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Synthesis and antimalarial testing of neocryptolepine analogues: Addition of ester function in SAR study of 2,11-disubstituted indolo [2,3-*b*]quinolines

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ABSTRACT

This report describes the synthesis, and *in vitro* and *in vivo* antimalarial evaluations of certain ester-modified neocryptolepine (5-methyl-5*H*-indolo[2,3-*b*]quinoline) derivatives. The modifications were carried out by introducing ester groups at the C2 and/or C9 position on the neocryptolepine core and the terminal amino group of the 3-aminopropylamine substituents at the C11 position with a urea/thiourea unit. The antiplasmodial activities of our derivative agents against two different strains (CQS: NF54, and CQR: K1) and the cytotoxic activity against normal L6 cells were evaluated. The test results showed that the ester modified neocryptolepine derivatives have higher antiplasmodial activities against both strains and a low cytotoxic activity against normal cells. The best results were achieved by compounds **9c** and **12b** against the NF54 strain with the IC_{50}/SI value as 2.27 nM/361 and 1.81 nM/321, respectively. While against K1 strain, all the tested compounds showed higher activity than the well-known antimalarial drug chloroquine. Furthermore, the compounds were tested for β-haematin inhibition and 12 were found to be more active than chloroquine (IC_{50} = 18 μM). Structure activity relationship studies exposed an interesting linear correlation between polar surface area of the molecule and β-haematin inhibition for this series. *In vivo* testing of compounds **7** and **8a** against NF54 strain on *Plasmodium berghei* female mice showed that the introduction of the ester group increased the antiplasmodial activity of the neocryptolepine core substantially.

1. Introduction

Malaria is a potentially deadly tropical disease caused by parasites of the genus *Plasmodium*, which is transmitted via the bites of infected mosquitoes [1]. According to the World Health Organization (WHO), this disease led to about 216 million malarial infected cases in 2010, and approximately 0.7 million died due to the non-availability of proper treatment, mostly involving children under 5 years old [2]. The *Plasmodium falciparum* species, which is the most virulent and deadly of the malaria parasites, is responsible in more than 90% of the cases. In spite of the intensive efforts to combat malaria, the incidence of malaria has not decreased, especially in the tropical and subtropical areas [3].

As one of the most effective and cheapest therapeutic agents, chloroquine (CQ) has been used for more than half a century as a specific drug for the treatment of malaria patients [4]. The unique endoperoxide structure of artemisinin emerged in the 1970s as the results of efforts to find a new drug from plants [5]. These drugs are still important regarding medical treatment and making efforts to find more active derivatives from the leads are still continued [6,7]. However, the *P. falciparum* strains resistant to CQ are still spreading in the endemic areas [8] and *in vitro* and *in vivo* resistances even against the most recently introduced artemisinin-based combination therapy (ACT) have also been demonstrated as therapy for uncomplicated *P. falciparum* infections [9]. Therefore, the development

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of new chemotherapeutic treatments for this disease is urgently needed.

The isolation of new lead compounds from plants is one of the strategies in the search for new drugs against infectious diseases [10–12]. The present work has been carried out as part of our ongoing program to develop novel antimalarial drugs based on the natural product neocryptolepine isolated from the roots of the climbing shrub *Cryptolepis sanguinolenta* [13] that is used in Central and West Africa in traditional medicine for the treatment of malaria [14].

The indoloquinoline alkaloids [15,16] such as cryptolepine (5-methyl-5*H*-indolo[2,3-*c*]quinoline) (**I**), neocryptolepine (5-methyl-5*H*-indolo[2,3-*b*]quinoline, cryptotackieine) (**II**), isocryptolepine (5-methyl-5*H*-indolo[3,2-*c*]quinoline, cryptosanguinolentine) (**III**), and the non-natural isoneocryptolepine (5-methyl-5*H*-indolo[2,3-*c*]quinoline) (**IV**) were reported to show potent antiplasmodial activity against chloroquine-resistant *P. falciparum* [17], as shown in Fig. 1. Due to the lower affinity of neocryptolepine for DNA and topoisomerase II inhibition compared to cryptolepine and isocryptolepine, the neocryptolepine core has been further used as the lead for developing an antimalarial agent [18].

In 2002, Pieters and the co-authors reported that the 2-halo-substituted neocryptolepines (2-bromo-, 2-chloro-, 2-fluoro-, and 2-iodo) are more active against *P. falciparum* than neocryptolepine and less cytotoxic, the most active and selective compound being 2-bromoneocryptolepine with an IC_{50} against the chloroquine-resistant *P. falciparum* of 4.0 μM while the IC_{50} on the MRC-5 cells is >32 μM. Furthermore, the 2,9-disubstituted derivatives with a cyano group at C9 and various substituents at C2 exhibited an antitrypanosomal activity in the micromole range in the absence of an obvious cytotoxicity [19]. Recently, El-Sayed et al. introduced aminoalkylamino groups at C8 or C11 of neocryptolepine skeleton by varying the substituents at C2, which showed an increased antiplasmodial activity of the neocryptolepine core with the *in vitro* selectivity index >500. The most active compounds bearing 5-methyl and 8-(N^1,N^1-diethylpentane-1,4-diamine) side chains showed an IC_{50} of 10 nM, which has the same potency as chloroquine and a selectivity index of 1800 [20].

In our previous paper describing the SAR study of the 5-methyl-5*H*-indolo[2,3-*b*]quinoline core, we modified and changed the ω-aminoalkylamino side chains at C11 by varying the electron-withdrawing or electron-donating nature of the C2 substituent. The compound bearing (3-(3-phenylureido)propyl)amino at C11 and Cl at C2 showed an antimalarial activity 4 times more potent than chloroquine (CQ) for CQS (NF54) with an IC_{50} of 2.2 nM and a selectivity index of 1400, and the compound substituted with ((3-phenylthioureido)pentyl)amino at C11 and MeO at C2 showed a much greater potency than CQ for CQR (K1) with an IC_{50} of 2.2 nM, a selectivity index of 1243 and a resistance index of 0.5 by K1/NF54 [21].

However, in spite of the significantly improved antimalarial activities of some compounds against the NF54 strain, testing of the *in vivo* drug screening model against *Plasmodium berghei* in Swiss mice were not satisfactory, as the neocryptolepine derivatives showed only some reduction in parasitaemia on day 4, with activities of 15.4% and 22.1%, respectively. Until now, the SAR studies of neocryptolepines (indolo[2,3-*b*]quinoline) have been conducted by changing the halogen, alkyl, nitro, alkoxy or alkylthio, amino, cyano groups as the substitution at the C2, C8, C9, and C11 positions [15]. Therefore, a search for a more effective group amenable to inducing a higher activity and less toxicity are urgently needed to improve the *in vivo* potency.

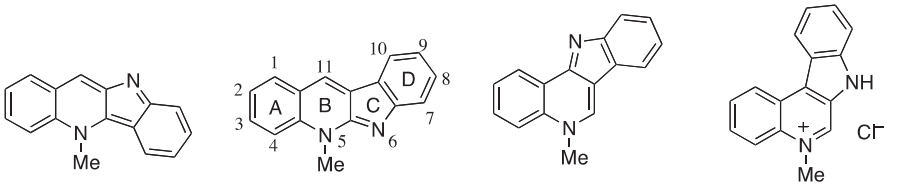
In our previous paper concerning the antiproliferative activity of neocryptolepine derivatives [22], we demonstrated that the introduction of an ester function on the indolo[2,3-*b*]quinoline core improved both the antiproliferative activity against several cell lines and the cytotoxicity index against the usual cell line [23]. A similar effect was also demonstrated on the activity of other plant-derived anticancer agents such as taxol [24], camptothecin [25], bryostatin [26], and 5-fluorouracil [27]. In addition, similar enhancement of the activities is demonstrated in the antiplasmodial and antitrypanosomal activities with bicyclic amides and esters of dialkylamino acids [28]. These improvements in the anticancer and antimalarial activity are believed to be due to increasing both the lipophilicity [29] and bioavailability of the drug by introducing an ester group into the core structure. Encouraged by these significant examples, we examined the introduction of an ester group to the neocryptolepine core and studied its influence on the antiplasmodial activity. In this study, we report the further investigation of the antimalarial potential of the neocryptolepine core. An ester group, which may act as a potential lipophilic site, was introduced at the C-2 position of the A-ring and/or the C-9 position of the D-ring in order to establish or extend the structure activity relationship (SAR) study for these regions.

2. Results and discussion

2.1. Chemistry

The C2- and C9-substituted 11-chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolines **6** were prepared using indole-3,5-dicaboxylate **2b** or 5-bromo-indole-3-carboxylate **2c** as the starting material. According to the method previously described by us [30], *N*-methylanilines were used for the three-step synthesis of **6**. The dimethyl 1*H*-indole-3,5-dicarboxylate (**2b**), a key starting compound, was first prepared by installation of ester group at the C-3 position of methyl 1*H*-indole-5-carboxylate (**1b**) using the reaction with trichloroacetyl chloride in the presence of pyridine, followed by alkaline treatment with KOH at reflux in MeOH.

The obtained 5-substituted methyl indole-3-carboxylate **2** was oxidatively combined with *N*-methylanilines **3** via chlorination with *N*-chlorosuccinimide (NCS) in the presence of 1,4-dimethylpiperazine, giving 2-(methyl(aryl)amino)-1*H*-indole-3,5-dicarboxylate **4**. The heating of **4** at 250 °C in diphenyl ether induced intramolecular acylation at the C-2 position of the aniline core, forming the tetracyclic indolo[2,3-*b*]quinolinone **5**. The treatment of **5** with $POCl_3$ afforded the desired chlorides **6** as a result of dehydrative chlorination. Addition-substitution at the C11 position smoothly proceeded by



Cryptolepine (**I**) Neocryptolepine (**II**) Isocryptolepine (**III**) Isoneocryptolepine (**IV**)

Fig. 1. Structures of indoloquinolines from *Cryptolepis sanguinolenta*.

heating with amines in THF to give various 11-aminoindolo[2,3-*b*]quinolines **7**–**9** (Scheme 1).

3. Pharmacology

3.1. Antiplasmodial activity and cytotoxicity

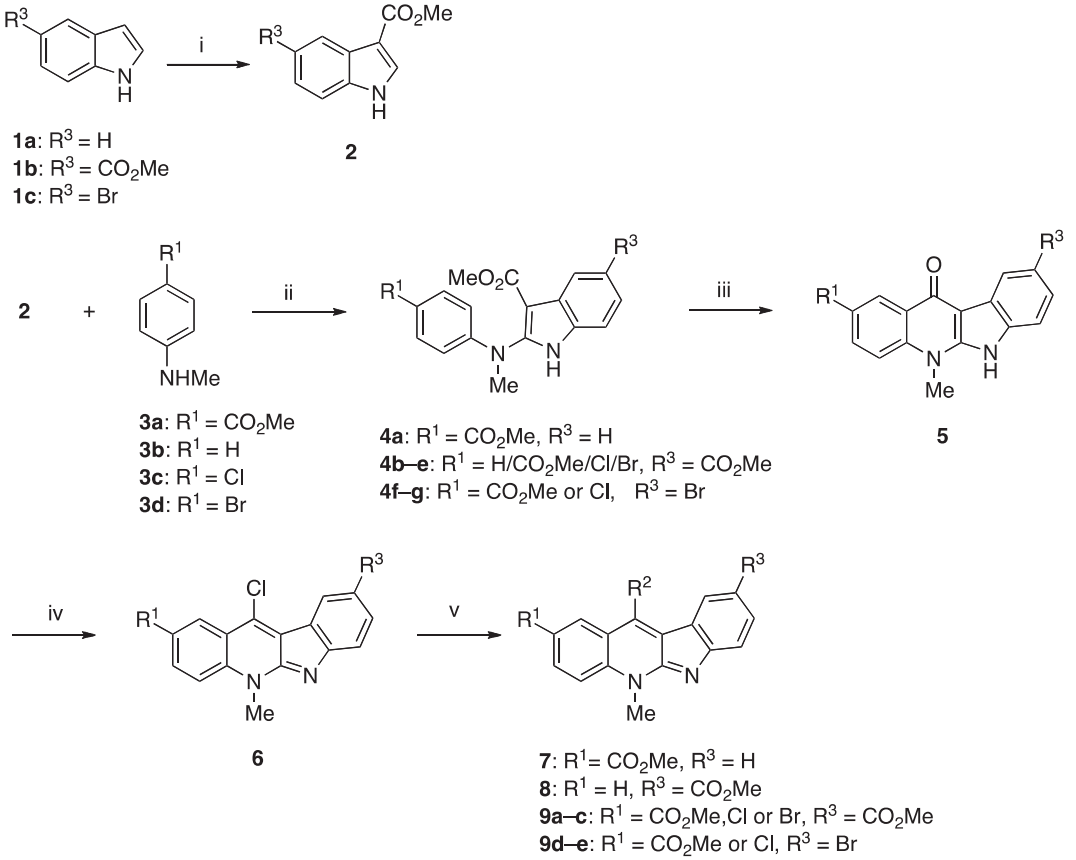
The introduction of an amino group at the C11 position and a halo group at the C2 position on the 5-methylindole[2,3-*b*]quinoline (neocryptolepine) core can significantly increase the antiplasmodial activities compared to the natural product against the chloroquine-sensitive *P. falciparum* Ghana strain based on a previous study [19]. Based on these facts, we introduced the amino group at the C11 position with the varying kinds of substituents at the C2 position and the C9 position for the SAR study of the neocryptolepine core.

The results of the studies on the antiplasmodial activity against *P. falciparum* (CQS: NF54) and the cytotoxicity towards L6 cells based on the synthesized derivatives **7**–**10** are summarized in Table 1 along with the results of the antimalarial drug, chloroquine. All the tested compounds, except the compound **8g**, have significant cytotoxic effect against the NF54 strains of *P. falciparum*. From the data of series **8**, we found that compound **8a**, with a 3-aminopropylamino group at the C11 position, contributed to the antimalarial activity most with an IC_{50} value of 24.8 nM. In addition, compared to the non-substituted compound **10**, which was synthesized in our previous study, the remarkable increase happened after introducing the ester group at the C2 or C9 position (compounds **7** and **8a**). Moreover, the ester group showed a higher contribution to the antiplasmodial activity than the Cl group that was regarded as the best motif at the C2 position. Therefore, we can confirm that the substitution of the ester group at either C2 or C9 position on the neocryptolepine core can both have a positive effect on the antimalarial activity. Due to this discovery, we have further synthesized several 2,9-disubstituted derivatives **9** by varying the halogen (−Cl, −Br) and ester group, and as expected, their antimalarial activity was improved and found higher than that of the well-known antimalarial agent, chloroquine, with a range of IC_{50} value from 2.16 to 9.52 nM and also an improved SI value that ranged from 56.4 to 361.

On the other hand, the solubility of an agent in an aqueous medium can significantly influence its further application, despite its high biological activity. We further tried to improve the solubility of the agents by transforming the ester group into a carboxylic acid substituent. The C9-ester substituted neocryptolepines were hydrolysed into the C9-carboxylic acid analogues by treating the methyl 11-amino-5*H*-indolo[2,3-*b*]quinoline-9-carboxylate **8** with NaOH(aq) in MeOH. The mixtures were refluxed overnight to afford the C9-carboxylic acid substituted indolo[2,3-*b*]quinolines **11** in good yields, as shown in Scheme 2. The evaluation results of their antimalarial activity are summarized in Table 2.

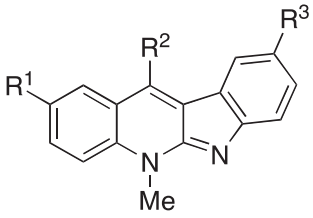
Unfortunately, it turned out that though the solubility of the hydrolysed derivatives **11** in water was improved, their antiplasmodial activity against *P. falciparum* dramatically decreased compared to their precursors **8a**, **8c** and **8d**. Similar effect was also mentioned on the comparable activity of the natural alkaloids cryptolepinoic acid and methyl cryptolepinoate [31].

Further modifications of the 11-amino neocryptolepine derivatives **7**–**9** at the terminal amino group of the lateral attachment were carried out as outlined in Scheme 3. The amino group was

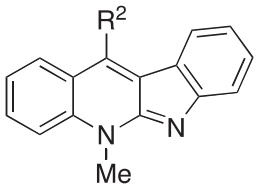


Scheme 1. Preparation of ester substituted 11-aminoneocryptolepines **7**–**9**. Reagents and conditions: (i) (a) pyridine, trichloroacetyl chloride, THF, (b) KOH, MeOH, reflux. (ii) (a) *N*-Chlorosuccinimide, 1,4-dimethylpiperazine, (b) trichloroacetic acid. (iii) Diphenyl ether, reflux. (iv) $POCl_3$, toluene, reflux. (v) Appropriate amines.

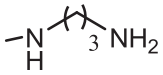
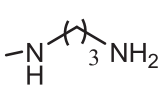
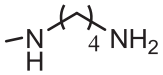
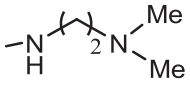
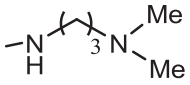
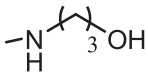
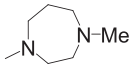
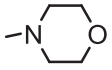
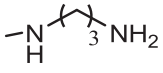
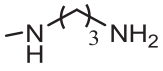
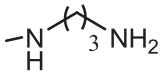
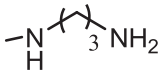
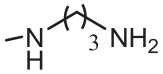
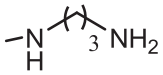
Table 1
The antiplasmodial activity against *P. falciparum* (CQS: NF54) and cytotoxicity towards L6 cells of the neocryptolepine derivatives **7**–**9**.



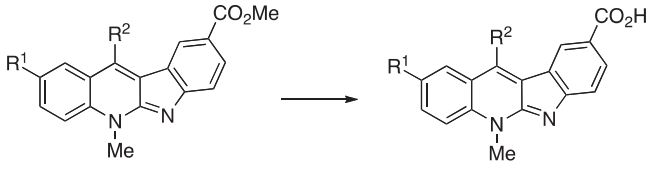
7–9



10[20]

Compound	R^1	R^2	R^3	Yield	L6 cells IC_{50} nM	NF54 IC_{50} nM	SI[a] L6/NF54	β-Haematin inhibition IC_{50} μM
Neocryptolepine					3194	1580	2.0	
10 [20]	H		H	91%	279	78.8	3.50	
8a	H		CO_2Me	92%	218	24.8	8.79	48.9
8b	H		CO_2Me	95%	436	55.8	7.81	
8c	H		CO_2Me	86%	315	42.5	7.41	51.5
8d	H		CO_2Me	87%	268	33.3	8.05	62.5
8e	H		CO_2Me	89%	639	278	9.49	122.8
8f	H		CO_2Me	91%	6190	750	8.25	105.5
8g	H		CO_2Me	90%	3810	1770	2.15	55.5
7	CO_2Me		H	93%	337	8.28	40.7	104.4
9a	CO_2Me		CO_2Me	95%	1390	9.52	146	13.0
9b	Cl		CO_2Me	88%	427	7.57	56.4	25.4
9c	Br		CO_2Me	83%	819	2.27	361	11.5
9d	CO_2Me		Br	96%	1050	4.54	232	10.2
9e	Cl		Br	91%	401	2.16	186	30.1
Podophylotoxin					14.5			
Chloroquine						9.40		18.4

[a] Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.).



Scheme 2. Hydrolysis of **8**. Reagents and conditions: MeOH/NaOH(*aq*).

transformed into urea/thiourea group by mixing with isocyanate or isothiocyanate in dried THF with stirring at 0 °C to room temperature. Their antiproliferative activities against *P. falciparum* and L6 cells are summarized in Table 3.

As shown in Table 3, after the transforming of the 3-aminopropylamine to the urea group, the cytotoxicity of all the synthesized urea derivatives **12** against the L6 cells decreased, especially the 2,9-diester substituted compound **12e** with a significant IC_{50} value of 26,500 nM. In addition, the mono-substituted derivatives **12a**–**d** at the C2 or C9 position had improved IC_{50} values ranging from 1.81 to 14.5 nM against *P. falciparum* (CQS: NF54). In addition, the thiourea group showed a higher contribution to the antiplasmodial activity against *P. falciparum* (CQS: NF54) than the urea group, when compared between agents **12c**/**12d** and agents **12e**/**12f**. On the other hand, the antiplasmodial activity against *P. falciparum* (CQS: NF54) of the C2 and C9 disubstituted ones **12e**–**i** slightly decreased. All these thiourea/urea derivatives showed significant activity against CQS (NF54), and some of them are higher than the well-known antimalarial drug chloroquine (IC_{50} = 9.1 nM). Compound **12b** gave the best result with an IC_{50} value of 1.81 nM and a high SI value of 321 among the compound **12** series.

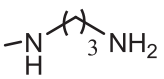
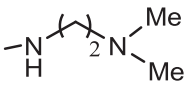
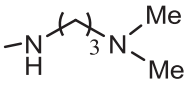
3.2. β-Haematin inhibition and QSAR

The formation of haemozoin in the acidic food vacuole of *P. falciparum* is vital for malaria parasite survival. Inhibiting this crystallisation process causes an accumulation of cytotoxic haematin, Fe(III)PPIX, an oxidation product of the haem molecule which is released upon the parasite's ingestion of haemoglobin [32]. Several well-known antimalarial drugs (e.g. chloroquine) are thought to interact with haematin at pH 4.8 through π-stacking, coordination and/or hydrogen bonding, blocking the growth face of haemozoin and slowing down the crystal formation rate [33]. Despite the development of resistance, now widely accepted to be the result of altered parasite transporter proteins in the food vacuole membrane, haemozoin inhibition remains a suitable parasite-specific drug target. Recently, neutral lipids have been shown to mediate haemozoin formation in the digestive vacuole [34,35]. Furthermore, synthetic haemozoin (β-haematin) can form under biomimetic conditions with detergent mediators, which imitate the neutral lipids. As a result, an assay has been developed which quantifies the ability of a compound to inhibit β-haematin formation by measuring the UV–vis absorbance of the haem–pyridine complex at 405 nm, formed when pyridine is added to free haematin [36,37].

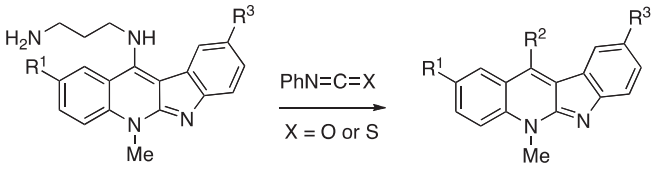
The compounds were tested for β-haematin inhibition (Tables 1 and 3) and many show high activity, with 12 samples having lower IC_{50} values than chloroquine (18 μM). Structure activity relationship studies exposed an interesting linear correlation (Fig. 2a) between polar surface area of the molecule and β-haematin inhibition for this series. Intriguingly, the direct relationship may suggest that the site at which these molecules block β-haematin growth is hydrophilic in nature. The neocryptolepine molecular structures bear similarities to the 4-aminoquinoline based antimalarials. *In vitro* studies with these drugs have revealed an increase in the amount of free haem in the parasite digestive vacuole, suggesting haemozoin inhibition as the mechanism of action [38]. Whilst the neocryptolepine series shows similar β-haematin inhibition activity, correlation analysis between whole cell NF54 IC_{50} values and β-haematin IC_{50}s revealed a very weak correlation (Fig. 2b). This is not entirely unexpected owing to the large number of factors that might influence a compound's ability to cross four membranes to reach the site of haemozoin formation (the red blood cell membrane, the parasitophorous vacuole membrane, the *P. falciparum* plasma membrane and the food vacuole membrane).

In order to explore factors that might influence activity in NF54 parasites, multiple regression analysis was carried out between the logged IC_{50} (log(NF54IC_{50})) and a range of predicted physical properties for each molecule in the series as well as measured β-haematin inhibition IC_{50} values (Fig. 2c). The statistically strongest correlation was observed for the 3-variable model with polar surface area (PSA), mean water of hydration (H_2O·hyd) and the natural log of the hydrogen bond donors (ln(HBD)). Fitted coefficients show a large dependence on the hydration term, such that molecules which are well hydrated have the greatest activity. Opposing this, with the second highest weighting, is the PSA term which, when decreased, increases the activity. Lastly, an increase in ln(HBD) results in greater activity. Upon entering the parasite membrane, a highly hydrated molecule would liberate many molecules of water, causing a favourable increase in entropy. A molecule with more hydrogen bond

Table 2
The antiplasmodial activity against *P. falciparum* (CQS: NF54) and cytotoxicity towards L6 cells of compounds **11**.

Compound	R^1	R^2	R^3	Yield	L6 cells IC_{50} nM	NF54 IC_{50} nM	SI[a] L6/NF54	Solubility in H_2O
11a	H		CO_2H	82%	113,000	1690	66.8	Freely
11b	H		CO_2H	79%	20,000	2550	7.84	Freely
11c	H		CO_2H	85%	66,200	2160	30.6	Freely

[a] Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.).



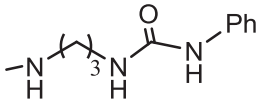
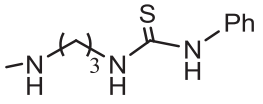
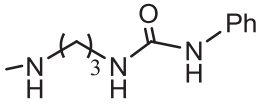
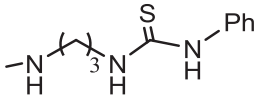
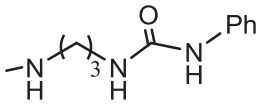
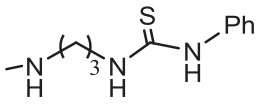
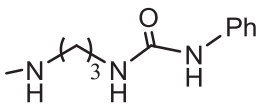
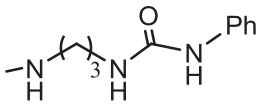
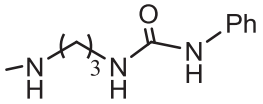
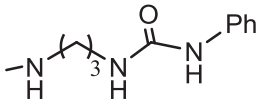
7–9 12

Scheme 3. Further modifications of the lateral amino group. Reagents and conditions: PhNCX, THF, 0–25 °C, 30 min.

donors would logically be better hydrated, and indeed there is a significant correlation between these two variables. However, should the molecule be too polar, it will not be able to pass through the lipophilic membrane. Thus a balance between hydration and polarity must be established for good activity. This correlation therefore appears to be physically feasible and could be used as a strategy for synthesizing derivatives with higher potency. For example, compounds with the best activity (**9c**–**12b**) all contain three HBDs, resulting in large H_2O·hyd and not more than one polar ester group. Furthermore the most active compound (**12b**) contains a thiourea which contributes significantly less to the PSA than the urea group without detrimentally affecting the number of waters of hydration and with no decrease in HBDs. In contrast, the least active compounds (**8e**–**g**) have a moderate PSA, less than one HBD and notably lower H_2O·hyd.

The series includes nine compounds containing the basic amino group, which is considered vital for pH trapping in the parasite acidic digestive vacuole. Previous work on 4-aminoquinolines suggests that these nine compounds should

Table 3
Antiplasmodial activity against *P. falciparum* (CQS: NF54) and cytotoxicity towards L6 cells of compounds **12**.

Compound	R^1	R^2	R^3	Yield	L6 cells	NF54	SI[a]	β-Haematin inhibition
					IC_{50} nM	IC_{50} nM	L6/NF54	IC_{50} μM
12a	CO_2Me		H	93%	754	4.16	181	23.0
12b	CO_2Me		H	84%	581	1.81	321	15.7
12c	H		CO_2Me	92%	2580	14.5	178	12.8
12d	H		CO_2Me	96%	2820	6.03	467	10.8
12e	CO_2Me		CO_2Me	90%	26,500	16.7	1590	4.6
12f	CO_2Me		CO_2Me	84%	627	14.4	43.5	7.5
12g	Cl		CO_2Me	89%	3340	13.6	246	14.9
12h	Br		CO_2Me	90%	6740	8.05	837	16.0
12i	CO_2Me		Br	83%	1120	7.15	157	10.1
12j	Cl		Br	89%	408	5.59	73	17.8
Podophylotoxin					14.5			
Chloroquine						9.40		18.4

[a] Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.).

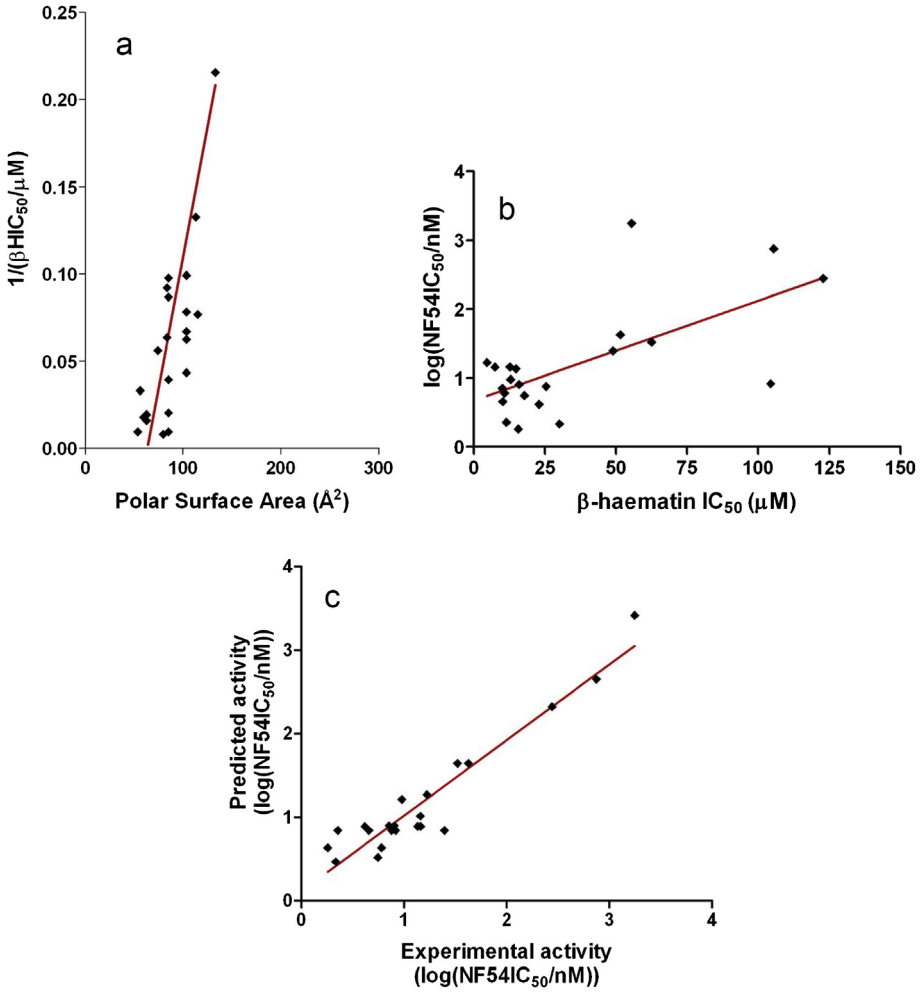


Fig. 2. Quantitative structure–activity relationships were found for the neocryptolepine series. (**a**) An increase in polar surface area (PSA) was found to improve β-haematin inhibition activity according to $1/\beta HIC_{50} = 0.001788(PSA) - 0.09485$, $r^2 = 0.58$, $P < 0.0001$. (**b**) A weak correlation ($r^2 = 0.43$ and $P = 0.001$) was observed for the log of NF54 activity with the β-haematin activity, showing that the biological activity of a compound depends on more factors than its ability to inhibit haemozoin, if this is indeed the target. (**c**) Predicted vs experimental NF54 activity based on multiple linear regression where $\log(NF54IC_{50}) = -0.299(H_2O.hyd) + 0.028(PSA) - 0.652(\ln(HBD)) + 3.108$ ($r^2 = 0.904$, $P < 0.0001$); statistically significant for both individual parameters ($t = 5.40, 5.78, 6.85, 4.34 > t_{crit} = 2.88$) and overall correlation ($F = 56.811 > F_{crit} = 5.09$) at 99% confidence level.

Table 4
Antiplasmodial activity against *P. falciparum* (CQS: NF54, CQR: K1) and cytotoxicity towards L6 cells of neocryptolepine derivatives.

NO	L6 cells IC_{50} nM[c]	NF54 IC_{50} nM[c]	SI[a] L6/NF54	K1 IC_{50} nM[c]	SI[a] L6/K1	RI[b] K1/NF54
Neocryptolepine	3194	1580	2.02	1696	1.88	1.07
7	337	8.28	40.7	22.1	15.2	2.67
8a	218	24.8	8.79	30.3	7.19	1.22
9a	1390	9.52	146	11.9	117	1.25
9b	427	7.57	56.4	20.2	21.1	2.67
9d	1050	4.54	232	15.9	66.0	3.5
9e	401	2.16	186	19.2	20.9	8.89
12a	754	4.16	181	56.1	13.4	13.5
12b	581	1.81	321	16.1	36.1	8.89
12c	2580	14.5	178	68.4	37.7	4.71
12d	2820	6.03	467	64.4	43.8	10.7
12e	26500	16.7	1590	40.8	650	2.44
12f	627	14.4	43.5	50.4	12.4	3.50
12g	3340	13.6	246	23.3	143	1.71
12i	1120	7.15	157	19.7	56.9	2.75
12j	408	5.59	73	16.8	24.3	3
Podophylotoxin	14.5					
Chloroquine		9.40		210		22.3

[a] Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.).
[b] Resistance Index is the ratio of IC_{50} for the resistant versus the sensitive strain (K1/NF54).
[c] The IC_{50} values are the means of two independent assays; the individual values vary less than a factor 2.

accumulate at the target site much more readily than those with no basic amino side chains and consequently display better activities [29,39]. Upon inspection, it is evident that the basic groups do not influence the NF54 activity and as a result, it appears unlikely that the target lies within the digestive vacuole. While it appears that haemozoin may not be the target of these compounds, the result is not completely definitive, because of the observation of a weak correlation as shown in Fig. 2b. Combrinck et al. [38] have shown the ability of antimalarials to increase free haem in cultured *P. falciparum* by using cell fractionation. Such a study could provide further evidence for the mechanism of action of this series.

We then selected some compounds with different substituents at the terminal amino group and the C2, C9 positions for further measurement of another strain – CQR (K1). As shown in Table 4, the RI provides a quantitative measurement of the antiplasmodial activity against the CQR strains relative to that against the CQS strains and reveals promising drug discovery leads [40]. Most of the tested compounds showed promising antimalarial activities against both strains and a low resistance index (RI). Gratifyingly, we found that all the compounds were significantly more active against the CQR (K1) than CQ, having IC_{50} values that ranged from 11.9 nM to 68.4 nM for K1 (CQ, IC_{50} = 210 nM) and RI values ranging from 1.22 to 13.5 (CQ, RI = 22.3).

3.3. In vivo antimalarial activity

The monoester substituted compounds **7** and **8a** with high antimalarial activities against the NF54 strain were subjected to an *in vivo* drug-screening model against *Plasmodium berghei* female mice in Switzerland. The *in vivo* study was carried out according to the standard protocol following the "4 Day Test" [41] by FACS analysis. Activity was calculated as the difference between the mean percent parasitaemia for the control (n = 5 mice) and treated groups expressed as a percent relative to the control group. After daily intraperitoneal dosing 20 mg/kg for four consecutive days, the neocryptolepine derivatives **7** and **8a** showed some reduction in parasitaemia on day 4 and day 7, with activities of 33.4% and 72.5%, which were improved remarkably after the introduction of the ester group with respect to the maximum activity of 22.1% at the dose of 50 mg/kg for the C2/C9 non-substituted compound 1-(3-(5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-ylamino)propyl)-3-phenylurea [20] in our previous study.

4. Conclusion

We have described the synthesis, *in vitro* and *in vivo* antimalaria evaluations of the C2 and/or C9 position ester-modified neocryptolepine derivatives against NF54 and K1 strains as well as the β-haematin inhibition. The cytotoxicity of these series of compounds against normal cell lines was also evaluated. Structure activity relationship studies were discussed by changing the kind of substituents at C2 and/or C9 and by introducing and modifying of side chains at C11 of the neocryptolepine core. Correlation between antiplasmodial activity and β-haematin activity was weak. Instead, factors likely to be involved in uptake of these compounds into the parasite appear to be most predictive of biological activity. The introduction of an ester group indeed significantly contributed to their high activities against *P. falciparum* and low cytotoxicity against the normal cell line, especially, to improving their *in vivo* activity greatly. The thiourea group at the terminal amino group at C11 also showed a contribution to the antiplasmodial activity.

5. Experimental

5.1. Chemistry: general and methods

The commercially obtained reagents were directly used without further purification. The ^{1}H NMR and ^{13}C NMR spectra were measured on the Varian INOVA-600 or Varian INOVA-400 spectrometer. High resolution mass spectra were obtained on a Bruker micrOTOF II-SKA spectrometer.

5.1.1. General procedure for the preparation of dimethyl 1H-indole-3,5-dicarboxylate (**2b**)

Pyridine (3.54 mmol) was added to a suspension of methyl 1*H*-indole-5-carboxylate (**1b**, 2.72 mmol) in anhydrous THF (6 mL) at 0 °C. A solution of trichloroacetyl chloride (3.54 mmol) in THF (6 mL) was added dropwise via an addition funnel over 1 h. The reaction mixture was then allowed to warm to room temperature to stir over night. The reaction mixture was quenched with 1 M HCl, dried over Na_2SO_4, and concentrated under vacuum. The resulting solids were then dissolved in MeOH (54 mL), and KOH(s) was added. The reaction mixture was heated to reflux for 5 h, stirred at ambient temperature for 1 h, followed by concentration under vacuum. The solids were purified by chromatography (SiO_2, 25% EtOAc/Hexanes). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.28 (s, 1H), 8.69 (d, J = 1.5 Hz, 1H), 8.23 (s, 1H), 7.83 (dd, J = 8.6, 1.7 Hz, 1H), 7.57 (dd, J = 8.6, 0.5 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.34 (s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.0, 164.4, 139.0, 134.3, 125.2, 123.3, 122.7, 112.5, 107.4, 51.9, 50.9.

5.1.2. Methyl 5-bromo-1H-indole-3-carboxylate (**2c**)

White solids; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.68 (s, 1H), 8.33 (d, J = 1.9 Hz, 1H), 7.92 (d, J = 3.0 Hz, 1H), 7.37 (dd, J = 8.6, 1.9 Hz, 1H), 7.29 (dd, J = 8.6, 0.4 Hz, 1H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.1, 134.6, 131.8, 127.4, 126.3, 124.2, 115.7, 112.9, 108.6, 51.3.

5.1.3. General procedure for the preparation of methyl 2-((4-(methoxycarbonyl)phenyl)(methyl)amino)-1H-indole-3-carboxylate (**4a**)

To the argon flushed solution of methyl indole-3-carboxylate (**2a**, 2.5 mmol) in CH_2Cl_2 (5 mL), a mixture of *N*-chlorosuccinimide (2.75 mmol) and *N*,*N*′-dimethylpiperazine (1.25 mmol) in CH_2Cl_2 (1 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 2 h, and a solution of trichloroacetic acid (0.63 mmol) and methyl 4-(methylamino)benzoate (**3a**, 5 mmol) in CH_2Cl_2 (2 mL) was then added. The reaction was allowed to warm to room temperature and further stirred for 3.5 h. The mixture was consecutively washed with saturated aqueous $NaHCO_3$, 1 N HCl, brine, and water. The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, Hexanes/EtOAc = 7/1) to give the white solids, mp: 217–219 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.25 (br s, 1H), 8.00 (d, J = 7.92 Hz, 1H), 7.81 (d, J = 8.80 Hz, 2H), 7.39 (d, J = 7.63 Hz, 1H), 7.29–7.15 (m, 2H), 6.73 (d, J = 8.80 Hz, 2H), 3.78 (s, 3H), 3.66 (s, 3H), 3.37 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 166.1, 163.5, 151.7, 145.3, 132.6, 130.7, 125.8, 122.7, 121.5, 121.0, 119.0, 112.9, 111.8, 98.4, 51.6, 50.6, 39.1.

5.1.4. Dimethyl 2-(methyl(phenyl)amino)-1H-indole-3,5-dicarboxylate (**4b**)

White solids, mp: 168–169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.09 (s, 1H), 8.80–8.75 (m, 1H), 7.85 (dd, J = 8.5, 1.7 Hz, 1H), 7.25–7.19 (m, 3H), 6.96–6.85 (m, 3H), 3.89 (s, 3H), 3.79 (s, 3H), 3.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.2, 164.3, 149.3, 146.5, 134.9, 129.3, 126.3, 124.0, 123.5(2C), 121.3, 117.1, 110.3, 97.6, 51.9, 51.0, 40.2.

ESI-HRMS: *m/z* calcd. for $C_{19}H_{17}N_2O_4$ $[M - H]^-$ 337.1188. Found 337.1166.

*5.1.5. Dimethyl 2-((4-(methoxycarbonyl)phenyl)(methyl)amino)-1H-indole-3,5-dicarboxylate (**4c**)*

White solids, mp: 234–236 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.58 (s, 1H), 8.84 (d, *J* = 1.3 Hz, 1H), 7.96 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.80–7.73 (m, 2H), 7.38 (d, *J* = 8.5 Hz, 1H), 6.70–6.63 (m, 2H), 3.93 (s, 3H), 3.82 (d, *J* = 3.5 Hz, 6H), 3.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.0, 167.3, 163.9, 150.9, 146.7, 135.0, 131.1, 125.7, 124.7, 124.4, 124.0, 120.6, 113.5, 110.9, 100.8, 52.0, 51.8, 51.2, 39.7. ESI-HRMS: *m/z* calcd. for $C_{21}H_{20}N_2O_6$ $[M + Na]^+$ 419.1219. Found 419.1217.

*5.1.6. Dimethyl 2-((4-chlorophenyl)(methyl)amino)-1H-indole-3,5-dicarboxylate (**4d**)*

White solids, mp: 188–189 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.79 (s, 1H), 8.70 (s, 1H), 7.92 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.21–7.16 (m, 2H), 6.82–6.77 (m, 2H), 3.93 (s, 3H), 3.84 (s, 3H), 3.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.01, 164.1, 148.4, 145.4, 134.8, 129.1, 126.1, 125.9, 124.3, 123.8(2C), 117.5, 110.4, 98.8, 52.1, 51.1, 40.1. ESI-HRMS: *m/z* calcd. for $C_{19}H_{18}ClN_2O_4$ $[M + H]^+$ 373.0960. Found 373.0965.

*5.1.7. Dimethyl 2-((4-bromophenyl)(methyl)amino)-1H-indole-3,5-dicarboxylate (**4e**)*

White solids, mp: 193–194 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.85 (s, 1H), 8.79 (s, 1H), 7.91 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.34–7.26 (m, 3H), 6.75–6.67 (m, 2H), 3.92 (s, 3H), 3.83 (s, 3H), 3.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.0, 164.0, 148.1, 145.9, 134.7, 132.1, 126.1, 124.4, 123.9(2C), 117.7, 113.2, 110.4, 99.1, 52.0, 51.1, 40.0. ESI-HRMS: *m/z* calcd. for $C_{19}H_{18}BrN_2O_4$ $[M + H]^+$ 417.0450. Found 417.0452.

*5.1.8. Methyl 5-bromo-2-((4-(methoxycarbonyl)phenyl)(methyl)amino)-1H-indole-3-carboxylate (**4f**)*

White solids, mp: 217–218 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.08 (s, 1H), 8.29 (d, *J* = 1.9 Hz, 1H), 7.81–7.76 (m, 2H), 7.37 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.22 (d, *J* = 8.6 Hz, 1H), 6.69–6.64 (m, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.42 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.3, 163.8, 151.0, 146.1, 131.1, 130.7, 127.8, 126.4, 124.4, 120.6, 115.7, 113.5, 112.5, 99.7, 51.9, 51.2, 39.7. ESI-HRMS: *m/z* calcd. for $C_{19}H_{18}BrN_2O_4$ $[M + H]^+$ 417.0450. Found 417.0457.

*5.1.9. Methyl 5-bromo-2-((4-chlorophenyl)(methyl)amino)-1H-indole-3-carboxylate (**4g**)*

White solids, mp: 193–194 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.36 (s, 1H), 8.24 (d, *J* = 1.9 Hz, 1H), 7.31 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.23–7.16 (m, 2H), 7.11 (d, *J* = 8.5 Hz, 1H), 6.79–6.75 (m, 2H), 3.82 (s, 3H), 3.41 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 164.0, 147.9, 145.5, 130.4, 129.2, 128.2, 125.9(2C), 124.1, 117.5, 115.5, 112.0, 97.9, 51.1, 40.1. ESI-HRMS: *m/z* calcd. for $C_{17}H_{15}BrClN_2O_2$ $[M + H]^+$ 393.0005. Found 393.0006.

*5.1.10. General procedure for the preparation of methyl 5-methyl-11-oxo-6,11-dihydro-5H-indolo[2,3-b]quinoline-2-carboxylate (**5a**)*

Methyl 2-((4-(*methoxycarbonyl*)*phenyl*)(*methyl*)*amino*)-1*H*-*indole*-3-*carboxylate* (**4a**, 1.5 mmol) was heated at reflux in diphenyl ether (8 mL) at 250 °C for 3 h. The solids were filtered and washed with diethyl ether to give pale grey solids, mp: >300 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.21 (s, 1H), 8.96 (d, *J* = 2.05 Hz, 1H), 8.28–8.12 (m, 2H), 7.84 (d, *J* = 8.80 Hz, 1H), 7.48 (d, *J* = 7.63 Hz, 1H), 7.36–7.17 (m, 2H), 3.98 (s, 3H), 3.91 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 171.0, 165.9, 146.9, 142.0, 134.7, 130.9, 127.8, 124.2, 123.7, 123.2, 122.5, 121.5, 120.3, 115.8, 111.0, 102.9, 52.1, 33.7.

*5.1.11. Methyl 5-methyl-11-oxo-6,11-dihydro-5H-indolo[2,3-b]quinoline-9-carboxylate (**5b**)*

Pale grey solids, mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.45 (s, 1H), 8.86–8.81 (m, 1H), 8.40 (d, *J* = 7.8 Hz, 1H), 7.94–7.86 (m, 1H), 7.78 (q, *J* = 9.1 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.42 (t, *J* = 7.1 Hz, 1H), 3.98 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.6, 166.9, 147.8, 139.2, 137.9, 131.5, 125.8, 124.7, 124.2, 123.8, 122.5, 122.0, 121.6, 115.5, 110.7, 102.1, 51.9, 33.4. ESI-HRMS: *m/z* calcd. for $C_{18}H_{13}N_2O_3$ $[M - H]^-$ 305.0932. Found 305.0867.

*5.1.12. Dimethyl 5-methyl-11-oxo-6,11-dihydro-5H-indolo[2,3-b]quinoline-2,9-dicarboxylate (**5c**)*

Pale grey solids, mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.53 (s, 0H), 8.91 (d, *J* = 2.1 Hz, 1H), 8.78 (d, *J* = 1.3 Hz, 1H), 8.17 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.89 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 3.95 (s, 3H), 3.91 (d, *J* = 4.7 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 171.1, 166.9, 165.9, 147.8, 142.0, 137.8, 131.2, 127.7, 124.6, 124.1, 123.4, 122.8(2C), 121.8, 116.1, 111.0, 102.7, 52.2, 52.0, 33.8. ESI-HRMS: *m/z* calcd. for $C_{20}H_{17}N_2O_5$ $[M + H]^+$ 365.1137. Found 365.1140.

*5.1.13. Methyl 2-chloro-5-methyl-11-oxo-6,11-dihydro-5H-indolo[2,3-b]quinoline-9-carboxylate (**5d**)*

Pale grey solids, mp: >300 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.54 (s, 1H), 8.81 (s, 1H), 8.29 (s, 1H), 7.92 (d, *J* = 8.3 Hz, 1H), 7.86 (d, *J* = 8.5 Hz, 1H), 7.80 (d, *J* = 9.0 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 1H), 3.98 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 170.3, 166.8, 147.9, 138.0(2C), 131.2, 126.9, 125.9, 124.6(2C), 123.6, 122.7, 121.8, 118.1, 111.0, 102.5, 51.9, 33.8. ESI-HRMS: *m/z* calcd. for $C_{18}H_{14}ClN_2O_3$ $[M + H]^+$ 341.0693. Found 341.0679.

*5.1.14. Methyl 2-bromo-5-methyl-11-oxo-6,11-dihydro-5H-indolo[2,3-b]quinoline-9-carboxylate (**5e**)*

Pale grey solids, mp: >300 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.57 (s, 1H), 8.82 (d, *J* = 1.3 Hz, 1H), 8.44 (d, *J* = 2.4 Hz, 1H), 7.96–7.87 (m, 2H), 7.81 (d, *J* = 9.1 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 3.99 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 170.2, 166.9, 147.9, 138.3, 138.1, 133.9, 127.8, 126.3, 124.6, 123.6, 122.7, 121.8, 118.3, 114.8, 111.0, 102.5, 51.9, 33.7. ESI-HRMS: *m/z* calcd. for $C_{18}H_{13}BrN_2O_3$ $[M + Na]^+$ 407.0007. Found 407.0009.

*5.1.15. Methyl 9-bromo-5-methyl-11-oxo-6,11-dihydro-5H-indolo[2,3-b]quinoline-2-carboxylate (**5f**)*

Pale grey solids, mp: >300 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.34 (s, 1H), 8.88 (d, *J* = 2.1 Hz, 1H), 8.22 (s, 1H), 8.15 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.79 (d, *J* = 9.0 Hz, 1H), 7.38 (d, *J* = 1.7 Hz, 2H), 3.92 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 171.1, 165.8, 147.2, 142.0, 133.6, 131.2, 127.7, 125.5(2C), 123.9, 122.7, 122.2, 115.9, 113.7, 113.0, 102.0, 52.2, 33.7. ESI-HRMS: *m/z* calcd. for $C_{18}H_{13}BrN_2O_3$ $[M + Na]^+$ 407.0007. Found 407.0007.

*5.1.16. 9-Bromo-2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11(6H)-one (**5g**)*

Pale grey solids, mp: >300 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.27 (s, 1H), 8.22 (dd, *J* = 5.2, 1.8 Hz, 2H), 7.77 (d, *J* = 9.1 Hz, 1H), 7.73 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.44–7.30 (m, 2H), 3.91 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 170.2, 147.3, 137.8, 133.7, 131.0, 126.7, 125.6(2C), 125.4, 124.6, 122.1, 117.8, 113.5, 112.9, 101.7, 33.6. ESI-HRMS: *m/z* calcd. for $C_{16}H_{10}BrClN_2O$ $[M + Na]^+$ 382.9563. Found 382.9565.

*5.1.17. General procedure for the preparation of methyl 11-chloro-5-methyl-5H-indolo[2,3-b]quinoline-2-carboxylate (**6a**)*

A suspension of methyl 5-methyl-11-oxo-6,11-dihydro-5*H*-indolo[2,3-*b*]quinoline-2-carboxylate (**5a**, 1 mmol) in phosphoryl

chloride was heated at reflux for 3 h and then poured into an ice-bath with stirring. Removal of the liquid was by filtration, and the solids were sequentially washed with aqueous saturated $NaHCO_3$, and water. The orange product was dried under vacuum over P_2O_5, mp: 281–283 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.95 (s, 1H), 8.29 (d, J = 8.22 Hz, 2H), 7.74–7.57 (m, 2H), 7.52 (t, J = 7.48 Hz, 1H), 7.22 (t, J = 7.48 Hz, 1H), 4.24 (s, 3H), 4.00 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.0, 155.1, 154.5, 139.2, 135.7, 131.3, 130.1, 128.2, 125.4, 123.9, 123.8, 123.5, 120.9, 118.5, 117.8, 114.3, 52.4, 33.4.

5.1.18. Methyl 11-chloro-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**6b**)

Orange solids, mp: 248–249 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.01 (s, 1H), 8.45 (d, J = 8.2 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.88 (t, J = 7.7 Hz, 1H), 7.81 (d, J = 8.6 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 4.43 (d, J = 7.3 Hz, 3H), 3.98 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.4, 157.6, 156.1, 137.1, 136.5, 131.5, 130.9, 126.0, 125.4, 123.4, 122.9(2C), 121.5, 119.2, 116.7, 114.4, 51.9, 33.4. ESI-HRMS: m/z calcd. for $C_{18}H_{14}ClN_2O_2$ $[M + H]^+$ 325.0744. Found 325.0797.

5.1.19. Dimethyl 11-chloro-5-methyl-5H-indolo[2,3-b]quinoline-2,9-dicarboxylate (**6c**)

Orange solids, mp: 278–280 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.14 (d, J = 1.8 Hz, 1H), 9.03 (d, J = 1.5 Hz, 1H), 8.51 (dd, J = 8.9, 1.8 Hz, 1H), 8.24 (dd, J = 8.4, 1.6 Hz, 1H), 7.91 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 4.55 (s, 3H), 4.06 (s, 3H), 3.99 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.2, 165.7, 156.4, 155.7, 139.1, 138.0, 132.2, 131.7, 128.6, 125.8, 125.1, 124.4, 123.0, 122.8, 119.2, 117.1, 115.0, 52.7, 52.1, 34.4. ESI-HRMS: m/z calcd. for $C_{20}H_{16}ClN_2O_4$ $[M + H]^+$ 383.0799. Found 383.0799.

5.1.20. Methyl 2,11-dichloro-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**6d**)

Orange solids, mp: 283–284 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.90 (d, J = 1.2 Hz, 1H), 8.33 (d, J = 2.3 Hz, 1H), 8.17 (dd, J = 8.4, 1.7 Hz, 1H), 7.73 (dd, J = 9.1, 2.3 Hz, 1H), 7.63 (dd, J = 21.2, 8.7 Hz, 2H), 4.33 (s, 3H), 3.97 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.3, 157.6, 155.9, 135.9, 135.1, 131.8, 131.6, 129.2, 125.7, 125.3, 124.7, 122.8, 122.3, 120.4, 117.0, 116.2, 52.1, 33.9. ESI-HRMS: m/z calcd. for $C_{18}H_{13}C_{l2}N_2O_2$ $[M + H]^+$ 359.0354. Found 359.0357.

5.1.21. Methyl 2-bromo-11-chloro-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**6e**)

Orange solids, mp: 289–290 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.06 (d, J = 1.5 Hz, 1H), 8.60 (d, J = 2.2 Hz, 1H), 8.27 (dd, J = 8.4, 1.7 Hz, 1H), 7.96 (dd, J = 9.1, 2.2 Hz, 1H), 7.74 (dd, J = 20.0, 8.7 Hz, 2H), 4.46 (s, 3H), 3.99 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.5, 158.5, 156.5, 135.7, 134.4, 131.7, 130.9, 128.8, 128.5, 125.9, 125.2, 123.3, 122.2, 120.8, 117.3, 116.3, 52.1, 33.6. ESI-HRMS: m/z calcd. for $C_{18}H_{13}BrClN_2O_2$ $[M + H]^+$ 402.9849. Found 402.9848.

5.1.22. Methyl 9-bromo-11-chloro-5-methyl-5H-indolo[2,3-b]quinoline-2-carboxylate (**6f**)

Orange solid, mp: >300 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.14 (d, J = 1.8 Hz, 1H), 8.53 (d, J = 1.9 Hz, 1H), 8.48 (dd, J = 8.9, 1.9 Hz, 1H), 7.84 (d, J = 9.0 Hz, 1H), 7.70–7.60 (m, 2H), 4.43 (s, 3H), 4.04 (d, J = 8.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.0, 155.4, 153.4, 139.6, 137.9, 137.2, 132.8, 132.0, 128.7, 126.5, 124.7, 124.3, 119.4, 118.7, 114.7, 113.6, 52.6, 33.6. ESI-HRMS: m/z calcd. for $C_{18}H_{13}BrClN_2O_2$ $[M + H]^+$ 402.9849. Found 402.9850.

5.1.23. 9-Bromo-2,11-dichloro-5-methyl-5H-indolo[2,3-b]quinoline (**6g**)

Orange solids, mp: 288–290 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.45 (d, J = 1.9 Hz, 1H), 8.37 (d, J = 2.3 Hz, 1H), 7.76 (dd, J = 9.1, 2.4 Hz, 1H), 7.66 (d, J = 9.1 Hz, 1H), 7.62 (dd, J = 8.4, 2.0 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 4.30 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 155.0, 153.6, 135.6, 135.5, 132.8, 131.8, 128.6, 126.5, 125.4, 125.0, 124.8, 120.08, 119.1, 115.9, 113.1, 33.5. ESI-HRMS: m/z calcd. for $C_{16}H_{10}BrCl_2N_2$ $[M + H]^+$ 378.9404. Found 378.9406.

5.2. General procedure for the preparation of 2,9-disubstituted 5-methyl-5H-indolo[2,3-b]quinolin-11-amine (**7**–**9**)

11-Chloro-5-methyl-5*H*-indolo[2,3-*b*]quinoline derivatives (**6a**–**g**, 1 mmol) was heated at reflux with a large excess of an appropriate amine in THF for 3–4 h. The reaction was monitored by TLC. Then the mixture was washed with water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by column chromatography using eluent changed from AcOEt to AcOEt-2 N ammonia in MeOH (10:1) to give the final product.

5.2.1. Methyl 11-(3-aminopropylamino)-5-methyl-5H-indolo[2,3-b]quinoline-2-carboxylate (**7**)

Yellow solids, mp: 149–150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.12 (d, J = 1.8 Hz, 1H), 8.25 (dd, J = 9.0, 1.8 Hz, 1H), 7.99 (d, J = 7.7 Hz, 1H), 7.90 (d, J = 9.1 Hz, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.09 (t, J = 7.0 Hz, 1H), 4.17 (s, 3H), 3.99 (t, J = 6.5 Hz, 2H), 3.93 (s, 3H), 2.67 (t, J = 6.3 Hz, 2H), 1.78–1.74 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 165.9, 156.2, 151.9, 148.1, 140.2, 130.2, 126.5, 124.5, 124.3, 122.1, 121.3, 118.5, 116.9, 115.3, 115.1, 103.7, 52.2, 47.4, 39.9, 33.1, 32.5. ESI-HRMS: m/z calcd. for $C_{21}H_{21}N_4O_2$ $[M - H]^-$ 361.1670. Found 375.1666.

5.2.2. Methyl 11-(3-aminopropylamino)-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**8a**)

Yellow solids, mp: 170–171 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.63 (d, J = 1.4 Hz, 1H), 8.52 (d, J = 7.7 Hz, 1H), 7.93–7.87 (m, 2H), 7.83 (t, J = 7.7 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.46 (t, J = 7.4 Hz, 1H), 4.19 (s, 3H), 4.00 (t, J = 6.6 Hz, 2H), 3.86 (s, 3H), 2.71 (t, J = 6.3 Hz, 2H), 1.84–1.78 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.4, 158.3, 155.7, 149.1, 137.5, 131.1, 125.6, 124.1(2C), 123.6, 121.2, 118.3, 116.0, 115.5(2C), 102.6, 51.6, 47.2, 33.1, 32.5. ESI-HRMS: m/z calcd. for $C_{21}H_{21}N_4O_2$ $[M - H]^-$ 361.1670. Found 361.1639.

5.2.3. Methyl 11-(4-aminobutylamino)-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**8b**)

Yellow oil; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.62 (t, J = 6.1 Hz, 1H), 8.53 (s, 1H), 7.92 (dd, J = 8.3, 7.0, 2H), 7.85 (t, J = 7.7 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 4.20 (s, 3H), 3.90 (t, J = 7.1 Hz, 2H), 3.87 (s, 3H), 2.67 (t, J = 7.4 Hz, 2H), 1.85–1.76 (m, 2H), 1.48 (d, J = 6.4 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.4, 158.4, 155.9, 149.0, 137.4, 131.2, 125.8, 124.0(2C), 123.7, 121.4, 118.4, 116.1, 115.7, 115.5, 103.0, 51.7, 47.3, 38.6, 32.5, 27.6, 24.9. ESI-HRMS: m/z calcd. for $C_{22}H_{25}N_4O_2$ $[M + H]^+$ 377.1978. Found 377.1978.

5.2.4. Methyl 11-(2-(dimethylamino)ethylamino)-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**8c**)

Yellow solids, mp: 172–173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.70 (s, 1H), 8.54 (d, J = 8.3 Hz, 1H), 7.97–7.91 (m, 2H), 7.86 (t, J = 7.8 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 7.26 (t, J = 4.5 Hz, 1H), 4.21 (s, 3H), 4.00 (dd, J = 10.8, 5.5 Hz, 2H), 3.87 (s, 3H), 2.65 (t, J = 6.1 Hz, 2H), 2.27 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 168.2, 157.4, 155.6, 149.3, 138.3, 130.5, 127.0, 125.3, 123.8, 122.5, 120.6, 119.7, 116.1, 115.6, 114.8, 106.0, 58.2, 51.5, 45.2, 44.7, 32.6. ESI-HRMS: m/z calcd. for $C_{22}H_{23}N_4O_2$ $[M - H]^-$ 375.1826. Found 375.1806.

*5.2.5. Methyl 11-(3-(dimethylamino)propylamino)-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**8d**)*

Yellow solids, mp: 105–106 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.65 (d, J = 1.4 Hz, 2H), 8.07 (dd, J = 8.4, 1.5 Hz, 1H), 8.00 (d, J = 8.3 Hz, 1H), 7.70 (dd, J = 11.9, 4.4 Hz, 2H), 7.65 (d, J = 8.6 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 4.23 (d, J = 1.2 Hz, 3H), 4.20–4.10 (m, 2H), 3.94 (s, 3H), 2.72–2.64 (m, 2H), 2.39 (s, 6H), 1.95–1.73 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 168.4, 158.3, 155.5, 149.0, 137.5, 130.3, 126.2, 124.1, 123.6, 123.4, 120.7, 119.1, 115.9, 115.8, 114.5, 103.0, 59.5, 51.5, 50.1, 45.4, 32.5, 26.3. ESI-HRMS: m/z calcd. for $C_{23}H_{25}N_4O_2[M-H]^-$ 389.1983. Found 389.1949.

*5.2.6. Methyl 11-(3-hydroxypropylamino)-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**8e**)*

Orange solids, mp: 223–224 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.60–8.50 (m, 2H), 7.94–7.88 (m, 2H), 7.86–7.81 (m, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.50–7.43 (m, 2H), 4.57 (t, J = 4.7 Hz, 1H), 4.19 (s, 3H), 3.97 (q, J = 6.5 Hz, 2H), 3.87 (s, 3H), 3.47 (dd, J = 10.8, 5.9 Hz, 2H), 1.90 (p, J = 6.4 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.4, 158.3, 155.8, 149.2, 137.4, 131.2, 125.7, 124.0(2C), 123.6, 121.3, 118.4, 116.0, 115.6(d), 103.1, 58.4, 51.6, 45.8, 40.0(overlap with DMSO-d_6 peaks), 33.6, 32.5. ESI-HRMS: m/z calcd. for $C_{21}H_{20}N_3O_3[M-H]^-$ 362.1492. Found 362.1483.

*5.2.7. Methyl 5-methyl-11-(4-methyl-1,4-diazepan-1-yl)-5H-indolo[2,3-b]quinoline-9-carboxylate (**8f**)*

Orange solids, mp: 179–180 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.93 (s, 1H), 8.47 (d, J = 8.2 Hz, 1H), 8.26–8.18 (m, 1H), 7.80 (dd, J = 3.7, 1.7 Hz, 2H), 7.71 (d, J = 8.4 Hz, 1H), 7.50 (ddd, J = 8.1, 4.2, 1.7 Hz, 1H), 4.37 (d, J = 0.8 Hz, 3H), 3.97 (d, J = 0.9 Hz, 3H), 3.93–3.87 (m, 2H), 3.78 (t, J = 6.1 Hz, 2H), 3.14 (d, J = 4.5 Hz, 2H), 3.05–2.90 (m, 2H), 2.63 (s, 3H), 2.39–2.23 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.1, 159.5, 157.8, 152.3, 138.5, 130.7, 129.7, 126.8, 125.7, 122.8, 122.0, 121.5, 121.0, 120.8, 116.8, 115.0, 61.3, 57.9, 52.9, 52.2, 51.9, 47.2, 33.3, 29.2. ESI-HRMS: m/z calcd. for $C_{24}H_{27}N_4O_2$ $[M+H]^+$ 403.2134. Found 403.2135.

*5.2.8. Methyl 5-methyl-11-morpholino-5H-indolo[2,3-b]quinoline-9-carboxylate (**8g**)*

Orange solids, mp: 213–215 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.01 (d, J = 1.6 Hz, 1H), 8.50 (d, J = 8.2 Hz, 1H), 8.20 (dd, J = 8.4, 1.7 Hz, 1H), 7.83 (d, J = 4.1 Hz, 2H), 7.74 (d, J = 8.4 Hz, 1H), 7.57–7.47 (m, 1H), 4.41 (s, 3H), 4.18–4.09 (m, 4H), 3.98 (s, 3H), 3.75–3.66 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.8, 158.4, 156.7, 151.3, 138.1, 131.0, 129.7, 126.5, 126.0, 122.4, 122.0, 121.0(2C), 120.7, 116.5, 115.1, 67.6, 51.9, 51.0, 33.7. ESI-HRMS: m/z calcd. for $C_{22}H_{22}N_3O_3$ $[M+H]^+$ 376.1661. Found 376.1662.

*5.2.9. Dimethyl 11-(3-aminopropylamino)-5-methyl-5H-indolo[2,3-b]quinoline-2,9-dicarboxylate (**9a**)*

Yellow solids, mp: 75–76 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.17 (s, 1H), 8.63 (s, 1H), 8.28 (d, J = 8.1 Hz, 1H), 7.97–7.89 (m, 2H), 7.55 (d, J = 8.4 Hz, 1H), 4.20 (s, 3H), 4.02 (t, J = 6.6 Hz, 2H), 3.94 (s, 3H), 3.87 (s, 3H), 2.75 (t, J = 6.3 Hz, 2H), 1.87–1.82 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.4, 165.9, 158.2, 155.4, 148.9, 140.2, 130.7, 126.6, 125.7, 124.1, 123.7, 122.0, 119.0, 116.4, 115.8, 115.2, 102.5, 52.3, 51.6(2C), 47.1, 32.8, 32.5. ESI-HRMS: m/z calcd. for $C_{23}H_{25}N_4O_4$ $[M+H]^+$ 421.1876. Found 421.1877.

*5.2.10. Methyl 11-(3-aminopropylamino)-2-chloro-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**9b**)*

Yellow solids, mp: 185–186 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.59 (d, J = 2.0 Hz, 1H), 8.54 (s, 1H), 7.89 (dd, J = 8.4, 1.4 Hz, 1H), 7.84 (d, J = 9.2 Hz, 1H), 7.79 (dd, J = 9.1, 2.1 Hz, 1H), 7.50 (d, J = 8.3 Hz, 1H), 4.12 (s, 3H), 3.93 (t, J = 6.7 Hz, 2H), 3.86 (s, 3H), 2.67 (t, J = 6.4 Hz, 2H), 1.86–1.77 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.4, 158.1, 155.8, 147.9, 136.1, 130.7, 125.8(2C), 123.9, 123.2, 118.6, 117.5, 116.7, 116.2, 104.6, 103.1, 51.8, 46.8, 39.9 (overlap with DMSO-d_6 peaks), 33.3, 32.6. ESI-HRMS: m/z calcd. for $C_{21}H_{22}ClN_4O_2$ $[M+H]^+$ 397.1431. Found 397.1432.

*5.2.11. Methyl 11-(3-aminopropylamino)-2-bromo-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylate (**9c**)*

Yellow solids, mp: 109–112 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.76 (s, 1H), 8.57 (s, 1H), 7.97–7.88 (m, 2H), 7.84 (d, J = 9.2 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 4.16 (s, 3H), 3.95 (t, J = 6.7 Hz, 2H), 3.86 (s, 3H), 2.67 (t, J = 6.4 Hz, 2H), 1.82 (t, J = 6.5 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.4, 158.1, 155.8, 147.9, 136.4, 133.4, 126.1, 125.9, 123.9(2C), 118.6, 117.7, 117.3, 116.2, 113.5, 103.1, 51.7, 46.6, 39.3, 33.1, 32.6, 32.3. ESI-HRMS: m/z calcd. for $C_{21}H_{22}BrN_4O_2$ $[M+H]^+$ 441.0926. Found 441.0927.

*5.2.12. Methyl 11-(3-aminopropylamino)-9-bromo-5-methyl-5H-indolo[2,3-b]quinoline-2-carboxylate (**9d**)*

Yellow solids, mp: 162–163 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.10 (d, J = 1.6 Hz, 1H), 8.24 (dd, J = 8.9, 1.7 Hz, 1H), 8.10 (d, J = 1.9 Hz, 1H), 7.88 (d, J = 9.0 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.39 (dd, J = 8.4, 1.9 Hz, 1H), 4.14 (s, 3H), 3.97 (t, J = 6.6 Hz, 2H), 3.92 (s, 3H), 2.71 (t, J = 6.2 Hz, 2H), 1.83–1.77 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 165.8, 156.5, 150.5, 149.0, 140.4, 130.7, 126.7(2C), 126.2, 124.0, 121.5, 118.5, 115.6, 114.9, 110.4, 102.3, 52.2, 47.5, 39.9 (overlap with DMSO-d_6 peaks), 32.9, 32.6. ESI-HRMS: m/z calcd. for $C_{21}H_{22}BrN_4O_2$ $[M+H]^+$ 441.0926. Found 441.0927.

*5.2.13. N-(3-Aminopropyl)-9-bromo-2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (**9e**)*

Yellow solids, mp: 152–154 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.59 (d, J = 1.8 Hz, 1H), 8.03 (d, J = 1.7 Hz, 1H), 7.86 (d, J = 9.2 Hz, 1H), 7.82 (dd, J = 9.1, 2.0 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.39 (dd, J = 8.4, 1.9 Hz, 1H), 4.12 (s, 3H), 3.91 (t, J = 6.6 Hz, 2H), 2.63 (t, J = 6.3 Hz, 2H), 1.77 (t, J = 6.5 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 156.6, 150.9, 148.1, 136.3, 130.7, 126.9, 126.1, 125.2, 124.2, 123.3, 118.3, 117.3, 116.5, 109.9, 102.9, 46.9, 39.5 (overlap with DMSO-d_6 peaks), 33.3, 32.5. ESI-HRMS: m/z calcd. for $C_{19}H_{19}BrClN_4$ $[M+H]^+$ 417.0482. Found 417.0480.

*5.3. General procedure for the preparation of 9-ester substituted 11-amino-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylic acid (**11a**–**c**)*

Methyl 11-amino-5-methyl-5*H*-indolo[2,3-*b*]quinoline-9-carboxylate **8a**, **8c** and **8d** was dissolved in MeOH and heated at reflux with 20% NaOH (aq.) overnight. The reaction was monitored by TLC. Then the mixture was neutralized with 1 N HCl (aq.) and concentrated to remove the MeOH under vacuum. The crude product was purified by reverse-phase chromatography using eluent changed from H_2O to H_2O/CH_3CN (50:1) to give carboxylic acid.

*5.3.1. 11-(3-Aminopropylamino)-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylic acid (**11a**)*

White solids, mp: >300 °C; ^{1}H NMR (600 MHz, D_2O) δ: 8.10 (d, J = 8.2 Hz, 1H), 7.90 (t, J = 7.5 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.58 (dd, J = 9.9, 4.9 Hz, 2H), 7.53 (s, 1H), 7.17 (dd, J = 7.9, 4.8 Hz, 1H), 3.69–3.57 (m, 5H), 2.93–2.83 (m, 2H), 2.06–1.91 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ: 168.9, 151.6, 146.9, 139.0, 135.8, 133.7, 127.4, 125.1, 123.7, 123.3, 122.4, 118.8, 116.2, 115.4, 111.0, 97.9, 44.8, 36.4, 34.3, 27.9. ESI-HRMS: m/z calcd. for $C_{20}H_{19}N_4O_2$ $[M-H]^-$ 347.1513. Found 347.1505.

*5.3.2. 11-(2-(Dimethylamino)ethylamino)-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylic acid (**11b**)*

White solids, mp: 276–280 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.03 (d, J = 8.0 Hz, 2H), 8.57 (s, 1H), 8.15 (dd, J = 19.0, 8.6 Hz, 2H), 8.04 (t, J = 7.8 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.71 (t, J = 7.7 Hz, 1H), 4.37 (dd, J = 11.4, 5.7 Hz, 2H), 4.30 (s, 3H), 3.55 (t, J = 5.5 Hz, 2H), 2.78 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.6, 152.5, 149.3, 140.71, 136.7, 133.3, 127.7, 125.2, 124.9, 124.5 (2C), 120.6, 116.9, 112.2, 99.5, 56.0, 43.1, 42.8, 35.7, 34.4. ESI-HRMS: m/z calcd. for $C_{21}H_{21}N_4O_2$ $[M - H]^-$ 361.1670. Found 361.1653.

*5.3.3. 11-(3-(Dimethylamino)propylamino)-5-methyl-5H-indolo[2,3-b]quinoline-9-carboxylic acid (**11c**)*

Pale Yellow solids, mp: 280–282 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.97 (s, 1H), 8.89 (d, J = 8.5 Hz, 1H), 8.52 (s, 1H), 8.12 (dd, J = 16.6, 8.6 Hz, 2H), 8.05–7.98 (m, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.70 (t, J = 7.7 Hz, 1H), 4.26 (s, 3H), 4.01 (dd, J = 12.9, 6.5 Hz, 2H), 3.15–3.04 (m, 2H), 2.70 (s, 6H), 2.31 (dt, J = 14.0, 7.2 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.7, 152.4, 149.3, 136.8, 133.2, 127.3, 124.9, 124.5 (2C), 124.2, 120.8, 116.9, 116.7, 112.2, 98.6, 53.7, 45.3, 42.1, 35.6, 25.1. ESI-HRMS: m/z calcd. for $C_{22}H_{23}N_4O_2$ $[M - H]^-$ 375.1826. Found 375.1828.

*5.4. General procedure for the preparation of 2,9-disubstituted 11-(3-(3-phenylureido)propylamino)-5H-indolo[2,3-b]quinolines (**12a–j**)*

2,9-Disubstituted 11-(3-aminopropylamino)-5-methyl-5*H*-indolo[2,3-*b*]quinolines (**7**–**9**, 50 mg) was completely dissolved in dry THF (1 mL), and then a solution of isocyanate (1.1 equiv.) and dry THF (1 mL) was added drop by drop under stirring at room temperature for 1 h. TLC monitoring was used to ensure the completion of reaction. After completion, the reaction mixture was evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography using AcOEt-2 N ammonia in MeOH (20:1 v/v) as an eluent to yield pure products as pale yellow solids.

*5.4.1. Methyl 5-methyl-11-(3-(3-phenylureido)propylamino)-5H-indolo[2,3-b]quinoline-2-carboxylate (**12a**)*

Pale yellow solids, mp: 210–211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.16 (d, J = 1.8 Hz, 1H), 8.40 (s, 1H), 8.26 (dd, J = 9.0, 1.6 Hz, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.91 (d, J = 9.0 Hz, 1H), 7.53 (d, J = 7.4 Hz, 1H), 7.48 (t, J = 6.0 Hz, 1H), 7.34 (dd, J = 8.6, 1.1 Hz, 2H), 7.32–7.26 (m, 1H), 7.22–7.16 (m, 2H), 7.14–7.06 (m, 1H), 6.92–6.82 (m, 1H), 6.17 (t, J = 5.8 Hz, 1H), 4.17 (s, 3H), 3.93 (s, 3H), 3.88 (dd, J = 13.2, 6.5 Hz, 2H), 3.15 (q, J = 6.5 Hz, 2H), 1.94–1.86 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 166.0, 156.3, 155.5, 152.1, 148.2, 140.4, 140.2, 130.4, 128.6, 126.4, 124.8, 124.2, 122.2, 121.4, 121.0, 118.6, 117.7, 117.0, 115.4(2C), 104.9, 52.2, 45.5, 36.5, 32.5, 31.6. ESI-HRMS: m/z calcd. for $C_{28}H_{26}N_5O_3$ $[M - H]^-$ 480.2036. Found 480.2024.

*5.4.2. Methyl 5-methyl-11-(3-(3-phenylthioureido)propylamino)-5H-indolo[2,3-b]quinoline-2-carboxylate (**12b**)*

Pale yellow solids, mp: 174–176 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.44 (s, 1H), 9.16 (d, J = 1.7 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.94 (dd, J = 16.7, 8.4 Hz, 2H), 7.72 (s, 1H), 7.53 (d, J = 7.4 Hz, 1H), 7.48 (s, 1H), 7.35–7.28 (m, 1H), 7.23 (d, J = 4.8, 4H), 7.16–7.10 (m, 1H), 7.07 (dt, J = 4.7, 3.5 Hz, 1H), 4.18 (s, 3H), 3.93 (s, 3H), 3.93–3.86 (m, 2H), 3.58–3.46 (m, 2H), 2.04–1.97 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 180.3, 171.6, 166.0, 156.2, 151.8, 148.2, 140.1, 138.9, 130.4, 128.7, 126.3, 124.9, 124.2(2C), 123.2, 122.3, 121.6, 118.8, 116.9, 115.4, 104.5, 52.3, 45.5, 41.4, 32.7, 30.3. ESI-HRMS: m/z calcd. for $C_{28}H_{28}N_5O_2S$ $[M + H]^+$ 498.1964. Found 498.1965.

*5.4.3. Methyl 5-methyl-11-(3-(3-phenylureido)propylamino)-5H-indolo[2,3-b]quinoline-9-carboxylate (**12c**)*

Pale yellow solids, mp: 209–211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.61 (d, J = 7.5 Hz, 1H), 8.57 (d, J = 1.5 Hz, 1H), 8.42 (s, 1H), 7.97–7.87 (m, 2H), 7.87–7.81 (m, 1H), 7.54 (t, J = 6.4 Hz, 2H), 7.48 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H), 7.33 (dd, J = 8.6, 1.1 Hz, 2H), 7.23–7.15 (m, 2H), 6.90–6.84 (m, 1H), 6.18 (t, J = 5.8 Hz, 1H), 4.20 (s, 3H), 3.91 (q, J = 6.6 Hz, 2H), 3.85 (s, 3H), 3.19 (q, J = 6.5 Hz, 2H), 1.97–1.90 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.4, 158.4, 155.9, 155.6, 149.2, 140.4, 137.4, 131.2, 128.6, 125.9, 123.9(2C), 123.7, 121.4, 121.1, 118.5, 117.8, 116.1, 115.8, 115.6, 103.5, 51.7, 45.3, 36.5, 32.6, 31.6. ESI-HRMS: m/z calcd. for $C_{28}H_{26}N_5O_3$ $[M - H]^-$ 480.2036. Found 480.2002.

*5.4.4. Methyl 5-methyl-11-(3-(3-phenylthioureido)propylamino)-5H-indolo[2,3-b]quinoline-9-carboxylate (**12d**)*

Pale yellow solids, mp: 172–175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.45 (s, 1H), 8.60 (d, J = 7.5 Hz, 1H), 8.57 (d, J = 1.4 Hz, 1H), 7.96–7.89 (m, 2H), 7.87–7.80 (m, 1H), 7.71 (s, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.52–7.45 (m, 2H), 7.23 (d, J = 4.3 Hz, 4H), 7.06 (dt, J = 8.6, 4.5 Hz, 1H), 4.20 (s, 3H), 3.93 (dd, J = 13.0, 6.5 Hz, 2H), 3.86 (s, 3H), 3.58–3.49 (m, 2H), 2.07–2.01 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 180.3, 167.4, 158.0, 155.3, 149.2, 138.9, 137.4, 131.3, 128.7, 125.9, 124.2(2C), 123.8(2C), 123.2, 121.4, 118.7, 115.8(2C), 115.5, 103.1, 51.7, 45.4, 41.3, 32.6, 30.4. ESI-HRMS: m/z calcd. for $C_{28}H_{28}N_5O_2S$ $[M + H]^+$ 498.1964. Found 498.1965.

*5.4.5. Dimethyl 5-methyl-11-(3-(3-phenylureido)propylamino)-5H-indolo[2,3-b]quinoline-2,9-dicarboxylate (**12e**)*

Pale yellow solids, mp: 228–229 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.22 (d, J = 1.7 Hz, 1H), 8.58 (d, J = 1.4 Hz, 1H), 8.38 (s, 1H), 8.29 (dd, J = 9.0, 1.8 Hz, 1H), 8.00 (d, J = 5.9 Hz, 1H), 7.97–7.90 (m, 2H), 7.56 (d, J = 8.4 Hz, 1H), 7.32 (dt, J = 8.8, 1.6 Hz, 2H), 7.20–7.15 (m, 2H), 6.89–6.83 (m, 1H), 6.16 (t, J = 5.7 Hz, 1H), 4.19 (s, 3H), 3.96–3.89 (m, 5H), 3.86 (s, 3H), 3.23–3.15 (m, 2H), 2.02–1.93 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 167.3, 165.9, 158.3, 155.5, 149.1, 140.4, 140.1, 130.8, 128.6, 126.3, 125.9, 124.0, 123.7, 122.2, 121.0, 119.1, 117.7, 116.5, 115.8, 115.4, 103.1, 52.3, 51.7, 45.2, 36.4, 32.8, 31.6. ESI-HRMS: m/z calcd. for $C_{30}H_{30}N_5O_5$ $[M + H]^+$ 540.2247. Found 540.2247.

*5.4.6. Dimethyl 5-methyl-11-(3-(3-phenylthioureido)propylamino)-5H-indolo[2,3-b]quinoline-2,9-dicarboxylate (**12f**)*

Pale yellow solids, mp: 118–119 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.44 (s, 1H), 9.21 (s, 1H), 8.56 (s, 1H), 8.31–8.23 (m, 1H), 7.94 (dd, J = 13.9, 8.2 Hz, 3H), 7.69 (s, 1H), 7.56 (dd, J = 8.3, 1.8 Hz, 1H), 7.26–7.19 (m, 4H), 7.06 (dt, J = 8.7, 4.4 Hz, 1H), 4.19 (d, J = 2.9 Hz, 3H), 3.94 (d, J = 0.7 Hz, 5H), 3.86 (s, 3H), 3.55 (s, 2H), 2.09–2.04 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 180.3, 171.5, 167.3, 165.8, 149.2, 140.0, 138.9, 130.9, 128.6, 126.3, 125.9, 124.2, 123.8, 123.1, 122.3, 119.4, 117.6, 116.2, 115.9, 115.5, 102.7, 52.3, 51.7, 45.3, 41.2, 33.0, 30.3. ESI-HRMS: m/z calcd. for $C_{30}H_{30}N_5O_4S$ $[M + H]^+$ 556.2019. Found 556.2019.

*5.4.7. Methyl 2-chloro-5-methyl-11-(3-(3-phenylureido) propylamino)-5H-indolo[2,3-b]quinoline-9-carboxylate (**12g**)*

Pale yellow solids, mp: 171–172 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.74 (d, J = 1.9 Hz, 1H), 8.52 (s, 1H), 8.41 (s, 1H), 7.95–7.88 (m, 2H), 7.84 (dd, J = 9.1, 2.1 Hz, 1H), 7.62 (s, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 7.8 Hz, 2H), 7.18 (t, J = 7.9 Hz, 2H), 6.87 (t, J = 7.3 Hz, 1H), 6.18 (t, J = 5.6 Hz, 1H), 4.15 (s, 3H), 3.90–3.86 (m, 2H), 3.85 (s, 3H), 3.20 (dd, J = 12.5, 6.4 Hz, 2H), 1.99–1.94 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 171.6, 167.3, 158.0, 155.5, 148.1, 140.4, 136.0, 130.9, 128.6, 126.1(2C), 123.9, 123.7, 123.1, 121.1, 118.9, 117.7(2C), 117.0, 116.1, 103.7, 51.7, 45.2, 36.4, 32.8, 31.5. ESI-HRMS: m/z calcd. for $C_{28}H_{27}ClN_5O_3$ $[M + H]^+$ 516.1802. Found 516.1805.

*5.4.8. Methyl 2-bromo-5-methyl-11-(3-(3-phenylureido) propylamino)-5H-indolo[2,3-b]quinoline-9-carboxylate (**12h**)*

Pale yellow solids, mp: 182–183 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.84 (d, J = 2.1 Hz, 1H), 8.51 (d, J = 1.4 Hz, 1H), 8.42 (s, 1H), 7.92 (ddd, J = 8.2, 5.9, 1.8 Hz, 2H), 7.81 (d, J = 9.2 Hz, 1H), 7.63 (t, J = 5.9 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.33 (dd, J = 8.5, 0.9 Hz, 2H), 7.19 (dd, J = 10.7, 5.1 Hz, 2H), 6.89–6.84 (m, 1H), 6.19 (t, J = 5.8 Hz, 1H), 4.13 (s, 3H), 3.89–3.83 (m, 5H), 3.20 (q, J = 6.5 Hz, 2H), 1.99–1.94 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 171.5, 167.3, 158.1, 155.5, 148.0, 140.4, 136.3, 133.5, 128.6, 126.0(2C), 123.9(2C), 121.1, 118.8, 117.8(2C), 117.5, 116.2, 113.8, 103.6, 51.7, 45.2, 36.4, 32.7, 31.5. ESI-HRMS: m/z calcd. for $C_{28}H_{27}BrN_5O_3$ $[M + H]^+$ 560.1297. Found 560.1295.

*5.4.9. Methyl 9-bromo-5-methyl-11-(3-(3-phenylureido) propylamino)-5H-indolo[2,3-b]quinoline-2-carboxylate (**12i**)*

Pale yellow solids, mp: 140–143 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.14 (d, J = 1.7 Hz, 1H), 8.38 (s, 1H), 8.26 (dd, J = 9.0, 1.7 Hz, 1H), 8.12 (d, J = 1.9 Hz, 1H), 7.90 (d, J = 9.0 Hz, 1H), 7.70 (t, J = 6.0 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.41 (dd, J = 8.4, 1.9 Hz, 1H), 7.34 (dd, J = 8.6, 1.0 Hz, 2H), 7.21–7.15 (m, 2H), 6.88–6.84 (m, 1H), 6.18 (t, J = 5.8 Hz, 1H), 4.14 (s, 3H), 3.92 (s, 3H), 3.86 (dd, J = 13.0, 6.6 Hz, 2H), 3.16 (dd, J = 5.7, 2.1 Hz, 2H), 1.94–1.90 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 165.9, 156.5, 155.5, 150.6, 149.2, 140.4(2C), 130.7, 128.6, 127.0, 126.8, 126.1, 123.9, 121.6, 121.0, 118.6, 117.7, 115.6, 114.9, 110.5, 103.4, 52.2, 45.5, 36.4, 32.6, 31.6. ESI-HRMS: m/z calcd. for $C_{28}H_{27}BrN_5O_3$ $[M + H]^+$ 560.1297. Found 560.1298.

*5.4.10. 1-(3-(9-Bromo-2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (**12j**)*

Pale yellow solids, mp: 145–148 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.67 (d, J = 2.2 Hz, 1H), 8.38 (s, 1H), 8.01 (d, J = 1.9 Hz, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.82 (dd, J = 9.1, 2.2 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.43–7.39 (m, 2H), 7.35–7.30 (m, 2H), 7.20–7.15 (m, 2H), 6.86 (tt, J = 7.4, 1.1 Hz, 1H), 6.17 (t, J = 5.8 Hz, 1H), 4.12 (s, 3H), 3.83 (q, J = 6.5 Hz, 2H), 3.14 (q, J = 6.5 Hz, 2H), 1.92–1.87 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 156.6, 155.5, 151.1, 148.1, 140.4, 136.3, 130.7, 128.6, 127.2, 125.9, 125.4, 124.2, 123.3, 121.0, 118.3, 117.7, 117.3, 116.6, 110.0, 103.9, 45.5, 36.4, 32.5, 31.5. ESI-HRMS: m/z calcd. for $C_{26}H_{24}BrClN_5O$ $[M + H]^+$ 536.0853. Found 536.0855.

6. Biological testing assay

6.1. Activity against P. falciparum

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay [42,43], using the chloroquine and pyrimethamine resistant *P. falciparum* K1 strain that originate from Thailand (Thaitong et al., 1983) [44] and strain susceptible to known antimalarial drugs (*P. falciparum* NF54) (Ponnudurai et al., 1981) [45], and all the test compounds were compared for activity with the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/l), $NaHCO_3$ (2.1 g/l), neomycin (100 U/mL), Albumax^R (5 g/l) and washed human red cells A^+ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4% CO_2, 3% O_2, 93% N_2. After 48 h 50 μL of ^{3}H-hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fibre filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate™ liquid scintillation counter (Wallac, Zürich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression (Huber 1993) [46] using Microsoft Excel.

6.2. In vitro cytotoxicity against L6 cells

Assays were performed in 96-well microtiter plates, each well containing 100 μL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L6 cells (a primary cell line derived from rat skeletal myoblasts) (Page et al., 1993 and Ahmed et al., 1994) [47,48]. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. After 70 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 μL of Alamar Blue was then added to each well and the plates incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression (Huber 1993) [46] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

6.3. Detergent mediated assay for β-haematin inhibition

The β-haematin formation inhibition assay method described by Carter et al. [32,36] was modified for manual liquid delivery. Three stock solutions of the samples were prepared by dissolving the pre-weighed compound in DMSO and after sonication, diluting with DMSO to give 20 mM, 2 mM and 0.4 mM solutions of each sample. These were delivered to a 96-well plate in duplicate to give concentrations ranging from 0 to 1000 μM (final concentration) with a total DMSO volume of 10 μL in each well after which deionised H_2O (70 μL) and NP-40 (20 μL; 30.55 μM) were added. A 25 mM haematin stock solution was prepared by sonicating haemin in DMSO for one minute and then suspending 178 μL of this in a 1 M acetate buffer (pH 4.8). The homogenous suspension (100 μL) was then added to the wells to give final buffer and haematin concentrations of 0.5 M and 100 μM respectively. The plate was covered and incubated at 37 °C for 5–6 h in a water bath. Analysis was carried out using the pyridine-ferrichrome method developed by Ncokazi and Egan [37]. A solution of 50% (v/v) pyridine, 30% (v/v) H_2O, 20% (v/v) acetone and 0.2 M HEPES buffer (pH 7.4) was prepared and 32 μL added to each well to give a final pyridine concentration of 5% (v/v). Acetone (60 μL) was then added to assist with haematin dispersion. The UV–vis absorbance of the plate wells was read on a SpectaMax plate reader. Sigmoidal dose–response curves were fitted to the absorbance data using GraphPad Prism v3.02 to obtain a 50% inhibitory concentration (IC_{50}) for each compound. Prediction of physical properties and multiple correlation analysis were carried out using the ChemSW Molecular Modeling Pro Plus v.6.36 software.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.072.

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