1.1 Hz, 1H), 7.20–7.15 (m, 1H), 7.10 (m, 1H), 6.93 (s, 1H), 4.73–4.63 (m, 1H, H-3), 4.46 (dd, J = 8.7, 7.5 Hz, 1H, H-2), 3.99 (dd, J = 8.8, 6.8 Hz, 1H, H-2), 3.72 (s, 3H, CH_3), 3.16 (dd, J = 14.7, 5.7 Hz, 1H, CH_2-indole), 2.71 (dd, J = 14.7, 8.9 Hz, 1H, CH_2-indole); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (C=O), 147.0, 137.8, 137.0, 134.6, 133.5, 131.1, 130.4, 129.0, 128.1, 127.4, 127.1, 124.6, 123.5, 121.8, 119.1, 119.0, 110.0, 109.3, 100.7, 76.3, 56.1, 32.8, 30.0 (NCH_3). HRMS-ESI(+) for $C_{26}H_{21}ClN_2O_2$ *m/z* 429.136432 $[M+H]^+$. Found *m/z* 429.1348.

(3*S*,9bR)-9b-(4-chlorophenyl)-3-((1-ethyl-1*H*-indol-3-yl) methyl)-2,3-dihydrooxazolo [2,3-a]isoindol-5(9b*H*)-one (9e) Following the general procedure, starting from compound **7b** (0.098 g, 0.236 mmol), sodium hydride, NaH 80 % anhydrous reagent (0.0125 g, 0.519 mmol) and iodoethane (0.0285 mL, 0.354 mmol, d = 1.94 g mL^{-1}) were added. The reaction was stirred at room temperature for 3 h. Eluent for flash chromatography: *n*-hexane/ethyl acetate 7:3. Recrystallization with the same combination of solvents. The product was obtained as a white light solid (0.0908, 87 %). mp: 56–58 °C. $[\alpha]_D^{25}$ = +72.1° (*c* = 0.43, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85–7.77 (m, 1H), 7.56–7.43 (m, 5H), 7.36–7.27 (m, 3H), 7.19 (m, 2H), 7.09 (t, J = 7.3 Hz, 1H), 7.00 (s, 1H), 4.68 (m, 1H, H-3), 4.44 (t, J = 8.2 Hz, 1H, H-2), 4.10 (dd, J = 14.4, 7.1 Hz, 2H, NCH_2), 3.98 (dd, J = 8.2, 6.9 Hz, 1H, H-2), 3.18 (dd, J = 14.8, 5.8 Hz, 1H, CH_2-indole), 2.70 (dd, J = 14.7, 9.3 Hz, 1H, CH_2-indole), 1.42 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (C=O), 147.0, 137.8, 136.1, 134.7, 133.5, 131.1, 130.4, 129.0 (2C), 128.2, 127.4 (2C), 125.3, 124.6, 123.5, 121.7, 119.1, 119.1, 110.1, 109.4, 100.7, 76.3, 56.2, 40.9 (NCH_2), 30.1, 15.6 (CH_3). HRMS-ESI(+) for $C_{27}H_{23}ClN_2O_2$ *m/z* 443.152082 $[M+H]^+$. Found *m/z* 443.1503.

(3*S*,9bR)-9b-(4-chlorophenyl)-3-((1-propyl-1*H*-indol-3-yl) methyl)-2,3-dihydrooxazolo [2,3-*a*] isoindol-5(9b*H*)-one (9f) Following the general procedure, starting from compound **7b** (0.0697 g, 0.168 mmol), sodium hydride, NaH, 80 % anhydrous reagent (0.0087 g, 0.369 mmol) and 1-bromopropane (0.023 mL, 0.369 mmol, d = 1.35 g mL^{-1}) were added. The reaction was stirred at room temperature for 3 h. Eluent for flash chromatography *n*-hexane/ethyl acetate 7:3. Recrystallization with the same combination of solvents. The product was obtained as a white solid (0.0653, 85 %). mp: 59–61 °C. $[\alpha]_D^{25} = +83.7°$ ($c = 0.43$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 (dd, $J = 5.7$, 2.7 Hz, 1H), 7.55–7.46 (m, 5H), 7.30 (dd, $J = 11.1$, 8.5 Hz, 3H), 7.19 (dd, $J = 8.2$, 5.3 Hz, 2H), 7.09 (dd, $J = 10.9$, 4.0 Hz, 1H), 6.98 (s, 1H), 4.67 (dt, $J = 12.6$, 6.3 Hz, 1H, H-3), 4.43 (dd, $J = 8.7$, 7.5 Hz, 1H, OCH_2), 3.98 (dt, $J = 8.9$, 7.0 Hz, 3H, OCH_2 and NCH_2), 3.19 (dd, $J = 14.4$, 5.6 Hz, 1H, CH_2-indole), 2.68 (dd, $J = 14.7$, 9.4 Hz, 1H, CH_2-indole), 1.80 (dt, $J = 14.4$, 7.2 Hz, 2H, CH_2), 0.89 (t, $J = 7.4$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (C=O), 147.0, 137.8, 136.4, 134.7, 133.5, 131.1, 130.4, 129.0 (2C), 128.1, 127.4 (2C), 126.1, 124.6, 123.5, 121.6, 119.1, 119.0, 109.9, 109.5, 100.7, 76.3, 56.1, 48.0 (NCH_2), 30.1, 23.6 (CH_2), 11.7 (CH_3). HRMS-ESI(+) for $C_{28}H_{25}ClN_2O_2$ *m/z* 457.167732 $[M+H]^+$. Found *m/z* 457.1650.

(3*S*,9bR)-9b-((4-fluorophenyl)-3-((1-methyl-1*H*-indol-3-yl) methyl)-2,3-dihydrooxazolo [2,3- *a*] isoindol-5(9b*H*)-one (9g): Following the general procedure, starting from compound **7c** (0.104g, 0.338 mmol), sodium hydride, NaH 95 %, (0.0126 g, 0.523 mmol) and methyl iodide (0.033 mL, 0.523 mmol, d = 2.28 g mL^{-1}) were added. The reaction was stirred at room temperature for 20 min. Eluent for flash chromatography *n*-hexane/ethyl acetate 6:4. Recrystallization with the same combination of solvents. The product was obtained as a white light solid (0.101 g, 94 %); mp: 73–75 °C. $[\alpha]_D^{22} = +151.1$ (c = 0.12, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 7.83–7.77 (m, 1H), 7.59–7.52 (m, 2H), 7.51–7.46 (m, 3H), 7.24 (d, $J = 1.0$ Hz, 1H), 7.21 (dd, $J = 1.7$, 0.9 Hz, 1H), 7.19 (dd, $J = 3.4$, 1.8 Hz, 1H), 7.12–7.06 (m, 2H), 7.03 (d, $J = 8.7$ Hz, 2H), 6.95 (s, 1H, CH-indole), 4.68 (ddd, J = 13.1, 10.9, 6.3 Hz, 1H, H-3), 4.45 (dd, $J = 8.7$, 7.5 Hz, 1H, H-2), 3.98 (dd, $J = 8.8$, 6.8 Hz, 1H, H-2), 3.72 (s, 3H, NCH_3), 3.17 (dd, $J = 14.5$, 5.9 Hz, 1H, CH_2-indole), 2.67 (dd, $J = 14.6$, 9.2 Hz, 1H, CH_2-indole). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (C=O), 163.0 (d, $J = 245.8$ Hz, C-F), 147.2, 137.0, 134.9 (d, $J = 3.0$ Hz), 133.5, 131.1, 130.3, 128.0, 127.8 (d, $J = 8.1$ Hz), 127.0, 124.5, 123.4, 121.8, 119.1, 118.9, 115.8 (d, $J = 21.8$ Hz), 110.1, 109.3, 100.7, 76.3, 56.0, 32.7, 30.0 (NCH_3). HRMS-ESI(+) for $C_{26}H_{21}FN_2O_2$ *m/z* 413.165983 $[M+H]^+$. Found *m/z* 413.1640.

(3*R*,9b*S*)-3-((1-methyl-1*H*-indol-3-yl)methyl)-9b-(*p*-tolyl)-2,3-dihydrooxazolo2,3-*a*] isoindol-5(9b*H*)-one (9h): Following the general procedure, starting from compound **7d** (0.150 g, 0.379 mmol), sodium hydride, NaH 95 %, (0.0182 g, 0.759 mmol) and methyl iodide (0.047 mL, 0.759 mmol, d = 2.28 g mL^{-1}) were added. The reaction was stirred at room temperature for 30 min. Eluent for flash chromatography *n*-hexane/ethyl acetate 6:4. Recrystallization with the same combination of solvents. The product was obtained as a white light solid (0.129 g, 84 %).; mp: 150–151 °C; $[\alpha]_D^{22} = +72.6$ (c = 0.13, CH_2Cl_2) ^1H NMR (300 MHz, $CDCl_3$) δ 7.82–7.77 (m, 1H), 7.51 (d, $J = 8.1$ Hz, 3H), 7.49–7.45 (m, 2H), 7.29–7.16 (m, 5H), 7.09 (ddd, $J = 8.0$, 6.8, 1.3 Hz, 1H), 6.97 (s, 1H, CH-indole), 4.67 (dq, $J = 9.2$, 6.8 Hz, 1H, H-3), 4.45 (dd, $J = 8.7$, 7.5 Hz, 1H, H-2), 3.99 (dd, $J = 8.7$, 6.8 Hz, 1H, H-2), 3.73 (s, 3H, NCH_3), 3.22 (ddd, J = 14.6, 6.0, 0.7 Hz, 1H, CH_2-indole), 2.66 (dd, $J = 14.6$, 9.2 Hz, 1H, CH_2-indole), 2.39 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (C=O), 147.5, 138.6, 137.0, 136.0, 133.3, 131.2, 130.1 (2C), 129.6, 128.0, 127.0, 125.8 (2C), 124.4, 123.5, 121.8, 119.0, 119.0, 110.4, 109.2, 101.1, 76.4, 55.9, 32.8 (NCH_3), 30.2, 21.3 (CH_3). HRMS-ESI(+) for $C_{27}H_{24}N_2O_2$ *m/z* 409.191054 $[M+H]^+$. Found *m/z* 409.1895.

(3*S*,9bR)-9b-((4-chloro-3-nitrophenyl)-3-((1-methyl-1*H*-indol-3-yl)methyl)-2,3-dihydrooxazolo [2,3-*a*] isoindol-5(9b*H*)-one (9i): Following the general procedure, starting from compound **7f** (0.0762 g, 0.166 mmol), sodium hydride 95 % anhydrous reagent (0.0080 g, 0.332 mmol) and methyl iodide (0.042 mL, 0.663 mmol, d = 2.28 g mL^{-1}) were added. The reaction was stirred at room temperature for 1 h. Eluent for flash chromatography: *n*-hexane/ethyl acetate 7:3. Recrystallization with the same mixture of solvents. The product was obtained as a light crystalline yellow solid (0.0548 g, 70 %). mp: 74–76 °C. $[\alpha]_D^{25} = +65.6°$ ($c = 0.32$, CH_2Cl_2) ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (dd, $J = 10.7$, 2.0 Hz, 1H), 7.84–7.81 (m, 1H), 7.55–7.48 (m, 3H), 7.28 (dd, $J = 8.4$, 2.0 Hz, 1H), 7.23–7.20 (m, 3H), 7.14–7.06 (m, 2H), 6.84 (s, 1H, CH-indole), 4.74–4.64 (m, 1H, H-3), 4.53 (dd, $J = 8.8$, 7.5 Hz, 1H, H-2), 4.10 (dd, $J = 14.0$, 7.9 Hz, 1H, H-2), 3.67 (s, 3H, NCH_3), 3.02 (ddd, $J = 14.9$, 7.8, 0.3 Hz, 1H, CH_2-indole), 2.88 (dd, $J = 14.8$, 8.0 Hz, 1H, CH_2-indole). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (C=O), 147.9, 146.4, 140.3, 137.0, 133.9, 132.1, 131.0, 130.9, 130.7, 128.2, 127.4, 126.9, 124.8, 123.5, 122.7, 121.9, 119.2, 119.0, 109.4, 109.3, 100.0, 75.9, 56.7, 32.7 (NCH_3), 29.3. HRMS-ESI(+) for $C_{26}H_{20}ClN_3O_4$ *m/z* 474.121510 $[M+H]^+$. Found *m/z* 474.1197.

(3*S*,9bR)-9b-(4-chloro-3-nitrophenyl)-3-((1-ethyl-1*H*-indol-3-yl)methyl)-2,3-dihydrooxazolo [2,3-*a*] isoindol-5(9b*H*)-one (9j) Following the general procedure, starting from compound **7f** (0.092 g, 0.200 mmol), sodium hydride, NaH 80 % anhydrous reagent (0.0106 g, 0.440 mmol) and iodoethane (0.024 mL, 0.300 mmol, d = 1.94 g mL^{-1}) were added. The reaction was stirred at room temperature for 3 h. Eluent for flash chromatography *n*-hexane/ethyl acetate 7:3. Recrystallization with the same combination of solvents. The product was obtained as a yellow solid (0.0813 g, 83 %). mp: 64–66 °C. $[\alpha]_D^{22} = +95.3$ (c = 0.11, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, $J = 2.00$ Hz, 1H), 7.85–7.82 (m, 1H), 7.55–7.50 (m, 3H), 7.34 (dd, $J = 8.4$ and 2.0 Hz, 1H), 7.29 (s, 1H), 7.27–7.25 (m, 1H), 7.21 (dd, $J = 6.8$ and 1.1 Hz, 1H), 7.17–7.13 (m, 1H), 7.09 (ddd, J = 7.9, 6.9 and 1.2 Hz, 1H), 6.93 (s, 1H), 4.75–4.66 (m, 1H, H-3), 4.50 (dd, $J = 8.8$, 7.4 Hz, 1H, OCH_2), 4.10–4.02 (m, 3H, OCH_2 and NCH_2), 3.08 (dd, $J = 14.7$, 5.2 Hz, 1H, CH_2-indole), 2.87 (dd, $J = 14.7$, 8.3 Hz, 1H, CH_2-indole), 1.40 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (C=O), 147.9, 146.4, 140.4, 136.0, 133.9, 132.1, 131.0, 130.9, 130.7, 128.3, 127.0, 125.6, 124.9, 123.5, 122.7, 121.8, 119.2, 119.1, 109.5, 109.4, 99.9, 76.0, 56.7, 40.8 (NCH_2), 29.4, 15.5 (CH_3). HRMS-ESI(+) for $C_{27}H_{22}ClN_3O_4$ *m/z* 488.137160 $[M+H]^+$. Found *m/z* 488.1355.

(3*R*,9b*S*)-9b-((4-chlorophenyl)-3-((1-methyl-1*H*-indol-3-yl) methyl)-2,3-dihydrooxazolo [2,3-*a*] isoindol-5(9b*H*)-one (10c): Following the general procedure, starting from compound **8b** (0.129 g, 0.310 mmol), sodium hydride, NaH 95 %, (0.0149 g, 0.621 mmol) and methyl iodide (0.0193 mL, 0.310 mmol, d = 2.28 g/mL^{-1}) were added. The reaction was stirred at room temperature for 30 min. Eluent for flash chromatography: *n*-hexane/ethyl acetate 7:3, and recrystallization using the same combination of solvents to obtain a white crystalline light solid (0.117 g, 88 %). The ^1H NMR spectrum was found to be similar to the one for **9d.** HRMS-ESI(+) for $C_{26}H_{21}ClN_2O_2$ *m/z* 429.136432 $[M+H]^+$. Found *m/z* 429.1350.

General procedure for the cyclization of *N*-alkylated oxazoloisoindolinones: To a solution of *N*-alkylated oxazoloisoindolinones (1.0 equiv.) in dry dichloromethane under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (4.0 equiv.) at room temperature. The reaction was stirred at room temperature for 20 min - 2 h. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane/ethyl acetate as eluent.

(7*S*,13b*S*)-7-(hydroxymethyl)-13,13b-dimethyl-7,8,13,13b-tetrahydro-5*H* benzo [1,2]indolizino [8,7-b]indol-5-one (11a) and (7*S*,13bR)-7-(hydroxymethyl)-13,13b-dimethyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino [8,7-b]indol-5-one (11b): To a solution of **9a** (0.0962 g, 0.289 mmol) in dry dichloromethane (10 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (145.40 μL, 1.16 mmol) at room temperature. The reaction was stirred at room temperature for 1 h 20 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using ethyl *n*-hexane/ethyl acetate (1:1) as eluent.

11a: Obtained as a white solid (0.037 g, 40 %); mp: 224–226 °C; $[\alpha]_D^{20}$ = - 83.2° (*c* = 0.39, MeOH); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 8.25 (d, *J* = 7.8 Hz, 1H), 7.75–7.68 (m, 2H), 7.54 (td, *J* = 7.6, 0.5 Hz, 1H), 7.42 (t, *J* 7.3 Hz, 2H), 7.17–7.12 (m,1H), 7.04–6.99 (m, 1H), 5.19 (t, *J* = 6.0 Hz, 1H, OH), 4.41–4.27 (m, 2H, OCH_2), 4.01 (s, 3H, NCH_3), 3.86–3.77 (m, 1H, H-7), 2.95–2.91 (m, 2H, CH_2- indole), 2.01 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) *δ* 168.3 (C=O), 148.1, 137.5, 137.2, 132.2, 131.5, 128.9, 125.5, 124.3, 123.2, 122.0, 119.3, 118.4, 109.7, 109.3, 66.2, 61.9 (CH_2OH), 55.0 (C-7), 32.5 (NCH_3), 24.7, 23.6 (CH_3). HRMS-ESI(+) for $C_{21}H_{20}N_2O_2$ *m/z* 333.159754 $[M+H]^+$. Found *m/z* 333.1587; RP-HPLC: t_R = 15.72 min.

11b: Obtained as a white solid (0.026 g, 28 %); mp: 254–256 °C; $[\alpha]_D^{20}$ = + 155.5° (c = 0.39, MeOH); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 8.22 (d, *J* = 8.3 Hz, 1H), 7.77–7.69 (m, 2H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.47–7.42 (m, 2H), 7.17 (t, *J* = 7.9 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 5.07–4.96 (m, 2H, OH and OCH_2), 4.11 (s, 3H, NCH_3), 3.65 (dd, *J* = 7.2, 5.8 Hz, 2H, H-7 and OCH_2), 2.98 (d, *J* = 15.8 Hz, 1H, CH_2-indole), 2.79 (dd, *J* 15.7, 6.8 Hz, 1H, CH_2-indole), 2.00 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) *δ* 168.1 (C=O), 148.9, 137.9, 134.6, 132.5, 130.4, 128.8, 126.0, 123.8, 123.4, 122.1, 119.2, 118.4, 109.8, 106.4, 63.8, 61.0 (CH_2OH), 50.1 (C-7), 33.1 (NCH_3), 27.5, 22.5 (CH_3). HRMS-ESI(+) for $C_{21}H_{20}N_2O_2$ *m/z* 333.159754 $[M+H]^+$. Found *m/z* 333.1589; RP-HPLC: t_R = 14.66 min.

(7*S*,13b*S*)-13-ethyl-7-(hydroxymethyl)-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (12a) and (7*S*,13bR)-13-ethyl-13b-7-(hydroxymethyl)-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (12b): To a solution of **9b** (0.261 g, 0.639 mmol) in dry dichloromethane (22.0 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (321.0 μL, 2.56 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using dichloromethane/methanol 2 % as eluent.

12a: Obtained as a white solid (0.18513 g, 71 %); mp: 273–275 °C; $[\alpha]_D^{20}$ = - 27.2° (*c* = 0.12, MeOH); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 7.79 (dd, *J* = 7.1, 6.0 Hz, 2H), 7.75–7.69 (m, 1H), 7.63 (td, *J* = 7.3, 1.0 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.42–7.35 (m, 3H), 7.24–7.17 (m, 1H), 7.11–7.05 (m, 1H), 6.89–6.85 (m, 2H), 4.98 (t, *J* = 6.0 Hz, 1H, OH), 4.30–4.22 (m, 3H, NCH_2 and OCH_2), 4.04–3.97 (m, 1H, OCH_2), 3.28–3.24 (m, 1H, H-7), 3.02 (dd, *J* = 13.9, 10.5 Hz 2H, CH_2-indole), 1.10 (t, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 168.2 (C=O), 148.1, 139.2, 137.0, 133.5, 133.2, 132.4, 129.5, 129.3, 128.9, 128.5, 126.7, 125.2, 124.5, 122.9, 120.0, 119.3, 112.5, 110.0, 75.7, 62.2 (CH_2OH), 54.2 (C-7), 40.8 (NCH_2), 23.8, 13.2 (CH_3). HRMS-ESI(+) for $C_{27}H_{24}N_2O_2$ *m/z* 409.191054 $[M+H]^+$. Found *m/z* 409.1900; RP-HPLC: t_R = 3.22 min.

12b: Obtained as a white solid (0.0768 g, 29 %); mp: 268–269 °C; $[\alpha]_D^{20}$ = + 16.5° (*c* = 0.12, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 8.02–7.96 (m, 1H), 7.59 (m, 3H), 7.54–7.48 (m, 1H), 7.41 (d, *J* = 8.3 Hz, 1H), 7.35–7.22 (m, 5H), 7.20–7.13 (m, 1H), 6.95 (d, *J* = 7.3 Hz, 2H), 5.21–5.10 (m, 1H, H-3), 4.45–4.35 (m, 2H, NCH_2), 3.40–3.23 (m, 3H, OCH_2 and CH_2-indole), 2.75 (dd, *J* = 16.0, 2.2 Hz, 1H, CH_2-indole), 1.27 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 170.3 (C=O), 148.9, 141.3, 136.8, 132.5, 132.3, 131.8, 129.4, 129.2, 129.0, 127.9, 126.8, 125.1, 124.5, 122.9, 119.9, 119.2, 109.9, 109.8, 63.0 (CH_2OH), 51.2 (C-7), 41.0 (NCH_2), 22.5, 15.4 (CH_3). HRMS-ESI(+) for $C_{27}H_{24}N_2O_2$ *m/z* 409.191054 $[M+H]^+$. Found *m/z* 409.1901; RP-HPLC: t_R = 2.45 min.

(7*S*,13b*S*)-7-(hydroxymethyl)-13b-phenyl-13-propyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (13a) and (7*S*,13bR)-7-(hydroxymethyl)-13b-phenyl-13-propyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5one (13b): To a solution of **9c** (0.053 g, 0.125 mmol) in dry dichloromethane (1.5 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (62 μL, 0.502 mmol) at room temperature. The reaction was stirred at room temperature for 20 min. After this period, the solvent was evaporated to dryness and the residue was purified by preparative chromatography using ethyl *n*-hexane/ethyl acetate (6:4) as eluent.

13a: obtained as a white solid (0,0368 g; 69 %); mp: 230–231 °C; $[\alpha]_D^{20}$ = - 54.5° (*c* = 0.22, MeOH). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 7.80 (d, *J* = 7.4 Hz, 1H), 7.77–7.69 (m, 1H), 7.68–7.58 (m, 2H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.42–7.31 (m, 3H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.08 (t, *J* = 7.3 Hz, 1H), 6.88 (dd, *J* = 7.4, 1.8 Hz, 2H), 4.29–4.23 (m, 1H, OCH_2), 4.18–4.09 (m, 1H, OCH_2), 4.10–4.01 (m, 2H, NCH_2), 3.31–3.26 (m, 1H, H-7), 3.06–2.94 (m, 2H, CH_2-indole), 1.73–1.58 (m, 1H, CH_2), 1.18–1.06 (m, 1H, CH_2), 0.85 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) *δ* 168.4 (C=O), 147.8, 139.0, 136.6, 134.1, 132.6, 132.3, 129.5, 129.0, 128.8, 127.9, 125.8, 125.2, 123.4, 122.4, 119.4, 118.8, 111.0, 110.4, 72.1, 61.7 (CH_2OH), 54.1 (C-7), 46.8 (NCH_2), 24.5 (CH_2), 22.4, 10.4 (CH_3). HRMS-ESI(+) for $C_{28}H_{26}N_2O_2$ *m/z* 423.206705 $[M+H]^+$. Found *m/z* 423.2052; RP-HPLC: t_R = 3.91 min.

13b: obtained as a white solid (0,011 g; 21 %); mp: 330–331 °C; $[\alpha]_D^{20}$ = + 9.56° (c = 0.42, MeOH). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 7.86 (d, *J* = 7.1 Hz, 1H), 7.73–7.68 (m, 1H), 7.64–7.59 (m, 1H), 7.56–7.50 (m, 1H), 7.30 (d, 2H, *J* = 6.1 Hz), 7.26–7.21 (m, 1H), 7.11–7.07 (m, 1H), 6.81 (d, *J* = 6.2 Hz, 1H), 4.81–4.79 (m, 1H, H-7), 4.27–4.23 (m, 2H, NCH_2), 3.06–2.91 (m, 3H, OCH_2, CH_2-indole), 2.72–2.68 (m, 1H, CH_2-indole), 1.75–1.63 (m, 1H, CH_2), 1.17–1.07 (m, 1H, CH_2), 0.88 (t, J = 7.0 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) *δ* 168.0 (C=O), 148.5, 140.9, 136.6, 132.5, 131.9, 131.3, 129.4, 128.5, 127.6, 126.2, 125.3, 123.5, 122.5, 119.4, 118.8, 110.4, 108.5, 69.3, 61.3 (CH_2OH), 50.0 (C-7), 47.1 (NCH_2), 22.7 (CH_2), 22.1, 10.3 (CH_3). HRMS-ESI(+) for $C_{28}H_{26}N_2O_2$ *m/z* 423.206705 $[M+H]^+$. Found *m/z* 423.2054; RP-HPLC: t_R = 2.93 min.

(7*S*,13b*S*)-13b-(4-chlorophenyl)-7-(hydroxymethyl)-13-methyl-7,8,13,13b-hexahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (14a) and (7*S*,13bR)-13b-(4-chlorophenyl)-7- (hydroxymethyl)-13-methyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (14b): To a solution of **9d** (0.1012 g, 0.245 mmol) in dry dichloromethane (12.2 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (129 μL, 1.03 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane/ethyl acetate (7:3) as eluent.

14a: Obtained as a white solid (0.0654 g, 60 %); m.p.: 284−285 °C; $[\alpha]_D^{20}$ = - 123.8° (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 7.92 (d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.72 (td, *J* = 7.6, 1.3 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 3H), 7.25–7.18 (m, 1H), 7.12–7.05 (m, 1H), 6.90 (d, *J* = 8.6 Hz, 2H), 4.96 (t, *J* = 6.0 Hz, 1H, OH), 4.30–4.20 (m, 1H, OCH_2), 4.11–4.00 (m, 1H, OCH_2), 3.73 (s, 3H, NCH_3), 3.22 (ddd, *J* = 12.1, 8.2, 2.6 Hz, 1H, H-7), 3.00 (qd, *J* = 15.3, 7.4 Hz, 2H, CH_2-indole). ^{13}C NMR (75 MHz, DMSO) *δ* 168.9 (C=O), 151.3, 148.2, 138.3, 138.1, 134.4, 134.2, 133.1, 132.7, 130.5, 130.0, 129.4, 125.9, 123.9, 122.9, 120.0, 119.1, 111.9, 110.4, 71.6, 62.2 (CH_2OH), 55.1 (C-7), 34.0 (NCH_3), 25.0. HRMS-ESI(+) for $C_{26}H_{21}ClN_2O_2$ *m/z* 429.136432 $[M+H]^+$. Found *m/z* 429.1357; RP-HPLC: t_R = 3.66 min.

14b: Obtained as a white solid (0.0312 g, 28 %); m.p.: 311–312 °C; $[\alpha]_D^{20}$ = + 36.0° (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) *δ* 7.87–7.84 (m, 2H), 7.71–7.64 (m, 1H), 7.64–7.59 (m, 1H), 7.53 (t, *J* = 7.9 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 7.27–7.21 (m, 1H), 7.12–7.07 (m, 1H), 6.86 (d, J = 8.2 Hz, 2H), 4.90 (ddd, *J* = 10.6, 6.1, 4.5 Hz, 1H, H-7), 4.73 (dd, *J* = 6.3, 4.3 Hz, 1H, OH), 3.89 (s, 3H, NCH_3), 3.15–3.04 (m, 2H, CH_2-indole and OCH_2), 2.92 (dd, *J* = 16.1, 6.9 Hz, 1H, CH_2-indole), 2.73–2.66 (m, 1H, OCH_2); ^{13}C NMR (75 MHz, DMSO) *δ* 168.0 (C=O), 148.6, 139.7, 137.8, 133.2, 132.9, 131.3, 130.9, 129.8, 129.4, 128.6, 126.0, 125.3, 123.5, 122.6, 119.5, 119.4, 118.7, 109.9, 108.5, 68.3, 61.0 (CH_2OH), 50.1 (C-7), 34.2 (NCH_3), 22.1. HRMS-ESI(+) for $C_{26}H_{21}ClN_2O_2$ *m/z* 429.136432 $[M+H]^+$. Found *m/z* 429.1337; RP-HPLC: t_R = 3.67 min.

(7*S*,13b*S*)-13b-(4-chlorophenyl)-13-ethyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-7-yl)methanol (15a) and (7*S*,13bR)-13b-(4-chlorophenyl)-13-ethyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-7-yl)methanol (15b): To a solution of **9e** (0.0305 g, 0.069 mmol) in dry dichloromethane (1.0 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (34 μL, 0.275 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane/ethyl acetate (7:3) as eluent.

15a: Obtained as a white solid (0.0202 g; 66 %); m.p.: 260–261 °C; $[\alpha]_D^{20}$ = - 142.4° (c = 0.19, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96–7.94 (m, 1H), 7.68–7.61 (m, 1H), 7.58–7.55 (m, 3H), 7.35 (d, 1H, *J* = 8.2 Hz), 7.29 (d, *J* = 6.9 and 1.1 Hz, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 7.19–7.14 (m, 1H), 6.82 (d, *J* = 8.7, 2H), 4.25 (qd, J = 7.2 and 1.6 Hz, 2H, NCH_2), 4.11 (dd, *J* = 13.1 and 1.2 Hz, 1H, OCH_2), 3.97 (dd, *J* = 13,0 and 4,7 Hz, 1H, OCH_2), 3.55 (dd, *J* = 14.6 and 12.2 Hz, 1H, CH_2-indole), 3.48–3.42 (m, 1H, H-7), 2.86 (dd, *J* = 14.6 and 2.7 Hz, 2H, CH_2-indole), 1.27 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0 (C=O), 147.7, 137.9, 137.0, 135.5, 133.1, 132.9, 132.6, 129.9, 129.7, 129.1, 126.6, 125.0, 124.7, 123.1, 120.1, 119.4, 112.7, 110.0, 72.7, 62.2 (CH_2OH), 54.3 (C-7), 40.8 (NCH_2), 23.8, 15.3 (CH_3). HRMS-ESI(+) for $C_{27}H_{23}ClN_2O_2$ *m/z* 443.152082 $[M+H]^+$. Found *m/z* 443.1507; RP-HPLC: t_R = 4.17 min.

15b: obtained as a white solid (0.0077g; 23 %); m.p.: 229–230 °C; $[\alpha]_D^{20}$ = + 12.5° (c = 0.34, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99–7.96 (m, 1H), 7.63–7.61 (m, 1H H-ar), 7.59–7.54 (m, 2H), 7.50–7.47 (m, 1H), 7.4 (d, *J* = 8.2 Hz, 1H), 7.30 (td, J = 7.0 and 1.1 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 2H), 7.16 (dd, *J* = 7.0 and 0.9 Hz, 1H), 5.12 (qd, J = 8.0 and 2.4 Hz, 1H, H-7), 4.37 (q, *J* = 7.14 Hz, 1H, NCH_2), 3,42 (dd, J = 11.2 and 7.0 Hz, 1H, OCH_2), 3.32–3,24 (m, 2H, OCH_2 and CH_2-indole), 2.78 (dd, *J* = 16,0 and 2.5 Hz, 1H, CH_2-indole), 1.24 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1 (C=O), 148.5, 136.8, 135.1, 132.6, 132.1, 131.5, 129.6, 129.4, 129.1, 126.7, 125.1, 124.6, 123.1, 120.1, 119.3, 110.0, 109.9, 69.6, 63.3 (CH_2OH), 51.2 (C-7), 40.9 (NCH_2), 22.5, 15.4 (CH_3). HRMS-ESI(+) for $C_{27}H_{23}ClN_2O_2$ *m/z* 443.152082 $[M+H]^+$. Found *m/z* 443.1489; RP-HPLC: t_R = 2.98 min.

(7*S*,13b*S*)-13b-(4-chlorophenyl)-13-propyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-7-yl)methanol (16a) and (7*S*,13bR)-13b-(4-chlorophenyl)-13-propyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-7-yl)methanol (16b): To a solution of **9f** (0.0452 g, 0.099 mmol) in dry dichloromethane (2,0 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (49 μL, 0.396 mmol) at room temperature. The reaction was stirred at room temperature for 20 min. After this period, the solvent was evaporated to dryness and the residue was purified by preparative chromatography using ethyl *n*-hexane/ethyl acetate (6:4) as eluent.

16a: obtained as a white solid (0.0308 g; 68 %); m.p.: 241–242 °C; $[\alpha]_D^{20}$ = - 87.7° (c = 0.22, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97–7.94 (m, 1H), 7.67–7.55 (m, 3H), 7.49 (d, *J* = 7,0 Hz, 1H), 7.34–7.29 (m, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 7.19–7.13 (m, 1H), 6.81 (d, *J* = 8.7 Hz, 2H), 4.13–4.07 (m, 2H, NCH_2), 4.06–4.03 (m, 1H, OCH_2), 3.96 (dd, *J* = 13.0 and 4.8 Hz, 1H, OCH_2), 3.56 (dd, *J* = 15.0 and 12.2 Hz, 1H, CH_2-indole), 3.47–3.41 (m, 1H, H-7), 2.85 (dd, *J* = 15.0 and 3.1 Hz, 1H, CH_2-indole), 1.93 (m, 1H, CH_2), 1.42–1.26 (m, 1H, CH_2), 0.96 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9 (C=O), 147.8, 138.0, 137.3, 135.4, 133.2, 132.9, 132.3, 129.9, 129.7, 129.1, 126.5, 124.8, 124.7, 123.0, 120.1, 119.4, 112.6, 110.0, 72.8, 62.2 (CH_2OH), 54.3 (C-7), 47.8 (NCH_2), 23.8, 22.9 (CH_2), 10.9 (CH_3). HRMS-ESI(+) for $C_{28}H_{25}ClN_2O_2$ *m/z* 457.167732 $[M+H]^+$. Found *m/z* 457.1645; RP-HPLC: t_R = 20.03 min.

16b: obtained as a white solid (0.0131g; 29 %); m.p.: 267–268 °C; $[\alpha]_D^{20}$ = + 11.1 (c = 0.17, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98–7.96 (m, 1H), 7.60–7.55 (m, 3H), 7.42–7.38 (m, 1H), 7.35 (s, 1H), 7.32–7.27 (m, 1H), 7.22 (d, *J* = 8.7 Hz, 2H), 7.14–7.14 (m, 1H), 6.86 (d, *J* = 8.7 Hz, 2H), 5.11–5.02 (m, 1H, H-7), 4.21–4.14 (m, 2H, NCH_2), 3.43 (dd, *J* = 11.2 and 6.7 Hz, 1H, OCH_2), 3.33–3.30 (m, 1H, OCH_2), 3.29–3.25 (m, 1H, CH_2-indole), 2.72 (dd, *J* = 16.0 and 3.3 Hz, 1H, CH_2-indole), 1.86–1.76 (m, 2H, CH_2), 1.34–1.26 (t, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2 (C=O), 148.5, 140.0, 137.1, 135.1, 132.2, 132.1, 131.8, 129.6, 129.3, 129.1, 126.5, 125.0, 124.7, 123.0, 120.1, 119.2, 110.1, 110.0, 69.7, 63.7 (CH_2OH), 51.4 (C-7), 47.7 (NCH_2), 23.2, 22.4 (CH_2), 10.9 (CH_3). HRMS-ESI(+) for $C_{28}H_{25}ClN_2O_2$ *m/z* 457.167732 $[M+H]^+$. Found *m/z* 457.1649; RP-HPLC: t_R = 18.04 min.

(7*S*,13b*S*)-13b-(4-fluorophenyl)-7-(hydroxymethyl)-13-methyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (17a) and (7*S*,13b*S*)-13b-(4-fluorophenyl)-7-(hydroxymethyl)-13-methyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (17b): To a solution of **9g** (0.1012 g, 0.245 mmol) in dry dichloromethane (8.80 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (123.3 μL, 0.981 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane/ethyl acetate (6:4) as eluent.

17a: Obtained as a white solid (0.0517 g, 51 %); mp: 247–250 °C; $[\alpha]_D^{20}$ = - 43.5° (c = 0.35, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 (d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.72 (td, *J* = 7.5, 1.3 Hz, 1H), 7.62 (td, *J* = 7.3, 0.5 Hz, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.21 (t, *J* = 8.6 Hz, 3H), 7.09 (t, *J* 7.1 Hz, 1H), 6.92 (dd, *J* = 8.8, 5.3 Hz, 2H), 4.97 (t, *J* = 6.0 Hz, 1H, OH), 4.26 (dt, *J* 12.4, 6.3 Hz, 1H, OCH_2), 4.09–4.01 (m, 1H, CH_2- OH), 3.73 (s, 3H, NCH_3), 3.41 (m, 1H, H-7), 3.00 (qd, *J* = 15.4, 7.7 Hz, 2H, CH_2-indole); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4 (C=O), 161.7 (d, *J* = 246.0 Hz, C-F), 147.9, 137.6, 135.0 (d, *J* = 2.7 Hz), 134.3, 132.6, 132.3, 130.4, 130.3, 129.4, 125.4 (d, *J* = 8.1 Hz, CH), 123.4, 122.4, 119.5, 118.6, 115.8 (d, *J* = 22.0 Hz, CH), 111.3, 109.9, 71.1, 61.7 (CH_2OH), 54.4 (C-7), 33.5 (NCH_3), 24.6. HRMS-ESI(+) for $C_{26}H_{21}FN_2O_2$ *m/z* 413.165983 $[M+H]^+$. Found *m/z* 413.1643; RP-HPLC: t_R = 6.35 min.

17b: Obtained as a white solid (0.0255 g, 25 %); mp: 259–261 °C; $[\alpha]_D^{20}$ = + 41.7° (c = 0.35, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.85 (d, *J* = 5.8 Hz, 2H), 7.65 (dt, *J* = 14.0, 7.3 Hz, 2H), 7.53 (t, *J* = 8.0 Hz, 2H), 7.29–7.21 (m, 1H), 7.12 (dd, *J* 17.6, 8.4 Hz, 3H), 6.92–6.82 (m, 2H), 4.88 (ddd, *J* = 10.6, 6.1, 4.5 Hz, 1H, H-7), 4.75 (dd, *J* = 6.3, 4.3 Hz, 1H, OH), 3.90 (s, 3H, NCH_3), 3.15–3.01 (m, 2H, CH_2- indole and OCH_2), 2.92 (dd, *J* = 15.1, 6.9 Hz, 1H, CH_2-indole), 2.68 (ddd, *J* 12.9, 7.3, 5.7 Hz, 1H, OCH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.0 (C=O), 161.3 (d, *J* = 246.2 Hz, C-F), 148.8, 137.7, 136.9 (d, *J* = 2.6 Hz, Cq), 132.9, 131.6, 130.9, 130.2 (d, *J* = 7.7 Hz, CH), 129.4, 126.0, 125.3, 123.5, 122.6, 119.4, 118.7, 115.4 (d, J = 21.0 Hz, CH), 110.0, 108.4, 68.3, 61.0 (CH_2OH), 50.1 (C-7), 34.2 (NCH_3), 22.1. HRMS-ESI(+) for $C_{26}H_{21}FN_2O_2$ *m/z* 413.165983 $[M+H]^+$. Found *m/z* 413.1630; RP-HPLC: t_R = 4.74 min.

(7*S*,13b*S*)-7-(hydroxymethyl)-13-methyl-13b-(*p*-tolyl)-7,8,13,13b-tetrahydro-5*H*-benzo [1,2] indolizino[8,7-*b*]indol-5-one (18a) and (7*S*,13bR)-7-(hydroxymethyl)-13-methyl-13b-(*p*-tolyl)-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (18b): To a solution of **9h** (0.0964 g, 0.236 mmol) in dry dichloromethane (8.8 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (119.0 μL, 0.944 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane/ethyl acetate (6:4) as eluent.

18a: Obtained as a white solid (0.0771 g, 80 %); mp: 247–250 °C; $[\alpha]_D^{20}$ = - 82.0° (c = 0.50, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (d, *J* = 7.7 Hz, 1H), 7.78 (d, *J* = 7.3 Hz, 1H), 7.70 (t, *J* = 7.3 Hz, 1H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.20 (t, *J* = 8.9 Hz, 3H), 7.08 (t, *J* = 7.4 Hz, 1H), 6.76 (d, *J* = 7.9 Hz, 2H), 5.00 (t, *J* = 5.8 Hz, 1H, OH), 4.33–4.22 (m, 1H, CH_2- OH), 4.05–3.95 (m, 1H, OCH_2), 3.72 (s, 3H, NCH_3), 3.09–2.90 (m, 2H, CH_2-

indole), 2.30 (s, 3H, p-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.9 (C=O), 148.6, 138.9, 138.1, 136.2, 135.1, 133.0, 132.8, 129.9, 129.8, 128.4, 126.0, 125.9, 123.8, 122.8, 119.9, 119.1, 111.6, 110.3, 72.0, 62.2 (CH_2OH), 54.4 (C-7), 33.9 (NCH_3), 25.1, 21.1 (CH_3). HRMS-ESI(+) for $C_{27}H_{24}N_2O_2$ *m/z* 409.191054 $[M+H]^+$. Found *m/z* 409.1885; RP-HPLC: t_R = 7.56 min.

18b: Obtained as a white solid (0.0120 g, 12 %); mp: 259–261 °C; $[\alpha]_D^{20}$ = + 74.5 (c = 0.48, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (d, *J* = 4.2 Hz, 1H), 7.86–7.83 (m, 1H), 7.69 (td, *J* = 7.6, 1.4 Hz, 1H), 7.65–7.59 (m, 1H), 7.53 (t, *J* = 7.9 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.28–7.21 (m, 1H), 7.13–7.06 (m, 1H), 6.86 (d, *J* = 8.2 Hz, 2H), 4.88 (ddd, *J* = 10.6, 6.1, 4.5 Hz, 1H, H-7), 4.76 (dd, *J* = 6.3, 4.3 Hz, 1H, OH), 3.89 (s, 3H, NCH_3), 3.14–3.01 (m, 2H, CH_2-indole and OCH_2), 2.92 (dd, *J* 16.1, 6.9 Hz, 1H, CH_2-indole), 2.72–2.63 (m, 1H, OCH_2), 2.28 (s, 3H, p-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.0 (C=O), 149.0, 137.9, 137.7, 137.6, 132.7, 132.0, 131.0, 129.2, 129.1, 127.7, 126.1, 125.2, 123.4, 122.4, 119.3, 118.6, 109.9, 108.2, 68.7, 60.3 (CH_2OH), 50.1 (C-7), 34.2 (NCH_3), 22.1, 20.6 (CH_3). HRMS-ESI(+) for $C_{27}H_{24}N_2O_2$ *m/z* 409.191054 $[M+H]^+$. Found *m/z* 409.1891; RP-HPLC: t_R = 5.33 min.

(7*S*,13b*S*)-13b-(4-chloro-3-nitrophenyl)-7-(hydroxymethyl)-13-methyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (19a) and (7*S*,13bR)-13b-(4-chloro-3-nitrophenyl)-7-(hydroxymethyl)-13-methyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (19b): To a solution of **9i** (0.0687 g, 0.145 mmol) in dry dichloromethane (6.1 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (85.5 μL, 0.680 mmol) at room temperature. The reaction was stirred at room temperature for 1 h 20 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane/ethyl acetate (6:4) as eluent.

19a: Obtained as a yellow solid (0.0117 g, 17 %); mp: 153–155 °C; $[\alpha]_D^{20}$ = - 39.5° (c = 0.35, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, *J* = 7.8 Hz, 1H), 7.82–7.70 (m, 3H), 7.65 (td, *J* = 7.4, 0.7 Hz, 1H), 7.57 (d, *J* 2.3 Hz, 1H), 7.55 (d, *J* 7.8 Hz, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.26–7.18 (m, 2H), 7.12–7.06 (m, 1H), 4.91 (t, *J* = 6.1 Hz, 1H, OH), 4.23–4.13 (m, 2H, OCH_2), 3.76 (s, 3H, NCH_3), 2.98 (ddd, *J* = 22.3, 16.8 9.3 Hz, 2H, CH_2-indole); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1 (C=O), 148.2, 147.5, 140.5, 138.3, 133.9, 133.5, 133.4, 132.7, 132.7, 130.3, 126.2, 125.9, 125.8, 125.7, 124.1, 123.1, 120.1, 119.3, 112.4, 110.6, 71.0, 62.0 (CH_2OH), 55.4 (C-7), 34.0 (NCH_3), 24.9. HRMS-ESI(+) for $C_{26}H_{20}ClN_3O_4$ *m/z* 474.121510 $[M+H]^+$. Found *m/z* 474.1193; RP-HPLC: t_R = 5.93 min.

19b: Obtained as a yellow pale solid (0.0460 g, 67 %); mp: 168–170 °C; $[\alpha]_D^{20}$ = + 53.7° (c = 0.41, MeOH); recrystallized in dichloromethane/*n*-hexane; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (d, *J* = 7.6 Hz, 1H), 7.87 (dd, *J* = 7.3, 1.0 Hz, 1H), 7.71 (td, *J* = 7.5, 1.5 Hz, 1H), 7.69 (d, *J* = 8.591 Hz, 1H), 7.64 (dd, *J* = 7.4, 0.7 Hz, 1H), 7.54 (dd, *J* = 7.8, 3.4 Hz, 3H), 7.26 (ddd, *J* = 8.3, 7.1, 1.0 Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 4.95–4.87 (m, 1H, H-7), 4.74 (dd, *J* = 5.7, 4.6 Hz, 1H, OH), 3.91 (s, 3H, NCH_3), 3.14 (td, *J* = 9.9, 6.0 Hz, 1H, CH_2-indole), 3.01–2.94 (m, 2H, CH_2- indole and OCH_2), 2.80 (dt, *J* = 10.3, 5.3 Hz, 1H, OCH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.3 (C=O), 147.8, 147.4, 141.9, 137.9, 133.3, 133.2, 131.9, 130.8, 130.4, 129.8, 125.9, 125.3, 125.2, 125.0, 123.7, 122.8, 119.5, 118.8, 110.2, 109.0, 67.7, 60.8 (CH_2OH), 50.2 (C-7), 34.2 (NCH_3), 22.0. HRMS-ESI(+) for $C_{26}H_{20}ClN_3O_4$ *m/z* 474.121510 $[M+H]^+$. Found *m/z* 474.1182; RP-HPLC: t_R = 4.70 min.

(7*S*,13b*S*)-13b-(4-chloro-3-nitrophenyl)-13-ethyl-7-(hydroxymethyl)-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (20a) and (7*S*,13bR)-13b-(4-chloro-3-nitrophenyl)-13-ethyl-7-(hydroxymethyl)-7,8,13,13b-tetrahydro-5H-benzo [1,2]indolizino[8,7-*b*]indol-5-one (20b): To a solution of **9j** (0.0711 g, 0.174 mmol) in dry dichloromethane (6.2 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (87.5 μL, 0.696 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane/ethyl acetate (6:4) as eluent.

20a: Obtained as a yellow solid (0.0097 g; 16 %); mp: 279–280 °C; $[\alpha]_D^{20}$ = + 8.6° (c = 0.23, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02–7.95 (m, 1H), 7.72–7.62 (m, 2H), 7.61–7.54 (m, 2H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.38 (m, 1H), 7.35 (s, 1H), 7.33–7.27 (m, 1H), 7.23–7.14 (m, 1H), 7.04 (dd, *J* = 8.5, 2.3 Hz, 1H), 5.37–5.21 (m, 1H), 4.37–3.89 (m, 4H), 3.63–3.32 (m, 4H), 2.89 (dd, *J* = 15.4, 3.3 Hz, 1H), 1.21 (t, *J* = 7.0 Hz, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.10 (C=O), 146.73, 140.60, 137.14, 133.05, 132.93, 132.68, 132,42, 131.40, 130.26, 128.11, 126.56, 125.16, 125.10, 124.70, 123.56, 120.45, 119.58, 113.40, 110.20, 71.91, 62.18 (CH_2OH), 54.75 (C-7), 29.84 (NCH_2), 23.73, 15.25 (CH_3). HRMS-ESI(+) for $C_{27}H_{22}ClN_3O_4$ *m/z* 488.137160 $[M+H]^+$. Found *m/z* 488.1339; RP-HPLC: t_R = 17.7 min.

20b: Obtained as a yellow solid (0.0396 g, 67 %); mp: 144–145 °C; $[\alpha]_D^{20}$ = −66.7° (c = 0.18; MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83–7.68 (m, 1H), 7.63 (t, *J* 6.8 Hz, 1H), 7.54 (d, *J* 7.8 Hz, 1H), 7.47 (d, *J* 8.3 Hz, 1H), 7.38 (dd, *J* = 8.3, 2.8 Hz, 1H), 7.24–7.17 (m, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 6.87 (dd, *J* = 7.4, 1.7 Hz, 1H), 4.98 (t, *J* = 5.9 Hz, 1H, OH), 4.25 (td, *J* = 14.2, 7.4 Hz, 3H, CH_2CH_3 and OCH_2), 4.05–3.97 (m, 1H, OCH_2), 3.28–3.24 (m, 1H, H-7) 3.05–2.94 (m, 2H, CH_2-indole), 1.10 (t, *J* = 6.9 Hz, 3H, CH_2CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.6 (C=O), 147.7, 146.7, 140.3, 136.5, 133.4, 132.9, 132.8, 132.4, 132.2, 129.9, 125.9, 125.8, 125.5, 123.6, 122.7, 119.6, 119.0, 111.7, 110.6, 70.8, 61.5 (CH_2OH), 50.2 (C-7), 24.2 (NCH_2), 14.8 (CH_3). HRMS-ESI(+) for $C_{27}H_{22}ClN_3O_4$ *m/z* 488.137160 $[M+H]^+$. Found *m/z* 488.1335; RP-HPLC: t_R = 16.7 min.

(7*R*,13bR)-7-(hydroxymethyl)-13-methyl-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (21a) and (7*R*,13b*S*)-7-(hydroxymethyl)-13-methyl-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (21b): To a solution of **10a** (0.0592 mg, 0.15 mmol) in dry dichloromethane (1.0 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (74 μL, 0.600 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness and the residue was purified by preparative thin layer chromatography using dichloromethane/methanol (95:5) as eluent.

21a: Obtained as a white solid (0.0295 g; 50 %); $[\alpha]_D^{20}$ = +159.2° (c = 0.21; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96–7.92 (m, 1H), 7.70–7.67 (m, 1H), 7.64–7.53 (m, 3H), 7.36–7.27 (m, 5H), 7.16 (ddd, J = 7.9, 5.8 and 2.2 Hz, 1H), 6.93–6.90 (m,1H), 5.60 (dd, *J* = 11.3 and 3.0 Hz, 1H, OH), 4.14–4.09 (m, 1H, OCH_2), 4.01–3.91 (m, 1H, OCH_2), 3.80 (s, 3H, CH_3), 3.56–3.53 (m, 1H, H-7 and CH_2-indole), 2.91–2.81 (m, 1h, CH_2-indole). HRMS-ESI(+) for $C_{26}H_{22}N_2O_2$ *m/z* 395.175404 $[M+H]^+$. Found *m/z* 395.1734; RP-HPLC: t_R = 6.01 min. The ^{13}C NMR spectrum was found to be similar to the one for **6a** [28].

21b: Obtained as a white solid (0.0195 g; 33 %); $[\alpha]_D^{20}$ = −34.9° (c = 0.14; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99–7.96 (m,1H), 7.65–7.60 (m, 1H), 7.58–7.51 (m, 3H), 7.37–7.28 (m, 5H), 7.20–7.14 (m, 1H), 7.00 (d, *J* = 7.0 Hz, 2H), 5.25 (m, 1H, OH), 3.94 (s, 3H, NCH_3), 3.52–3.33 (m, 3H, OCH_2 and H-7), 3.26 (dd, *J* = 16.1 and 7.5 Hz, 1H, CH_2-indole), 2.81 (dd, *J* = 16.1 and 0,8 Hz, 1H, CH_2-indole). HRMS-ESI (+) for $C_{26}H_{22}N_2O_2$ *m/z* 395.175404 $[M+H]^+$. Found *m/z* 395.1730; RP-HPLC: t_R = 4.02 min. The ^{13}C NMR spectrum was found to be similar to the one for **6b** [28].

(7*R*,13bR)-13-ethyl-7-(hydroxymethyl)-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (22a) and (7*R*,13b*S*)-13-ethyl-7-(hydroxymethyl)-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino[8,7-*b*]indol-5-one (22b): To a solution of **10b** (0.0963 g, 0.235 mmol) in dry dichloromethane (1.0 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (116.4 μL, 0.943 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness

and the residue was purified by preparative thin layer chromatography using dichloromethane/methanol (95:5) as eluent.**22a**: Obtained as a white solid (0.0584 g; 61 %). $[\alpha]_D^{20} = +156.1^\circ$ (c = 0.21; CH_2Cl_2); The ^1H NMR spectrum was found to be similar to the one for **12a**; HRMS-ESI(+) for $C_{27}H_{24}N_2O_2$ *m/z* 409.191054 $[M+H]^+$. Found *m/z* 409.1886. **22b**: Obtained as a white solid (0.0228 g; 24 %). $[\alpha]_D^{20} = -2.56^\circ$ (c = 0.11; CH_2Cl_2); The ^1H NMR spectrum was found to be similar to the one for **12b**; HRMS-ESI(+) for $C_{27}H_{24}N_2O_2$ *m/z* 409.191054 $[M+H]^+$. Found *m/z* 409.1885.

(7*R*,13bR)-13b-(4-chlorophenyl)-7- (hydroxymethyl)-13-methyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino [8,7-*b*] indol-5-one (23a) and (7*R*,13b*S*)-13b-(4-chlorophenyl)-7-(hydroxymethyl)-13-methyl-7,8,13,13b-tetrahydro-5*H*-benzo [1,2]indolizino [8,7-*b*] indol-5-one (23b): To a solution of **10c** (0.0903 g, 0.211 mmol) in dry dichloromethane (9.2 mL) under an atmosphere of N_2, was added $BF_3{\cdot}OEt_2$ (105.8 μL, 0.842 mmol) at room temperature. The reaction was stirred at room temperature for 15 min. After this period, the solvent was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane/ethyl acetate (1:1) as eluent.**23a**: Obtained as a white solid (0.0433 g, 46 %); $[\alpha]_D^{20} = +159.9^\circ$ (c = 1.0; $CHCl_3$); The ^1H NMR spectrum was found to be similar to the one for **14a**; HRMS-ESI(+) for $C_{26}H_{21}ClN_2O_2$ *m/z* 429.136432 $[M+H]^+$. Found *m/z* 429.1338. **23b**: Obtained as a white solid (0.0395 g, 44 %); $[\alpha]_D^{20} = -14.4^\circ$ (c = 1.0; $CHCl_3$); The ^1H NMR spectrum was found to be similar to the one for **14b**; HRMS-ESI(+) for $C_{26}H_{21}ClN_2O_2$ *m/z* 429.136432 $[M+H]^+$. Found *m/z* 429.1352.

4.2. Biology

4.2.1. Evaluation of in vitro antiplasmodial activity against P. Falciparum blood stage infection

Human red blood cells infected with ~1 % parasitemia of ring-stage *P. falciparum* synchronized with 5 % sorbitol were incubated with tested compounds in 96-well plates at 37 °C for 48 h in RPMI-1640 medium, supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4, 0.5 % Albumax, and 100 mM hypoxanthine under an atmosphere of 3 % O_2, 5 % CO_2, 91 % N_2. After 48 h, the cells were fixed in 2 % paraformaldehyde, transferred into PBS with 100 mM NH_4Cl, 0.1 % Triton X-100, and 1 nm YOYO-1, and then analyzed in a flow cytometer (FACSort, Becton Dickinson, Franklin Lakes, NJ, USA; $\lambda_{ex} = 488$ nm, $\lambda_{em} = 520$ nm) to count infected erythrocytes. IC_{50} values were calculated using GraphPad PRISM 5 software (La Jolla, CA, USA).

4.2.2. Cytotoxicity against mammalian cells (CC50)

In vitro cytotoxicity was assayed against two mammalian cell lines, J774 (murine macrophages) and HepG2 (human hepatocellular carcinoma). The cell lines were maintained in RPMI-1640 (HepG2) or DMEM (J774) containing 10 % fetal bovine serum and supplemented with L-glutamine, vitamins, and amino acids in 75-cm^2 flasks at 37 °C, with the medium changed twice weekly. Cell cultures from 60 % to 80 % confluence were trypsinized, washed in complete medium, and 4×10^4 cells were plated in 100 μL per well with complete medium in 96-well flat-bottom white plates for 24 h at 37 °C prior to the addition of the compounds. Triplicate aliquots of compound and the reference drugs (stock solution in DMSO) covering six different concentrations at 2-fold dilutions were added to the wells. Following incubation for 72 h at 37 °C, plates were maintained at room temperature, the culture medium was removed, and 100 μL volume of CellTiter-Glo reagent was added to each well. The bioluminescence was measured using a microplate reader Filtermax F5 Multi-Mode instrument (Molecular Devices) and Softmax software.CC50 data were obtained from at least two independent experiments for each cell line and analyzed using Prism 8.4.0. The selectivity index was estimated using the CC_{50}s from mammalian cells divided by the IC_{50} obtained against the asexual blood stage of *P. falciparum*.

4.2.3. Drug-induced hemolysis

Fresh and uninfected human O^+ erythrocytes (uRBC) were washed three times with sterile phosphate-buffered saline (PBS), adjusted for 1.5 % hematocrit, and 100 μL was dispensed in a 96-well round-bottom plate. Then, 100 μL complex previously diluted in DMSO and suspended in PBS were dispensed in the respective wells. Each compound was assayed in triplicate at 10 μM. Untreated cells received 100 μL PBS containing 0.5 % DMSO (negative control), while positive controls received saponin (Sigma-Aldrich) at 1 % v/v. Plates were incubated for 1 h at 37 °C under 5 % CO_2. Plates were centrifuged at 1500 rpm for 10 min and 100 μL supernatant was transferred to another plate, in which absorbance was measured at 540 nm using a SpectraMax 190 (Molecular Devices, San Jose, USA). Percentage hemolysis was calculated in comparison to positive and negative controls and plotted against drug concentration generated using Prism. One single experiment was performed.

4.2.4. Evaluation of In vitro activity against the liver stage of P. Berghei infection and viability studies in Huh-7 cells

Huh-7 cells, a human hepatoma cell line, were cultured in 1640 RPMI 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* hepatic infection *in vitro* was determined by measuring the luminescence of Huh-7 cell lysates 48 h after infection with a firefly luciferase-expressing *P. berghei* line, PbGFP-Luccon, as previously described [30,33]. Briefly, 1×10^4 cells/well were seeded in 96-well plates the day before drug treatment and infection. Tested compounds were prepared by preparing 2.5, 5 or 10 mM stock solutions of the compounds in DMSO, and were subsequently diluted in culture medium to the desired concentration. Medium was replaced by fresh medium containing the appropriate concentration of each compound 1 h prior to infection with 10,000 sporozoites/well, freshly obtained through disruption of salivary glands of infected female *Anopheles stephensi* mosquitoes, followed by centrifugation at 1700*g* for 5 min. Parasite load was determined 48 h after infection by luminescence measurement using Biotium's Firefly Luciferase Assay Kit. The effect of the compounds on the viability of Huh-7 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 IC_{50} values were determined using GraphPad software.

4.2.5. In vivo blood schizontocidal activity

Male Swiss albino mice (4–6 weeks) were infected by intraperitoneal injection of 10^6 *P. berghei*-infected erythrocytes (strain ANKA/GFP/Luc) and randomly divided into groups of four. Each drug was solubilized in DMSO/saline (10:90 v/v) prior to administration. Treatment was initiated 24 h post-infection and given daily for three consecutive days by intraperitoneal injection of 100 μL. Mice treated with chloroquine under the same schedule were employed as positive controls, while untreated infected mice were used as negative controls. Parasitemia was assessed at 4, 5, 6, 7, and 8 days post-infection (DPI), and survival was assessed at 30 DPI. To ensure a humane endpoint, mice displaying symptoms of severe anemia were euthanized prior the 30 DPI follow-up. The percentage reduction of parasitemia was calculated as [(mean vehicle group) – (mean treated group)/(mean vehicle group)] × 100 %). Experiments were conducted in the year 2023 in accordance with the recommendations of the Ethical Issues Guidelines issued by National Council for controlling animal testing (CONCEA, Brazil), and were approved (reference number 008/2020) by the local animal ethics committee at Fiocruz Bahia (IGM, Salvador, Brazil).

CRediT authorship contribution statement

Paulo A.F. Pacheco: Writing – review & editing, Writing – original draft, Investigation. **Ricardo J.F. Ferreira:** Writing – review & editing, Writing – original draft, Investigation. **Diana Fontinha:** Writing – review & editing, Investigation. **Caroline Conceição Sousa:** Writing – review & editing, Investigation. **Jenny Legac:** Investigation. **Valentina Barcherini:** Writing – review & editing, Investigation. **Philip J. Rosenthal:** Writing – review & editing, Supervision. **Miguel Prudêncio:** Writing – review & editing, Supervision, Funding acquisition. **Diogo R.M. Moreira:** Writing – review & editing, Supervision, Funding acquisition. **Maria M.M. Santos:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by National Funds (FCT/MEC, Fundação para a Ciência e Tecnologia and Ministério da Educação e Ciência) through UIDB/04138/2020 (iMed.Ulisboa), project PTDC/QUI-QOR/1304/2020, and fellowship 2022.11539.BD (R.F.). The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project Nº 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC). The authors acknowledge the financial support from FCT and Portugal 2020 to the Portuguese Mass Spectrometry Network (Rede Nacional de Espectrometria de Massa – RNEM; LISBOA-01-0145-FEDER-402-022125). M.P. acknowledges the "la Caixa" Foundation for grant HR-848 and the European Union Horizon Europe programme for grant 101080744. D.F. acknowledges FCT/MEC for grant 2022.02624.PTDC. D.R.M.M. acknowledges the support of CNPq (grants no. 305732/2019–6 and 440227/2022–4, Brazil) and INOVA Fiocruz (grant number VPPIS-004-FIO-22-2-97-30, Brazil). C.C.S. was supported through a FAPESB and a CAPES Print FIOCRUZ doctoral scholarship (Finance Code 001, Brazil).

Abbreviations used

ABS	asexual blood stages
CQ	chloroquine
Et	ethyl
Me	methyl
P	*Plasmodium*
Ph	phenyl
Pr	Propyl

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmcr.2025.100258.

Data availability

Data will be made available on request.

References

[1] K. Plewes, S.J. Leopold, H.W.F. Kingston, A.M. Dondorp, Malaria: what's new in the management of malaria? Infect. Dis. Clin. 33 (1) (2019) 39–60, https://doi.org/10.1016/j.idc.2018.10.002.
[2] E.A. Ashley, A. Pyae Phyo, C.J. Woodrow, Malaria, Lancet 391 (10130) (2018) 1608–1621, https://doi.org/10.1016/s0140-6736(18)30324-6.
[3] M. Prudêncio, J.C. Costa, Research funding after COVID-19, Nat. Microbiol. 5 (8) (2020) 986, https://doi.org/10.1038/s41564-020-0768-z.
[4] W.H. Organization, Wolrd Malaria Report, 2023.
[5] A.A. Escalante, M.A. Pacheco, Malaria molecular epidemiology: an evolutionary genetics perspective, Microbiol. Spectr. 7 (4) (2019), https://doi.org/10.1128/microbiolspec.AME-0010-2019.
[6] S. Sato, Plasmodium-a brief introduction to the parasites causing human malaria and their basic biology, J. Physiol. Anthropol. 40 (1) (2021) 1–13, https://doi.org/10.1186/s40101-020-00251-9.
[7] S. Basu, P.K. Sahi, Malaria: an update, Indian J. Pediatr. 84 (7) (2017) 521–528, https://doi.org/10.1007/s12098-017-2332-2.
[8] P.F. Pimenta, A.S. Orfano, A.C. Bahia, A.P. Duarte, C.M. Ríos-Velásquez, F.F. Melo, F.A. Pessoa, G.A. Oliveira, K.M. Campos, L.M. Villegas, N.B. Rodrigues, R. Nacif-Pimenta, R.C. Simões, W.M. Monteiro, R. Amino, Y.M. Traub-Cseko, J.B. Lima, M. G. Barbosa, M.V. Lacerda, An overview of malaria transmission from the perspective of Amazon Anopheles vectors, Mem. Inst. Oswaldo Cruz 110 (1) (2015) 23–47, https://doi.org/10.1590/0074-02760140266.
[9] A.F. Cowman, J. Healer, D. Marapana, K. Marsh, Malaria, Biol. Dise. Cell 167 (3) (2016) 610–624, https://doi.org/10.1016/j.cell.2016.07.055.
[10] C.J. Merrick, Hypnozoites in Plasmodium: do parasites parallel plants? Trends Parasitol. 37 (4) (2021) 273–282, https://doi.org/10.1016/j.pt.2020.11.001.
[11] E.A. Lopes, M.M. Santos, M. Mori, Pharmacological treatment of malaria, in: Antiprotozoal Drug Development and Delivery, Springer, 2022, pp. 219–240.
[12] K.K. Dayananda, R.N. Achur, D.C. Gowda, Epidemiology, drug resistance, and pathophysiology of Plasmodium vivax malaria, J. Vector Borne Dis. 55 (1) (2018) 1–8, https://doi.org/10.4103/0972-9062.234620.
[13] A. Bartoloni, L. Zammarchi, Clinical aspects of uncomplicated and severe malaria, Mediterr. J. Hematol. Infect. Dis. 4 (1) (2012) e2012026, https://doi.org/10.4084/mjhid.2012.026.
[14] D.A. Milner Jr., Malaria pathogenesis, Cold Spring Harb. Perspect Med. 8 (1) (2018) a025569, https://doi.org/10.1101/cshperspect.a025569.
[15] S. Yeung, Malaria-update on antimalarial resistance and treatment approaches, Pediatr. Infect. Dis. J. 37 (4) (2018) 367–369, https://doi.org/10.1097/inf.0000000000001887.
[16] K. Haldar, S. Bhattacharjee, I. Safeukui, Drug resistance in Plasmodium, Nat. Rev. Microbiol. 16 (3) (2018) 156–170, https://doi.org/10.1038/nrmicro.2017.161.
[17] M.B. Markus, Malaria eradication and the hidden parasite reservoir, Trends Parasitol. 33 (7) (2017) 492–495, https://doi.org/10.1016/j.pt.2017.03.002.
[18] M.P. Anthony, J.N. Burrows, S. Duparc, J.J. Moehrle, T.N. Wells, The global pipeline of new medicines for the control and elimination of malaria, Malar. J. 11 (2012) 316, https://doi.org/10.1186/1475-2875-11-316.
[19] J.N. Burrows, S. Duparc, W.E. Gutteridge, R. Hooft van Huijsduijnen, W. Kaszubska, F. Macintyre, S. Mazzuri, J.J. Möhrle, T.N.C. Wells, New developments in anti-malarial target candidate and product profiles, Malar. J. 16 (1) (2017) 26, https://doi.org/10.1186/s12936-016-1675-x.
[20] E.A. Lopes, M.M.M. Santos, M. Mori, Antimalarial drugs: what's new in the patents? Expert Opin. Ther. Pat. 33 (3) (2023) 151–168, https://doi.org/10.1080/13543776.2023.2203814.
[21] P.M. Cheuka, P. Njaria, G. Mayoka, E. Funjika, Emerging drug targets for antimalarial drug discovery: validation and insights into molecular mechanisms of function, J. Med. Chem. 67 (2) (2024) 838–863, https://doi.org/10.1021/acs.jmedchem.3c01828.
[22] A.S. Surur, S.A. Huluka, M.L. Mitku, K. Asres, Indole: the after next scaffold of antiplasmodial agents? Drug Des. Dev. Ther. 14 (2020) 4855–4867, https://doi.org/10.2147/dddt.S278588.
[23] P.A.F. Pacheco, M.M.M. Santos, Recent progress in the development of indole-based compounds active against malaria, trypanosomiasis and leishmaniasis, Molecules 27 (1) (2022) 319, https://doi.org/10.3390/molecules27010319.
[24] P.N. Kalaria, S.C. Karad, D.K. Raval, A review on diverse heterocyclic compounds as the privileged scaffolds in antimalarial drug discovery, Eur. J. Med. Chem. 158 (2018) 917–936, https://doi.org/10.1016/j.ejmech.2018.08.040.
[25] M. Condello, E. Pellegrini, G. Multari, F.R. Gallo, S. Meschini, Voacamine: alkaloid with its essential dimeric units to reverse tumor multidrug resistance, Toxicol. Vitro 65 (2020) 104819, https://doi.org/10.1016/j.tiv.2020.104819.
[26] S.A. Bouwman, R. Zoleko-Manego, K.C. Renner, E.K. Schmitt, G. Mombo-Ngoma, M.P. Grobusch, The early preclinical and clinical development of cipargamin (KAE609), a novel antimalarial compound, Trav. Med. Infect. Dis. 36 (2020) 101765, https://doi.org/10.1016/j.tmaid.2020.101765.
[27] A.B. Oliveira, M.F. Dolabela, F.C. Braga, R.L. Jácome, F.P. Varotti, M.M. Póvoa, Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids, An. Acad. Bras. Cienc. 81 (4) (2009) 715–740, https://doi.org/10.1590/s0001-37652009000400011.
[28] N.A. Pereira, Â. Monteiro, M. Machado, J. Gut, E. Molins, M.J. Perry, J. Dourado, R. Moreira, P.J. Rosenthal, M. Prudêncio, M.M. Santos, Enantiopure indolizinoindolones with *in vitro* activity against blood- and liver-stage malaria parasites, ChemMedChem 10 (12) (2015) 2080–2089, https://doi.org/10.1002/cmdc.201500429.
[29] V. Barcherini, J. Almeida, E.A. Lopes, M. Wang, E.S.D. Magalhães, M. Mori, S. Wang, L. Saraiva, M.M.M. Santos, Potency and selectivity optimization of tryptophanol-derived oxazoloisoindolinones: novel p53 activators in human colorectal cancer, ChemMedChem 16 (1) (2021) 250–258, https://doi.org/10.1002/cmdc.202000522.
[30] I.H. Ploemen, M. Prudêncio, B.G. Douradinha, J. Ramesar, J. Fonager, G.J. van Gemert, A.J. Luty, C.C. Hermsen, R.W. Sauerwein, F.G. Baptista, M.M. Mota, A. P. Waters, I. Que, C.W. Lowik, S.M. Khan, C.J. Janse, B.M. Franke-Fayard, Visualisation and quantitative analysis of the rodent malaria liver stage by real

time imaging, PLoS One 4 (11) (2009) e7881, https://doi.org/10.1371/journal.pone.0007881.

[31] M. Rottmann, C. McNamara, B.K. Yeung, M.C. Lee, B. Zou, B. Russell, P. Seitz, D. M. Plouffe, N.V. Dharia, J. Tan, S.B. Cohen, K.R. Spencer, G.E. González-Páez, S. B. Lakshminarayana, A. Goh, R. Suwanarusk, T. Jegla, E.K. Schmitt, H.P. Beck, R. Brun, F. Nosten, L. Renia, V. Dartois, T.H. Keller, D.A. Fidock, E.A. Winzeler, T. T. Diagana, Spiroindolones, a potent compound class for the treatment of malaria, Science 329 (5996) (2010) 1175–1180, https://doi.org/10.1126/science.1193225.

[32] V. Barcherini, J.B. Loureiro, A. Sena, C. Madeira, P. Leandro, L. Saraiva, A.M. M. Antunes, M.M.M. Santos, Metabolism-guided optimization of tryptophanol-derived isoindolinone p53 activators, Pharmaceuticals 16 (2) (2023) 146, https://doi.org/10.3390/ph16020146.

[33] M. Prudêncio, M.M. Mota, A.M. Mendes, A toolbox to study liver stage malaria, Trends Parasitol. 27 (12) (2011) 565–574, https://doi.org/10.1016/j.pt.2011.09.004.