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Synthesis, β-haematin inhibition, and in vitro antimalarial testing of isocryptolepine analogues: SAR study of indolo[3,2-*c*]quinolines with various substituents at C2, C6, and N11

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ABSTRACT

A series of indolo[3,2-*c*]quinolines were synthesized by modifying the side chains of the ω-aminoalkylamines at the C6 position and introducing substituents at the C2 position, such as F, Cl, Br, Me, MeO and NO_2, and a methyl group at the N11 position for an SAR study. The in vitro antiplasmodial activities of the 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 compounds **6k** and **6l** containing the branched methyl groups of 3-aminopropylamino at C6 with a Cl atom at C2 exhibited a very low cytotoxicity with IC_{50} values above 4000 nM, high antimalarial activities with IC_{50} values of about 11 nM for CQS (NF54), IC_{50} values of about 17 nM for CQR (K1), and RI resistance indices of 1.6. Furthermore, the compounds were tested for β-haematic inhibition, and QSAR revealed an interesting linear correlation between the biological activity of CQS (NF54) and three contributing factors, namely solubility, hydrophilic surface area, and β-haematin inhibition for this series. In vivo testing of **6l** showed a reduction in parasitaemia on day 4 with an activity of 38%.

1. Introduction

Malaria is a mortal disease caused by *Plasmodium* parasites, which are spread between humans by infected mosquitoes.[1] In 2012, malaria caused about 219 million clinical cases and approximately 0.66 million deaths.[2] Currently, a number of effective antimalarial drugs and treatments are available, but drug resistance remains a problem because of the rapid evolution and adaptation of the malaria parasite.[3] Moreover, current antimalarial drugs are still unaffordable for underdeveloped countries where people are the most vulnerable.[4] Therefore, novel and economical alternatives are needed.

Chloroquine (CQ, **I**), historically, the most effective and cheapest therapeutic agent, found long-lasting use for more than half a century as a specific drug for the treatment of malaria patients.[5] The structurally unique artemisinin with an endoperoxide function was introduced in the 1970s as a result of a search for a new drug from Chinese herbal medicines.[6] However, the *Plasmodium falciparum* strains resistant to CQ are now widespread in endemic areas[7,8] and evidence of in vitro and in vivo resistance even against the most recently introduced artemisinin-based combination therapy (ACT) has also now been demonstrated for the therapy of uncomplicated *P. falciparum* infections.[9,10]

The mechanism of action of chloroquines against the malaria parasites was explored by one of our groups.[11] It was argued that the intra-erythrocytically active antimalarial agents act by binding to haematin,[12–14] blocking β-haematin formation and leaving toxic haematin in the parasite.[15] This suggests that the mode of action of such intra-erythrocytically active antimalarials is to simply complex haem and prevent the conversion to β-haematin. Based on these results, the design of antimalarial drugs has been envisaged based on the 4-aminoquinoline cores and a search for new antimalarial agents with the chloroquine-related core has continued to be actively pursued.[16–18]

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0968-0896/

Due to the appearance of resistance to chloroquine, other chloroquine-related antimalarial drugs with quinoline motifs have been proposed.[19,20] Examples include amodiaquine (**II**) (trade names Camoquin, Flavoquine)[21] and amopyroquine (**III**)[22], both of which contain a 4-aminoquinoline core. Amodiaquine has been shown to be more effective than chloroquine in treating chloroquine-resistant *Plasmodium falciparum* malaria infections.

The other leading antimalarial drug with a quinoline motif is mefloquine (**IV**).[23] Mefloquine remains effective against human malaria parasites. These close analogues of chloroquine as well as certain chloroquine analogues, such as lumefantrine, maintain activity against chloroquine-resistant parasite strains, Indeed, resistance to chloroquine is not believed to arise from any change in the drug target, but rather primarily from mutations in a membrane protein, PfCRT, found in the parasite digestive vacuole that is thought to transport chloroquine away from its site of action.[24,25] This effect is compound specific. As a result, it may be possible to design new antimalarials based on the same mechanism of action as that of chloroquine.

Finding new lead compounds from plant products is one of the pivotal strategies in the search for new drugs against infectious diseases.[26,27] In this regard, the constituents of the roots of the climbing shrub *Cryptolepis sanguinolenta*, growing in some African countries, is promising since an aqueous macerate or decoction of this root is used for the treatment of endemic disease such as malaria fever.[28,29] Among the known constituents of this plant, cryptolepine (**V**),[30,31] neocryptolepine (**VI**),[32] and isocryptolepine (**VII**)[33]are attractive as the lead candidates for developing more active derivatives (see Fig. 2), since these compounds show antimalarial activities though with a weak potency.[34,35]

In particular, the structure of isocryptolepine (**VII**) has attracted our attention, since this compound can be envisaged to possess the 4-aminoquinoline subunit, which can be recognized by dissecting out the tetracyclic indolo[4,3-*c*]quinoline core of **VIII** at the bond indicated by the wavy line.[36]

Isocryptolepine (**VII**) and its C2-, C3-, C8-, or C9-substituted derivatives were evaluated for their in vitro antiplasmodial activity against the CQS and CQR strains of *P. falciparum*.[37] All the modified compounds showed a higher activity than the parent isocryptolepine (**VII**), and 8-bromo-2-chloroisocryptolepine was the most potent derivative with a selectivity index >100 versus the mammalian cell. On the other hand, the derivatives of the indolo[3,2-*c*]quinoline structures **VIII** were examined with their 5N-oxide for antimalarial activities by varying the substituents at C3, C6, C8, and N11.[38,39]

Among the tested derivatives, 3-chloro-11-(dimethylamino-3-propyl)-8-methoxyindolo[3,2-*c*]quinoline 5N-oxide had the strongest blood *schizontocidal* antimalarial activity against the *P. berghei* strain in vitro.[38] Later, Go et al. developed indolo[3,2-*c*]quinoline with a 4-methylpiperazino group, which was about 104 times more active in vitro than chloroquine,[40,41] but its cytotoxicity was not determined.

These examples suggested that the indolo[3,2-*c*]quinoline core has an inherent potential as a lead antimalarial agent and the introduction of substituents to the appropriate positions in this motif could improve its biological activities.

As part of our ongoing program aimed at finding antimalarial agents based on the plant-derived lead compounds from *Cryptolepis sanguinolenta*,[42–44] we have prepared a series of indolo[3,2-*c*]quinoline derivatives by varying the substituents at C6 and N11. The antimalarial activities and the cytotoxicity were improved by modification of the substituents at C6 and the effect of the *N*-methyl group at N11 was also investigated. Furthermore, the compounds were tested for β-haematin inhibition[45] for a better understanding of the mechanism of action of the indolo[3,2-*c*]quinoline derivatives. The most favorable compound was submitted for in vivo drug screening against the *Plasmodium berghei* malaria model in Swiss mice.

2. Chemistry

Since Timari's first accomplishment of the total synthesis of isocryptolepine in 1997,[46] a variety of synthetic schemes was proposed for this structural motif and its analogues comprising either a pyridine or an indole ring closure in the final stage.[47–51] For example, a one-step procedure for the construction of 5,11-dehydroindolo[3,2-*c*]quinoline-6-one using isatin and 2-aminobenzylamine was described by Bergman in 2003,[52] in which isatin consist of a 2(1*H*)-quinolone unit and 2-aminobenzylamine formed the indole part. Later, Tzeng and co-workers[53] applied this method to the synthesis of a series of 11H-indolo[3,2-*c*]quinoline derivatives modified with an amino group at the C6 position. Mohan applied the Fischer indole synthesis for construction of the isocryptolepines in 2005,[54] which highlighted the one-step synthesis with a good yield, but only one example was demonstrated. A similar approach was later reported by Kumar in 2009[55] using 1-methyl-1,2,3,4-tetrahydroquinolin-4-one and phenylhydrazine. Furthermore, a multistep method was described by Choshi and co-workers[56] for isocryptolepine using the methyl 2-(indol-2-yl)benzoate, in which the microwave-assisted tandem Curtius rearrangement and electrocyclic ring closure of an aza 6π-electron system was employed as the key operation.

In our approach to construct a series of indolo[3,2-*c*]quinoline derivatives, the 5,11-dihydro-indolo[3,2-*c*]quinolin-6-ones (**3**) were chosen as the key intermediates. As shown in Scheme 1, compound **3**, obtained from **1** and **2**by heating in acetic acid, was then converted to 6-chloro-11*H*-indolo[3,2-*c*]quinoline (**4**) by dehydrative chlorination with $POCl_3$, and then amino groups were introduced at the C6 of **4** by the ArSN reaction with various amines. According to this protocol, various R^1 substituents at the C2 position, such as F, Cl, Br, Me, MeO and NO_2, can be introduced using isatin bearing the respective substituents. Appropriate amines, comprising ω-aminoalkylamines, ω-(*N*,*N*-dialkylamino)alkylamines, ω-hydroxyalkylamines, cyclic secondary amines, and arylamines, reacted with **4** by conventional heating or by applying MW irradiation in a short time, yielding the 6-amino-11*H*-indolo[3,2-*c*]quinolines **5**. Some terminal amino groups of **5** were further transformed into urea **6** (X = O, S), sulfonamides **7** and carbonyl-amides **8** as shown in Scheme 2.

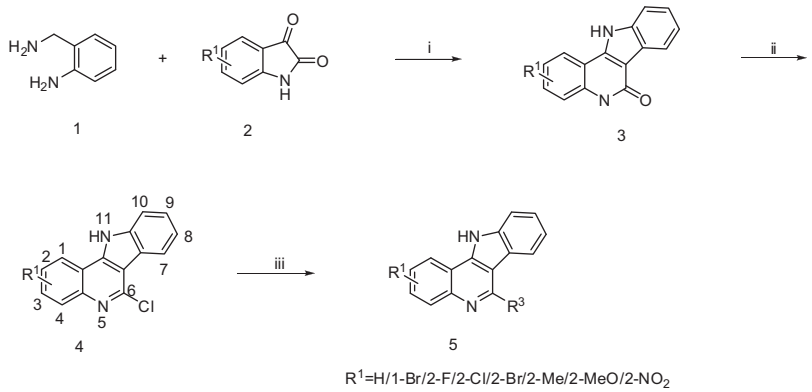
We then obtained the *N*-methylated analogues **10**. The methylation of **4** by heating with NaH followed by MeI produced the 6-chloro-11-methyl-indolo[3,2-*c*]quinoline (**9**), which was heated with the appropriate amines under MW irradiation to yield **10**. The terminal amino group was coupled by the treatment of **10** with the isocyanate to give **11** as shown in Scheme 3.

3. Results and discussion

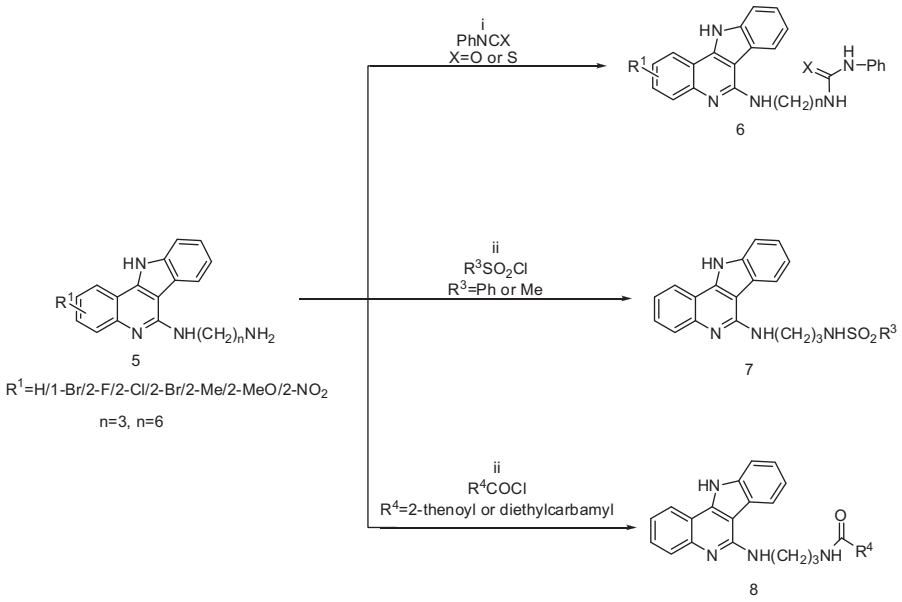
3.1. Antiplasmodial activity and cytotoxicity

As a result of the initial screening of the C6-substituted indolo[3,2-*c*]quinolines, prepared from **4a** using various amines, trends arising from the effect of the C6-substituents on the in vitro antiplasmodial activity against the CQS (NF54) cells were elucidated. Thus, the ω-aminoalkylamino groups of **5a**–**5g** are more effective than the alkylamino group of **5h**–**5j**, and the benzeneamino group of **5l** is less effective (Table 1).

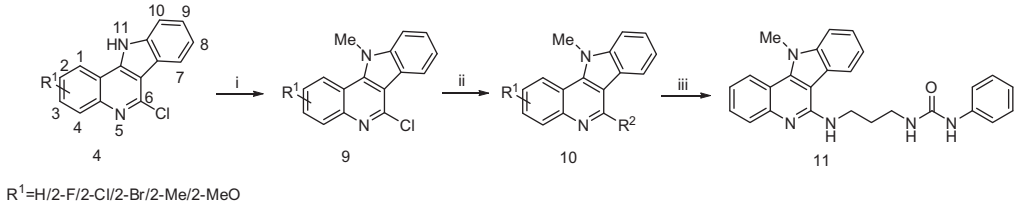
In the next step, the cooperative effect of the substituent at the C2 position was screened using halogens and a methyl group as a substituent on the 6-(3-aminopropylamino)indolo[3,2-*c*]quinolines. 2-Chloro-substituted **5p** had the highest activity relative to



Scheme 1. Synthesis of indolo[3,2-*c*]quinolines with amino group at the C-6 position. Reagents and conditions: (i) AcOH, reflux, 8–20 h; (ii) $POCl_3$, 130 °C, 8 h; (iii) appropriate amines, 90–140 °C, 20 min–4 h.



Scheme 2. Synthesis of indolo[3,2-*c*]quinolines by further modifications of the terminal amino group. Reagents and conditions: (i) THF, rt; (ii) THF, Et_3N, rt.



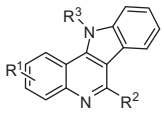
Scheme 3. Synthesis of indolo[3,2-*c*]quinolines by N-11 methylation and amination at the C6 position. Reagents and conditions: (i) MeI, NaH, 0 °C–rt, 4 h; (ii) appropriate amines, 140 °C, 24 h; (iii) THF, rt.

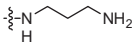
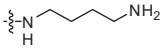
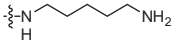
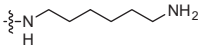
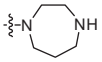
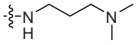
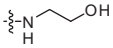
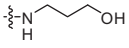
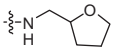
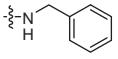
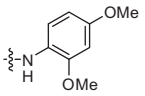
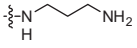
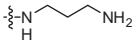
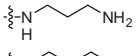
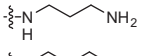
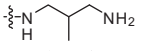
the 2-bromo-substituted **5n** and 2-fluoro-substituted **5m**, while the 2-methyl-substituted **5o** was the least effective among the tested compounds.

We also examined the effect of branched 3-aminopropylamino-substituents at the C6 position against the CQS (NF54) cell. Thus, the (3-amino-2-methylpropylamino) and (3-amino-2,2-dimethylpropylamino)-substituted **5q** and **5r** showed a slightly decreased activity against CQS (NF54), while the toxicity against the L6 cells decreased to half that of the non-branched (3-aminopropylamino)-substituted **5p**.

We next examined the ω-modification of the 6-(ω-aminoalkylamino)-substituted indolo[3,2-*c*]quinolines using phenylisocyanate and phenylthioisocyanate (Table 2). A remarkable improvement in the activity against CQS (NF54) was attained with

Table 1
Antiplasmodial activity against *P. falciparum* (CQS, NF54; CQR, K1) and cytotoxicity toward L6 Cells of indolo[3,2-*c*]quinoline derivatives **4** and **5**



No.	R^1	R^2	R^3	L6 cells IC_{50}[c] nM	NF54 IC_{50}[c] nM	SI[a] L6/NF54	K1 IC_{50}[c] nM	SI[a] L6/K1	RI[b] K1/NF54	β-Haematin inhibition μM
Isocryptolepine				1190			780	1.5		
4a	H	Cl	H	192956.1	10209.7	18.9	NT[d]			>1000
5a	H		H	626.8	13.7	45.8	82.7	7.6	6.0	116.3
5b	H		H	739.1	36.1	20.5	NT[d]			62.0
5c	H		H	816.5	12.6	64.8	25.1	32.5	2.0	81.0
5d	H		H	1182.1	15.0	78.8	90.2	13.1	6.0	49.6
5e	H		H	1776.4	26.4	67.3	NT[d]			62.4
5f	H		H	4108.7	63.2	65.0	NT[d]			171.0
5g	H		H	606.1	9.4	64.3	25.1	24.1	2.66	83.6
5h	H		H	890.6	140.6	6.3	NT[d]			200
5i	H		H	717.4	226.5	3.2	NT[d]			286.7
5j	H		H	2051.1	113.4	18.1	NT[d]			147.8
5k	H		H	2934.5	18.6	157.8	NT[d]			30.6
5l	H		H	15213.0	316.7	48.0	NT[d]			44.1
5m	2-F		H	638.9	13.0	49.1	NT[d]			14.3
5n	2-Br		H	891.0	8.1	110.0	NT[d]			19.4
5o	2-Me		H	322.0	36.1	8.9	NT[d]			60.9
5p	2-Cl		H	1120.7	6.2	180.8	67.7	16.6	10.9	12.4
5q	2-Cl		H	1918.4	29.5	65.0	59.0	32.5	2	12.7
5r	2-Cl		H	1839.3	25.5	72.1	51.0	36.1	2	20.7
Podophylotoxin				14.5						
Chloroquine					9.4		209.5		22.3	30–33
Artemisinin					4.3		2.8		0.7	
Amodiaquine										11–14

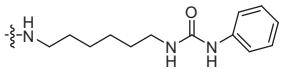
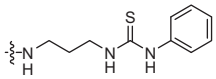
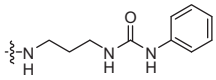
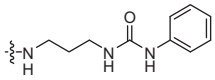
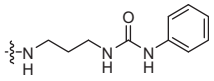
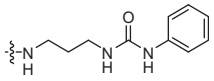
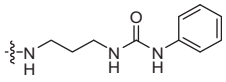
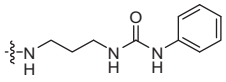
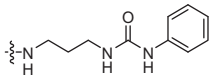
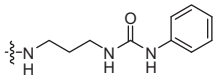
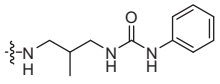
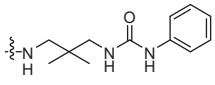
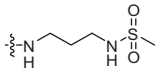
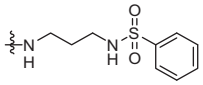
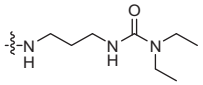
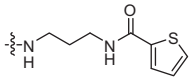
[a] Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.).
[b] Resistance index is the ratio of IC_{50} for the resistant versus the sensitive strain (K1/NF54).
[c] The IC_{50} values are the means of two independent assays; the individual values vary by less than a factor of 2.
[d] Not tested.

the 6-(3-phenylureido)propylamino)-substituted **6c** having an IC_{50} 2.4 nM, compared to 13.7 nM of the unmodified **5a**. In contrast, the activity of the 6-(3-phenylthioureido)propylamino)-substituted **6b** slightly decreased to 23.5 nM compared to 13.7 nM of **5a**. Subsequently, the effect of substituents, such as 2-F, 1-Br, 2-Br, 2-Me, 2-MeO, 2-NO_2, at the C1 or C2 position of the 6-(3-phenylureido)propylamino)-substituted indolo[3,2-*c*]quinolines was examined. However, distinct synergistic effects of the substituents at the C1 or C2 were not found. Indeed, the activity was weaker for all these derivatives.

Modification of the 3-aminopropylamino group at C6 with other groups, such as methanesulfonyl **7a**, benzenesulfonyl **7b**, *N*,*N*-diethylamidoyl **8a**, or 2-thiophenoyl **8b**, were tested. However, no significant improvements in the in vitro antiplasmodial activity were achieved, though the cytotoxicity was improved by the ω-modifications.

As a result of the valuation of the substituent effects at the C2 and C6 positions, and modification and branching of their pendant groups at C6, we have succeeded in improving the SI values to about 377 with IC_{50} values of about 11 nM for

Table 2
Antiplasmodial activity against *P. falciparum* (CQS, NF54; CQR, K1) and cytotoxicity toward L6 Cells of indolo[3,2-*c*]quinoline derivatives **6**, **7** and **8**

No.	R^1	R^2	R^3	L6 cells IC_{50}[c] nM	NF54 IC_{50}[c] nM	SI[a] L6/NF54	K1 IC_{50}[c] nM	SI[a] L6/K1	RI[b] K1/NF54	β-Haematin inhibition μM
Isocryptolepine				1190			780	1.5		
6a	H		H	861.5	22.1	39.0	24.4	35.3	1.1	16.2
6b	H		H	249.1	23.5	10.6	49.3	5.1	2.1	18.5
6c	H		H	1152.6	2.4	480.3	53.7	21.5	22.4	23.9
6d	2-F		H	1504.2	25.7	58.5	49.2	30.6	1.9	22.7
6e	1-Br		H	3624.2	288.7	12.6	NT[d]			22.0
6f	2-Br		H	1281.1	22.5	56.9	36.9	34.7	1.6	13.9
6g	2-Me		H	965.7	23.6	40.9	42.5	22.7	1.8	17.2
6h	2-MeO		H	1517.8	17.1	88.8	19.6	77.4	1.1	14.8
6i	2-NO_2		H	4642.7	24.2	191.8	NT[d]			15.2
6j	2-Cl		H	2568.0	27.0	95.1	50.0	51.4	1.9	14.4
6k	2-Cl		H	4105.3	10.9	376.6	17.5	234.6	1.6	14.6
6l	2-Cl		H	4004.4	10.6	377.8	16.9	235.9	1.6	11.7
7a	H		H	1063.9	54.3	19.6	NT[d]			118.5
7b	H		H	810.6	72.0	11.3	92.9	8.7	1.3	24.2
8a	H		H	3568.8	41.1	86.8	NT[d]			39.2
8b	H		H	3495.6	72.4	48.3	84.9	41.2	1.2	24.1

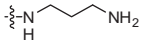
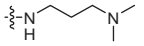
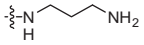
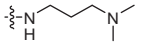
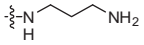
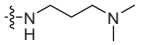
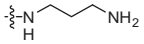
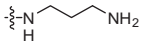
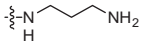
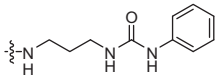
[a] Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.).
[b] Resistance index is the ratio of IC_{50} for the resistant versus the sensitive strain (K1/NF54).
[c] The IC_{50} values are the means of two independent assays; the individual values vary by less than a factor of 2.
[d] Not tested.

compounds **6k** and **6l**, whose C6-(3-aminopropylamino) group was modified with phenylthiourea and branched with methyl groups.

In this study, we also investigated the effect of the introduction of a methyl group at the C11 position. A series of 11-methyl-indolo [3,2-*c*]quinoline derivatives (**10** and **11**) was investigated (Table 3). Their antimalarial activity was not improved relative to the compound **5** series, and they exhibited a higher cytotoxicity against the L6 cells.

We then selected some compounds for testing against the CQR (K1) strain of *P. falciparum*. All the tested compounds showed a potent antimalarial activity against the CQS strains (IC_{50} value ranging from 16 to 91 nM), and low RI values between 1 and 6. The RI provides a quantitative measurement of the antiplasmodial

Table 3
Antiplasmodial activity against *P. falciparum* (CQS, NF54; CQR, K1) and cytotoxicity toward L6 Cells of indolo[3,2-*c*]quinoline derivatives **9**, **10** and **11**

No.	R^1	R^2	R^3	L6 cells IC_{50} nM	NF54 IC_{50} nM	SI[a] L6/NF54	K1 IC_{50} nM	SI[a] L6/K1	RI[b] K1/NF54	β-Haematin inhibition μM
Isocryptolepine				1190			780	1.5		
9	H	Cl	Me	17284.0	7386.0	2.3	NT[d]			>1000
10a	H		Me	279.2	72.3	3.9	85.4	3.3	1.2	24.2
10b	H		Me	258.7	273.7	0.9	NT[d]			~1000
10c	H		Me	700.8	93.2	7.5	NT[d]			>1000
10d	2-Br		Me	824.5	31.3	26.3	NT[d]			26.6
10e	2-Br		Me	576.2	58.3	9.9	NT[d]			41.2
10f	2-Br		Me	457.0	48.6	9.4	NT[d]			39.9
10g	2-Cl		Me	619.8	35.4	17.5	NT[d]			28.0
10h	2-Cl		Me	425.2	95.4	4.5	NT[d]			38.4
10i	2-Cl		Me	313.4	40.9	7.7	NT[d]			25.9
10j	2-F		Me	291.6	52.7	5.5	NT[d]			25.8
10k	2-Me		Me	191.6	31.4	6.1	NT[d]			29.5
10l	2-MeO		Me	137.6	32.9	4.2	NT[d]			56.9
11	H		Me	107.7	7.3	14.8	NT[d]			22.7

[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 by less than a factor of 2.
[d] Not tested.

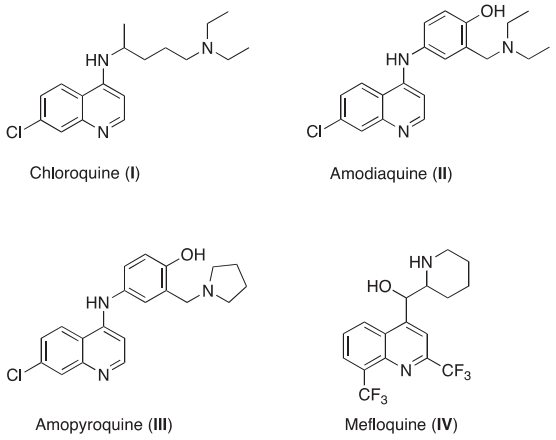


Figure 1. Chloroquine and its related antimalarial agents.

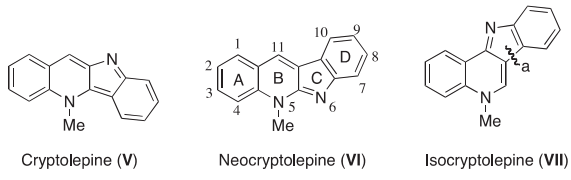


Figure 2. Structures of indoloquinolines from *Cryptolepis sanguinolenta.*

activity against the CQR strains relative to that against the CQS strains and reveals promising drug discovery leads.[57] The introduction of the MeO group at the C2 position (**6h**) was more effective in improving the antiplasmodial activity against the CQR (K1) strains than the other groups with an IC_{50} value of 19.6 nM. It is very interesting that **6k** and **6l** containing branched methyl groups at the C6-side chain exhibited significantly increased cytotoxicity values ((IC_{50} = 4100 or 4000 nM) relative to compound **6j**, and antimalarial activity increased especially against the CQR (K1) strains.

3.2. β-Haematin inhibition and QSAR

Inspection of the activity data revealed the importance of the R^2 amine side chain for high β-haematin and parasite activity in this series. This is evident from the weak parasite activity determined for **4a** and **9**, both of which contain only a chloro substituent at the R^2 position (Fig. 3-green). Interestingly, these compounds also have very a weak β-haematin activity, which suggests that the amine side chain is vital for the molecular interaction with haem at pH 4.8. This may well be due to the hydrogen bonding capabilities of the amine group which could assist the interaction of the fused-ring system with haem in order to prevent the formation of β-haematin. A scattered but statistically significant correlation (r^2 = 0.41, P < 0.0001) was observed between the log of the IC_{50} for β-haematin inhibition, log(βHi), and the log(IC_{50}) of the biological activity against the NF54 strain (Fig. 4a). This direct linear trend showed that while haemozoin inhibition is likely a contributing factor to the parasite activity and hence a probable mechanism of action, there are, as expected, other factors which likely play a significant role.

In order to define these factors, a linear regression with combinations of the predicted physical properties for each compound was carried out. Figure 4b shows the best statistical fit that contains physically relevant coefficients. The result of the analysis

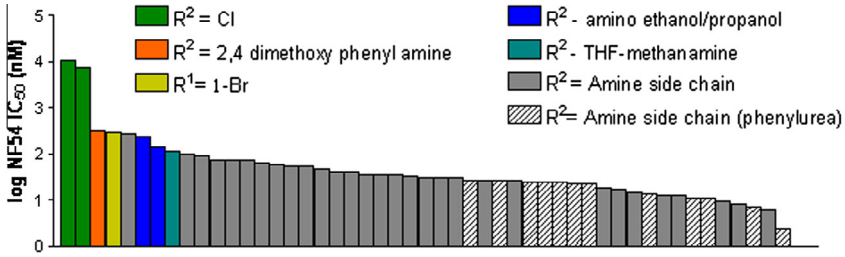


Figure 3. All compounds arranged by increasing biological activity. The least active compounds are those with Cl at the C6 position (**4a**, **9**), the 2,4-dimethoxy phenyl ring on the side chain (**5l**), the compound with a R^1 = bromo at the C1 position (**6e**) (as opposed to the C2 position **6f**) and an alcohol (**5i** and **5h**) or tetrahydrofuran (THF) group on the amine side chain (**5j**). On the other hand, the compounds containing phenylurea on the amine side chain show the greatest activity (**6a**–**6d**, **6f**–**6l** and **11**).

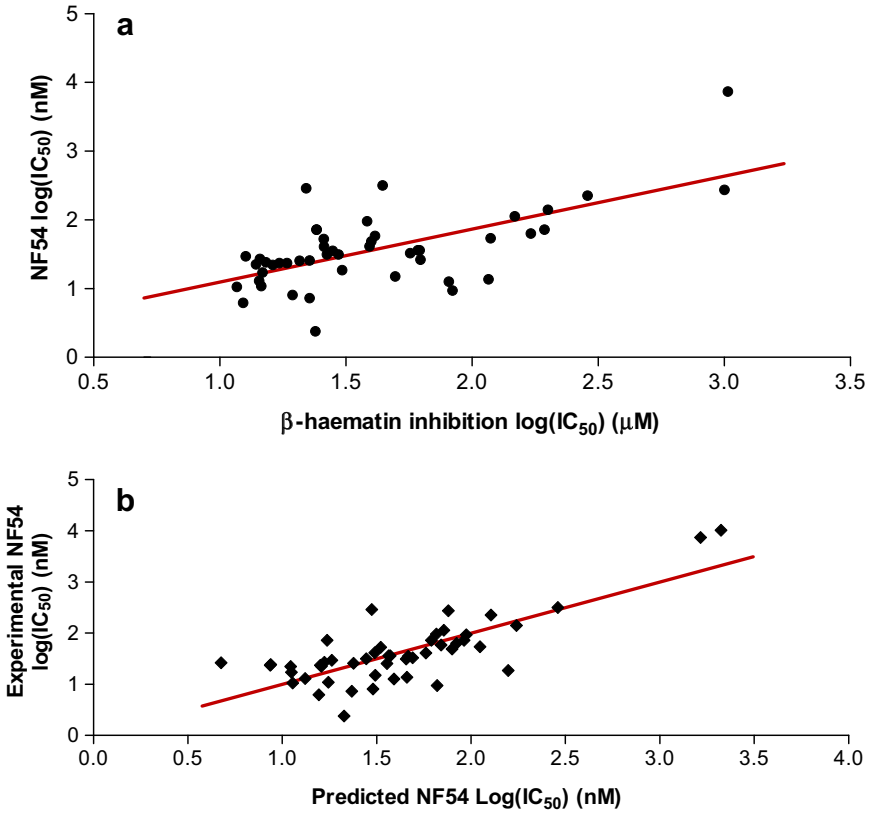


Figure 4. Linear correlation analysis revealed (a) a relationship between activities such that $\log(NF54IC_{50}) = 0.772^{*}\log(\beta HiIC_{50}) + 0.32$, $r^2 = 0.41$, $P < 0.0001$. (b) QSAR analysis between biological activity and predicted physical properties improved the statistics; $\log(NF54IC_{50}) = 0.193^{*}(Sol.) - 0.027^{*}(\%Hydrophil.) - 10.069^{*}(1/\beta Hi) - 2.208$, $r^2 = 0.61$, $P < 0.0001$, statistically significant both for individual parameters ($t = 4.13, 4.99, 3.87 > t_{crit} = 2.69$) and overall correlation ($F = 23.01 > F_{crit} = 4.25$) at 99% confidence level.

predicted that the biological IC_{50} correlates with the sum of terms related to the solubility, hydrophilicity and $1/\beta HiIC_{50}$. By incorporating these extra parameters, the statistics were improved so that less scatter was observed ($r^2 = 0.61$). The equation for the predicted NF54 $\log(IC_{50})$ indicates that an increase in the solubility parameter (Sol.) decreased the activity, a result that has previously been observed in other series,[42] while increasing the percentage of the hydrophilic surface area (%Hydrophil.) on the molecule increases the activity. These parameters represent the hydrophobic/hydrophilic balance required for optimal biological activity. Furthermore, a lower βHi IC_{50} results in a lower logNF54 IC_{50} as expected. The compounds with the highest solubility are the hydroxylated **5h** and **5i** (Fig. 1-blue), which, together with their slightly higher βHi IC_{50}s, could account for their weak activity. The compounds with R^2 = Cl (Fig. 3-green) also have a high solubility and low% hydrophilic surface area, however, their lack of activity is primarily due to their lack of β-haematin inhibition. It is important to note that when these two compounds are excluded, the trend in Figure 4b remains statistically significant. The other compound with a lower activity is **5l** which possesses a relatively small% hydrophilic surface area, indicating that the compound is slightly too hydrophobic for optimal activity. Figure 3 also demonstrates that generally, the phenylurea moiety on the amine side chain results in the highest biological activity. Further investigation of the phenylurea-containing compounds reveals that they have a moderate solubility (average phenylurea Sol. = average non-phenylurea Sol.), however, the average% hydrophilic area is 8% higher for the phenylurea compounds which could explain their greater NF54 activity.

3.3. In vivo antimalarial activity

Compound **6l** with the lowest cytotoxicity (IC_{50} above 4004 nM), low resistant index of 1.6 and strong antiplasmodial

activity in vitro (11 nM) was selected for an in vivo drug testing model against *Plasmodium berghei* in mice. The in vivo study was carried out according to the standard protocol following the '4 Day Test' by the FACS analysis. The activity was calculated as the difference between the mean percent parasitaemia for the control (n = 5 mice) and treated groups expressed as a percentage relative to the control group. After daily intraperitoneal dosing at 50 mg/kg for four consecutive days, this compound showed a significant reduction in parasitaemia on day 4 with an activity of 38%. While not strongly active in vivo, this result suggests that modifications to improve the water solubility might produce an analogue with an improved in vivo activity.

4. Conclusion

We have prepared a series of indolo[3,2-*c*]quinolines by varying the substituents at the C2 position, and modifying the terminal amino group of the C6-aminoalkylamino side chain with phenylisocyanate. A methyl group was then introduced at N11 by methylation. All the synthesized derivatives showed a potent antiplasmodial activity against CQS (NF54), and CQR (K1) strains in vitro. Compared to **5a**, the 2-chloro-substituted derivative **5p** was the most effective, and the urea derivatives **6c** had an increased activity against the CQS strain (NF54). The sulfonyl derivatives **7** and carbonyl derivatives **8** were less effective. Compounds **6k** and **6l** containing branched methyl groups exhibited significantly higher cytotoxicity values, and antimalarial activity especially increased against the CQR strain (K1). Compounds **10** and **11** bearing an N11-methyl group did not show an improved antimalarial activity and they exhibited a higher cytotoxicity against L6 cells. Most of the compounds have a very effective β-haematin inhibition activity. The linear correlation analysis revealed there were three contributing factors, namely, water solubility, hydrophilic surface area, and β-haematin inhibition that influence the biological activity of this series against CQS (NF54) parasites.

5. Experimental

5.1. Chemistry

5.1.1. General

The commercially obtained reagents were used without further purification. Column chromatographies were achieved on a silica gel column (230–400 mesh) using a gradient solvent system (*n*-hexane/ ethyl acetate as the eluent unless otherwise specified). The ^{1}H NMR and ^{13}C NMR spectra were measured on the Varian INOVA-600 spectrometer with DMSO-d_6 as the solvent unless otherwise indicated. Chemical shifts (δ ppm) were determined using tetramethylsilane (TMS) as the internal reference. Melting points were determined on a J-Science RFS-10 hot stage microscope. High resolution mass spectra were obtained on a Bruker micrOTOF II-SKA spectrometer. Purity was verified using HPLC systems. HPLC was performed on Waters e2695 Separations Module using Waters 2998 Photodiode Array (PDA) detector equipped with a Symmetry C18 column (4.6 × 150 mm, 5 μm). Water (A) and ACN (B) were used as eluents. A 100–10%, B, 35 min gradient was used with a flow rate of 1 ml/min. 0.1%TFA was added to solvent A and B. 254 nm was used as wavelength.

5.1.2. General procedure for the synthesis of 6-aminoindolo[3,2-*c*]quinolines 5 and 10

6-Chlorooindolo[3,2-*c*]quinoline **4** or **9** (0.4 mmol) and an excess of the appropriate amine (2.0 mmol) heated together at 90–100 °C for 20 min–24 h. Completion of the reaction was monitored by TLC. The reaction mixture then was added to water, and the formed solids were collected by filtration. The crude product was purified by flash chromatography using AcOEt-2N ammonia in MeOH (10:1 V/V) as the eluent to yield pure **5** or **10** as solids.

5.1.2.1. N^1-(11*H*-Indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (5a). Yield: 93%, mp: >230 °C decompose; ^{1}H NMR δ 8.42 (d, J = 7.9 Hz, 1H), 8.25 (d, J = 7.9 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.48 (dd, J = 8.2, 7.0 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.27 (dt, J = 14.6, 7.4 Hz, 2H), 7.07 (s, 1H), 3.80 (t, J = 5.9 Hz, 2H), 2.75 (t, J = 5.7 Hz, 2H), 1.83 (m, 2H); ^{13}C NMR δ 153.4, 146.6, 141.1, 138.5, 128.4, 126.6, 124.3, 122.0, 121.9, 121.0 (2C), 120.5, 114.5, 111.8, 103.1, 40.5 39.6, 32.0. HPLC purity 99.1%. HRMS (ESI) calcd for $C_{18}H_{18}N_4$ $[M-H]^-$ Exact Mass: 289.1453, found 289.1456.

5.1.2.2. N^1-(11*H*-Indolo[3,2-*c*]quinolin-6-yl)butane-1,4-diamine (5b). Yield: 78%, mp: 166–168 °C; ^{1}H NMR δ 8.40 (d, J = 7.6 Hz, 1H), 8.25 (d, J = 7.6 Hz, 1H), 7.66 (d, J = 6.9 Hz, 2H), 7.48 (t, J = 7.3 Hz, 1H), 7.41 (t, J = 7.3 Hz, 1H), 7.27 (dt, J = 17.1, 7.2 Hz, 2H), 6.62 (s, 1H), 3.72 (s, 2H), 2.64 (t, J = 6.6 Hz, 2H), 1.78 (m, 2H), 1.52 (dd, J = 13.5, 6.7 Hz, 2H); ^{13}C NMR δ 153.4, 146.6, 141.2, 138.5, 128.4, 126.7, 124.3, 122.0, 121.9, 121.2, 121.1, 120.5, 114.5, 111.8, 103.2, 42.0, 40.8, 31.4, 27.3. HPLC purity 99.9%. HRMS (ESI) calcd for $C_{19}H_{20}N_4$ $[M-H]^-$ Exact Mass: 303.1610, found 303.1614.

5.1.2.3. N^1-(11*H*-Indolo[3,2-*c*]quinolin-6-yl)pentane-1,5-diamine (5c). Yield: 80%, mp: 168–169 °C; ^{1}H NMR δ 8.41 (d, J = 7.6 Hz, 1H), 8.26 (m, 1H), 7.66 (d, J = 7.2 Hz, 2H), 7.49 (t, J = 7.1 Hz, 1H), 7.41 (t, J = 7.4 Hz, 1H), 7.28 (dt, J = 14.9, 7.2 Hz, 2H), 6.56 (s, 1H), 3.72 (s, 2H), 2.56 (s, 2H), 1.77 (d, J = 5.4 Hz, 2H), 1.43 (s, 4H); ^{13}C NMR δ 153.4, 146.6, 141.2, 138.5, 128.4, 126.7, 124.3, 122.0, 121.9, 121.2, 121.1, 120.5, 114.5, 111.8, 103.2, 42.2, 41.0, 33.8, 29.8, 24.6. HPLC purity 99.3%. HRMS (ESI) calcd for $C_{20}H_{22}N_4$ $[M-H]^-$ Exact Mass: 317.1766, found 317.1761.

5.1.2.4. N^1-(11*H*-Indolo[3,2-*c*]quinolin-6-yl)hexane-1,6-diamine (5d). Yield: 78%; ^{1}H NMR δ 8.38 (d, J = 7.9 Hz, 1H), 8.23 (d, J = 7.7 Hz, 1H), 7.64 (d, J = 8.1 Hz, 2H), 7.47 (dd, J = 11.2, 4.0 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 7.27 (dt, J = 18.5, 7.4 Hz, 2H), 6.54 (d, J = 5.5 Hz, 1H), 3.69 (dd, J = 13.1, 6.6 Hz, 2H), 2.51 (d, J = 5.9 Hz, 2H), 1.75 (t, J = 6.9 Hz, 2H), 1.40 (m, 6H); ^{13}C NMR δ 153.4, 146.6, 141.2, 138.5, 128.4, 126.7, 124.3, 121.9, 121.8, 121.2, 121.1, 120.5, 114.5, 111.8, 103.2, 42.1, 40.9, 33.8, 29.9, 27.1, 26.8. HPLC purity 97.8%. HRMS (ESI) calcd for $C_{21}H_{24}N_4$ $[M-H]^-$ Exact Mass: 331.1923, found 331.1916.

5.1.2.5. *N*-(3-(4-(3-Aminopropyl)piperazin-1-yl)propyl)-11*H*-indolo[3,2-*c*]quinolin-6-amin (5e). Yield: 47%. ^{1}H NMR δ 12.47 (s, 1H), 8.36 (d, J = 8.0 Hz, 1H), 8.23 (dd, J = 7.9, 1.1 Hz, 1H), 7.63 (dd, J = 10.8, 8.2 Hz, 2H), 7.48 (d, J = 1.4 Hz, 1H), 7.41 (s, 1H), 7.33–7.22 (m, 2H), 6.64 (s, 1H), 3.72 (d, J = 6.1 Hz, 2H), 2.53 (t, J = 6.7 Hz, 4H), 2.44 (t, J = 6.7 Hz, 6H), 2.34– 2.23 (m, 4H), 1.95–1.85 (m, 2H), 1.54–1.42 (m, 2H); ^{13}C NMR δ 153.4, 146.5, 141.1, 138.5, 128.4, 126.6, 124.3, 121.93, 121.8, 121.2 (2C), 120.9, 114.5, 111.8, 103.2, 57.1 (2C), 56.3, 53.6 (2C), 53.2 (2C), 30.7, 26.5 (2C). HPLC purity 98.1%. HRMS (ESI) calcd for $C_{25}H_{32}N_6$ $[M-H]^-$ Exact Mass: 415.2610, found 415.2609.

5.1.2.6. 6-(1,4-Diazepan-1-yl)-11*H*-indolo[3,2-*c*]quinoline (5f). Yield: 83%, mp: 216–218 °C; ^{1}H NMR δ 12.61 (s, 1H), 8.35 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.56 (dd, J = 8.1, 7.1 Hz, 1H), 7.42 (m, 2H), 7.31 (t, J = 7.5 Hz, 1H), 3.86 (s, 2H), 3.80 (d, J = 2.3 Hz, 2H), 2.98 (d, J = 2.3 Hz, 2H), 2.88 (t, J = 5.6 Hz, 2H), 1.88 (s, 2H); ^{13}C NMR δ 157.6, 145.1, 142.7, 138.9, 128.6, 127.7, 124.6, 123.0, 122.5, 122.1, 122.0, 120.7, 115.5, 112.0, 106.0, 55.4, 50.7, 49.1,

48.6, 31.1. HPLC purity 100%. HRMS (ESI) calcd for $C_{20}H_{20}N_4$ $[M-H]^-$ Exact Mass: 315.1610, found 315.1608.

5.1.2.7. N^1-(11*H*-Indolo[3,2-*c*]quinolin-6-yl)-N^3,N^3-dimethylpropane-1,3-diamine (5g). Yield: 90%; ^{1}H NMR δ 12.45 (s, 1H), 8.25 (t, J = 8.1 Hz, 2H), 7.66 (d, J = 8.0 Hz, 2H), 7.48 (t, J = 7.6 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.26 (t, J = 7.4 Hz, 1H), 7.14 (s, 1H), 3.75 (dd, J = 11.4, 5.9 Hz, 2H), 2.46 (t, J = 6.2 Hz, 2H), 2.24 (s, 6H), 1.89 (dd, J = 12.7, 6.3 Hz, 2H); ^{13}C NMR δ 153.4, 146.7, 141.2, 138.6, 128.5, 126.7, 124.3, 122.0, 122.0, 121.2, 120.6, 120.5, 114.6, 112.0, 103.2, 58.9, 45.8 (2C), 41.0, 26.7. HPLC purity 99.9%. HRMS (ESI) calcd for $C_{20}H_{22}N_4$ $[M-H]^-$ Exact Mass: 317.1766, found 317.1759.

5.1.2.8. 2-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)ethanol (5h). Yield: 35%. ^{1}H NMR δ 12.50 (s, 1H), 8.35 (d, J = 7.9 Hz, 1H), 8.26 (d, J = 7.8 Hz, 1H), 7.66 (t, J = 8.8 Hz, 2H), 7.51 (t, J = 7.6 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.30 (dd, J = 17.0, 7.8 Hz, 2H), 6.64 (d, J = 5.1 Hz, 1H), 5.41 (s, 1H), 3.80 (m, 2H), 3.75 (t, J = 5.2 Hz, 2H); ^{13}C NMR δ 153.7, 146.2, 141.3, 138.6, 128.7, 126.4, 124.5, 122.1, 121.9, 121.5, 121.1, 120.7, 114.6, 112.0, 103.2, 61.5, 44.2. HPLC purity 100%. HRMS (ESI) calcd for $C_{17}H_{15}N_3O$ $[M-H]^-$ Exact Mass: 276.1137, found 276.1141.

5.1.2.9. 3-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)propan-1-ol (5i). Yield: 96%, mp: 199–201 °C; ^{1}H NMR δ 12.51 (s, 1H), 8.36 (d, J = 7.9 Hz, 1H), 8.26 (d, J = 7.8 Hz, 1H), 7.65 (dd, J = 13.0, 8.3 Hz, 2H), 7.50 (t, J = 7.4 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 7.29 (dd, J = 14.6, 7.3 Hz, 2H), 6.80 (s, 1H), 3.81 (m, 2H), 3.60 (t, J = 5.7 Hz, 2H), 1.89 (m, 2H); ^{13}C NMR δ 153.5, 146.0, 141.1, 138.5, 128.5, 126.1, 124.3, 121.9, 121.8, 121.2, 120.8, 120.5, 114.4, 111.8, 102.9, 59.4, 38.6, 32.9. HPLC purity 100%. HRMS (ESI) calcd for $C_{18}H_{17}N_3O$ $[M-H]^-$ Exact Mass: 290.1293, found 290.1296.

5.1.2.10. *N*-((Tetrahydrofuran-2-yl)methyl)-11*H*-indolo[3,2-*c*]quinolin-6-amine (5j). Yield: 42%, mp: 185–187 °C; ^{1}H NMR δ 12.48 (s, 1H), 8.30 (m, 1H), 8.25 (m, 1H), 7.67 (dd, J = 10.4, 5.0 Hz, 2H), 7.50 (t, J = 7.5 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.29 (dt, J = 17.5, 7.5 Hz, 2H), 6.49 (t, J = 5.3 Hz, 1H), 4.30 (m, 1H), 3.86 (m, 1H), 3.77 (dd, J = 7.8, 3.5 Hz, 2H), 3.66 (d, J = 7.3 Hz, 1H), 1.93 (m, 2H), 1.79 (m, 2H); ^{13}C NMR δ 153.3, 146.4, 141.2, 138.6, 129.0, 126.64, 124.7, 122.0, 121.8, 121.3, 120.9, 120.6, 114.6, 111.9, 103.0, 77.6, 67.6, 44.9, 29.1, 25.6. HPLC purity 100%. HRMS (ESI) calcd for $C_{20}H_{19}N_3O$ $[M-H]^-$ Exact Mass: 316.1450, found 316.1444.

5.1.2.11. *N*-Benzyl-11*H*-indolo[3,2-*c*]quinolin-6-amine (5k). Yield: 48%, mp: 205–207 °C; ^{1}H NMR δ 12.51 (s, 1H), 8.53 (d, J = 7.9 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.52 (d, J = 8.0 Hz, 2H), 7.44 (t, J = 7.6 Hz, 1H), 7.33–7.26 (m, 5H), 7.18 (t, J = 7.0 Hz, 1H), 5.01 (d, J = 5.8 Hz, 2H); ^{13}C NMR δ 153.0, 146.4, 142.0, 141.4, 138.6, 128.5 (3C), 127.9 (2C), 126.8, 126.7, 124.4, 122.0, 121.9, 121.3, 121.2, 120.6, 114.7, 111.9, 103.1, 44.0. HPLC purity 100%. HRMS (ESI) calcd for $C_{22}H_{17}N_3$ $[M-H]^-$ Exact Mass: 322.1344, found 322.1336.

5.1.2.12. *N*-(2,4-Dimethoxyphenyl)-11*H*-indolo[3,2-*c*]quinolin-6-amine (5l). Yield: 96%, mp: 220–221 °C; ^{1}H NMR δ 12.70 (s, 1H), 8.94 (d, J = 8.8 Hz, 1H), 8.34 (d, J = 7.6 Hz, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.97 (s, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.59 (dd, J = 11.1, 4.0 Hz, 1H), 7.50 (t, J = 7.5 Hz, 1H), 7.44 (t, J = 7.4 Hz, 1H), 7.40 (t, J = 7.4 Hz, 1H), 6.76 (d, J = 2.6 Hz, 1H), 6.65 (dd, J = 8.8, 2.6 Hz, 1H), 4.03 (s, 3H), 3.79 (s, 3H); ^{13}C NMR δ 155.1, 150.5, 149.7, 145.8, 141.4, 138.8, 128.9, 127.3, 124.9, 124.1, 122.6, 122.1, 121.4, 121.2, 120.5, 119.6, 115.1, 112.6, 104.6, 103.6, 99.2, 56.8, 55.8. HPLC purity 99.8%. HRMS (ESI) calcd for $C_{23}H_{19}N_3O$ $[M-H]^-$ Exact Mass: 368.1399, found 368.1398.

5.1.2.13. N^1-(2-Fluoro-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (5m). Yield: 87%, mp: 222–224 °C; ^{1}H NMR δ 8.42 (d, J = 8.0 Hz, 1H), 8.01 (dd, J = 9.6, 3.0 Hz, 1H), 7.65 (dd, J = 9.0, 5.3 Hz, 2H), 7.442 (m, 1H), 7.36 (m, 1H), 7.33 (m, 1H), 7.07 (s, 1H), 3.76 (d, J = 5.2 Hz, 2H), 2.73 (t, J = 6.2 Hz, 2H), 1.81 (m, 2H); ^{13}C NMR δ 156.2 (J = 237.1 Hz), 153.0, 143.4, 140.6 (J = 3.6 Hz), 138.5, 128.5 (J = 8.4 Hz,), 124.6, 121.7, 121.2, 120.7, 116.8 (J = 24.1 Hz), 114.4 (J = 9.6 Hz), 111.9, 106.2 (J = 23.1 Hz), 103.6, 40.5, 39.7, 32.9. HPLC purity 99.6%. HRMS (ESI) calcd for $C_{18}H_{17}FN_4$ $[M-H]^-$ Exact Mass: 307.1359, found 307.1364.

5.1.2.14. N^1-(2-Bromo-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (5n). Yield: 82%, mp: 181–183 °C; ^{1}H NMR δ 8.50 (s, 1H), 8.44 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 8.1 Hz, 1H), 7.58 (m, 2H), 7.42 (t, J = 7.5 Hz, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.25 (s, 1H), 3.79 (s, 2H), 2.75 (t, J = 6.1 Hz, 2H), 1.81 (m, 2H); ^{13}C NMR δ 153.7, 145.3, 140.0, 138.6, 1301.0, 128.640, 124.7, 124.2, 121.6, 121.2, 120.78, 116.1, 112.8, 112.0, 103.6, 40.5, 39.7, 32.8. HPLC purity 96.3%.

5.1.2.15. N^1-(2-Methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (5o). Yield: 61%, mp: 229–231 °C; ^{1}H NMR δ 8.38 (d, J = 7.8 Hz, 1H), 8.03 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.38 (t, J = 7.4 Hz, 1H), 7.31 (d, J = 8.2 Hz, 1H), 7.26 (t, J = 7.4 Hz, 1H), 3.76 (d, J = 5.8 Hz, 2H), 2.73 (t, J = 5.6 Hz, 2H), 2.46 (s, 3H), 1.84–1.76 (m, 2H); ^{13}C NMR δ 153.1, 144.8, 141.0, 138.6, 130.1, 129.9, 126.5, 124.2, 122.0, 121.4, 121.1, 120.5, 114.4, 111.9, 103.24, 40.5, 39.7, 33.0, 21.5. HPLC purity 96.2%. HRMS (ESI) calcd for $C_{19}H_{21}N_4$ $[M+H]^+$ Exact Mass: 305.1766, found 305.1745.

5.1.2.16. N^1-(2-Chloro-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (5p). Yield: 95%, mp: >230 °C decompose; ^{1}H NMR δ 8.45 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 2.2 Hz, 1H), 7.65 (dd, J = 15.5, 8.5 Hz, 2H), 7.48–7.40 (m, 2H), 7.30 (t, J = 7.5 Hz, 1H), 7.24 (s, 1H), 3.79 (d, J = 4.2 Hz, 2H), 2.75 (t, J = 6.1 Hz, 2H), 1.81 (m, J = 6.2 Hz, 2H); ^{13}C NMR δ 153.6, 145.1, 140.1, 138.6, 128.4, 128.3, 124.8, 124.7, 121.7, 121.2, 121.1, 120.8, 115.4, 112.0, 103.6, 40.5, 39.8, 32.9. HPLC purity 98.5%. HRMS (ESI) calcd for $C_{18}H_{18}ClN_4$ $[M+H]^+$ Exact Mass: 325.1220, found 325.1192.

5.1.2.17. N^1-(2-Chloro-11*H*-indolo[3,2-*c*]quinolin-6-yl)-2-methylpropane-1,3-diamine (5q). Yield: 97%, mp: 214–216 °C; ^{1}H NMR δ 8.42 (d, J = 8.0 Hz, 1H), 8.32 (d, J = 2.5 Hz, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.55 (s, 1H), 7.45 (dd, J = 8.8, 2.5 Hz, 1H), 7.44–7.40 (m, 1H), 7.32–7.25 (m, 1H), 3.77 (dd, J = 7.7, 5.3 Hz, 1H), 3.54 (dd, J = 8.5, 4.1 Hz, 1H), 2.71 (dd, J = 12.4, 4.6 Hz, 1H), 2.59 (dd, J = 12.4, 7.0 Hz, 1H), 1.98 (t, J = 3.9 Hz, 1H), 0.97 (d, J = 6.9 Hz, 3H); ^{13}C NMR δ 153.6, 145.1, 140.1, 138.6, 128.4, 128.3, 124.8, 124.7, 121.67, 121.1, 121.1, 120.8, 115.3, 112.0, 103.6, 47.4, 46.5, 35.6, 17.0. HPLC purity 99.5%. HRMS (ESI) calcd for $C_{19}H_{20}ClN_4$ $[M+H]^+$ Exact Mass: 339.1376, found 339.1352.

5.1.2.18. N^1-(2-Chloro-11*H*-indolo[3,2-*c*]quinolin-6-yl)-2,2-dimethylpropane-1,3-diamine (5r). Yield: 71%, mp: 226–228 °C; ^{1}H NMR δ 8.38 (d, J = 7.9 Hz, 1H), 8.38 (m, 1H), 7.81 (s, 1H), 7.66 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 8.9 Hz, 1H), 7.47–7.40 (m, 2H), 7.30 (t, J = 7.5 Hz, 1H), 3.63 (s, 2H), 2.55 (s, 2H), 0.99 (s, 6H); ^{13}C NMR δ 153.9, 145.1, 140.1, 138.7, 128.5, 128.2, 124.9, 124.8, 121.7, 1212, 121.1, 121.0, 115.4, 112.1, 103.7, 51.9, 50.6, 24.7,

14.6 (2C). HPLC purity 98.6%. HRMS (ESI) calcd for $C_{20}H_{22}ClN_4$ $[M+H]^+$ Exact Mass: 353.1533, found 353.1518.

5.1.2.19. N^1-(11-Methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamin (10a). Yield: 62%; ^{1}H NMR δ 8.52 (d, J = 8.2 Hz, 1H), 8.45 (d, J = 7.9 Hz, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.71 (d, J = 8.2 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.05 (s, 1H), 4.33 (d, J = 9.1 Hz, 3H), 3.79 (d, J = 5.6 Hz, 2H), 2.74 (t, J = 6.0 Hz, 2H), 1.82 (dd, J = 12.2, 6.0 Hz, 2H); ^{13}C NMR δ 153.2, 147.5, 140.6, 140.0, 128.2, 127.2, 124.4, 122.8, 121.1, 121.0, 120.9, 120.7, 115.1, 110.3, 103.4, 40.4, 39.7, 33.9, 32.9. HPLC purity 88.2%.

5.1.2.20. 2,2-Dimethyl-N^1-(11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (10b). Yield: 52%, mp: 127–129 °C; ^{1}H NMR δ 8.52 (d, J = 8.3 Hz, 1H), 8.42 (d, J = 7.9 Hz, 1H), 7.82 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.61 (s, 1H), 7.54–7.44 (m, 2H), 7.34 (t, J = 7.5 Hz, 1H), 7.27 (dd, J = 8.1, 7.0 Hz, 1H), 4.34 (s, 3H), 3.64 (s, 2H), 2.53 (s, 2H), 0.99 (s, 6H); ^{13}C NMR δ 153.4, 147.4, 140.5, 140.0, 128.1, 127.0, 124.4, 122.8, 121.0, 121.0, 120.8, 120.7, 115.1, 110.3, 103.4, 51.9, 50.4, 35.9, 33.9, 24.7 (2C). HPLC purity 99.2%.

5.1.2.21. N^1,N^1-Dimethyl-N^3-(11-methyl-11H-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamin (10c). Yield 82%, mp: 158–160 °C; ^{1}H NMR δ 8.53 (d, J = 8.3 Hz, 1H), 8.30 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 7.52 (d, J = 0.9 Hz, 1H), 7.47 (d, J = 7.3 Hz, 1H), 7.37 (d, J = 7.8 Hz, 1H), 7.28 (s, 1H), 7.15 (s, 1H), 4.35 (s, 3H), 3.75 (d, J = 5.6 Hz, 2H), 2.46 (t, J = 6.3 Hz, 2H), 2.25 (s, 6H), 1.88 (m, 2H); ^{13}C NMR δ 153.1, 147.5, 140.5, 139.9, 128.1, 127.3, 124.4, 122.8, 121.1, 120.9, 120.7, 120.4, 115.1, 110.4, 103.4, 58.9, 45.8 (2C), 41.1, 33.9, 26.6. HPLC purity 99.8%.

5.1.2.22. N^1-(2-Bromo-11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (10d). Yield: 58%, mp: 153–155 °C; ^{1}H NMR δ 8.55 (s, 1H), 8.46 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.61 (t, J = 8.1 Hz, 2H), 7.48 (d, J = 7.7 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 7.21 (s, 1H), 4.30 (s, 3H), 3.77 (d, J = 3.9 Hz, 2H), 2.74 (t, J = 6.2 Hz, 2H), 1.81 (m, 2H); ^{13}C NMR δ 153.4, 146.2, 140.0, 139.3, 130.8, 129.1, 124.8, 124.6, 121.1, 121.0, 120.3, 116.4, 112.9, 110.4, 103.7, 40.4, 39.8, 33.6, 32.6. HPLC purity 90.5%.

5.1.2.23. N^1-(2-Bromo-11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)-2,2-dimethylpropane-1,3-diamine (10e). Yield: 61%, mp: 217–219 °C; ^{1}H NMR δ 8.54 (s, 1H), 8.42 (d, J = 7.9 Hz, 1H), 7.81 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 6.1 Hz, 2H), 7.49 (t, J = 7.7 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H), 4.30 (s, 3H), 3.62 (s, 2H), 2.55 (s, 2H), 0.99 (s, 6H); ^{13}C NMR δ 153.6, 146.1, 140.0, 139.2, 130.8, 129.0, 124.7, 124.6, 121.2, 120.9, 120.4, 116.5, 112.8, 110.4, 103.8, 52.0, 50.6, 35.4, 33.6, 24.7 (2C). HPLC purity 93.1%.

5.1.2.24. N^1-(2-Bromo-11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)-N^3,N^3-dimethylpropane-1,3-diamine (10f). Yield: 53%, mp: 132–134 °C; NMR δ 8.56 (d, J = 1.7 Hz, 1H), 8.30 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.65 (m, 2H), 7.49 (d, J = 7.3 Hz, 1H), 7.38 (d, J = 7.9 Hz, 1H), 7.28 (s, 1H), 4.32 (s, 3H), 3.74 (d, J = 5.3 Hz, 2H), 2.46 (t, J = 6.3 Hz, 2H), 2.24 (s, 6H), 1.81 (m, 2H); ^{13}C NMR δ 153.4, 146.3, 140.0, 139.3, 130.9, 129.3, 124.9, 124.7, 121.2, 120.6, 120.4, 116.5, 113.0, 110.6, 103.8, 58.9, 45.9 (2C), 41.15, 33.7, 26.5. HPLC purity 93.0%.

5.1.2.25. N^1-(2-Chloro-11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (10g). Yiel: 67%, mp: 171–173 °C; ^{1}H NMR δ 8.46 (d, J = 7.9 Hz, 1H), 8.43 (s, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.49 (m, 2H), 7.34 (t, J = 7.4 Hz, 1H), 7.20 (s, 1H), 4.31 (s, 3H), 3.78 (d, J = 5.3 Hz, 2H), 2.74 (t, J = 6.1 Hz, 2H), 1.81 (m, 2H); ^{13}C NMR δ 153.4, 146.0, 140.0, 139.5, 128.9, 128.2, 124.9, 124.8, 121.7, 121.2, 121.1, 120.4, 115.8, 110.5, 103.8, 40.6, 39.7, 33.6, 32.8. HPLC purity 98.0%.

5.1.2.26. N^1-(2-Chloro-11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)-2,2-dimethylpropane-1,3-diamine (10h). Yield: 67%, mp: 180–182 °C; ^{1}H NMR δ 8.46 (d, J = 2.3 Hz, 1H), 8.44 (d, J = 7.9 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.9 Hz, 1H), 7.51 (dd, J = 6.5, 2.4 Hz, 2H), 7.35 (s, 1H), 4.35 (s, 3H), 3.62 (d, J = 4.4 Hz, 2H), 2.54 (s, 2H), 0.99 (s, 6H); ^{13}C NMR δ 153.6, 146.0, 140.1, 139.4, 128.7, 128.2, 124.8, 124.8, 121.7, 121.3, 120.9, 120.5, 115.8, 110.5, 103.9, 52.1, 50.7, 35.5, 33.6, 24.7 (2C). HPLC purity 96.5%.

5.1.2.27. N^1-(2-Chloro-11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)-N^3,N^3-dimethylpropane-1,3-diamine (10i). Yield: 61%, mp: 119–121 °C; ^{1}H NMR δ 8.42 (d, J = 2.3 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.9 Hz, 1H), 7.49 (dd, J = 8.9, 2.4 Hz, 2H), 7.37 (s, 1H), 7.26 (s, 1H), 4.31 (s, 3H), 3.73 (d, J = 5.2 Hz, 2H), 2.45 (t, J = 6.3 Hz, 2H), 2.24 (s, 6H), 1.90–1.83 (m, 2H); ^{13}C NMR δ 153.3, 146.0, 140.0, 139.4, 128.9, 128.2, 124.9, 124.8, 121.7, 121.1, 120.5, 120.4, 115.7, 110.5, 103.8, 58.8, 45.8(2C), 41.1, 33.6, 26.5. HPLC purity 100%.

5.1.2.28. N^1-(2-Fluoro-11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (10j). Yield: 36%, mp: 116–118 °C; ^{1}H NMR δ 8.44 (d, J = 7.9 Hz, 1H), 8.18 (dd, J = 10.8, 2.4 Hz, 1H), 7.77 (t, J = 10.0 Hz, 1H), 7.70 (dd, J = 9.1, 5.8 Hz, 1H), 7.45 (dd, J = 16.9, 9.1 Hz, 1H), 7.36 (ddd, J = 10.7, 8.5, 2.6 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 4.29 (d, J = 9.8 Hz, 3H), 3.75 (t, J = 5.9 Hz, 2H), 2.74 (t, J = 6.1 Hz, 2H), 1.86–1.78 (m, 2H); ^{13}C NMR δ 156.1(J = 237.1 Hz), 152.8, 144.2, 140.0, 139.9(J = 3.0 Hz), 128.8 (J = 9.1 Hz), 124.7, 121.2, 121.0, 120.4, 116.6 (J = 24.1 Hz), 114.6 (J = 10.6 Hz) 110.3, 107.3 (J = 24.2 Hz), 103.8, 40.0, 39.5, 33.4, 32.3. HPLC purity 99.2%.

5.1.2.29. N^1-(2,11-Dimethyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamin (10k). Yield: 46%, mp: 112–114 °C; ^{1}H NMR δ 8.45 (d, J = 7.8 Hz, 1H), 8.32 (s, 1H), 7.82 (d, J = 8.3 Hz, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 7.39–7.32 (m, 2H), 4.37 (s, 3H), 3.78 (t, J = 6.1 Hz, 2H), 2.75 (t, J = 5.8 Hz, 2H), 2.51 (s, 3H), 1.86–1.79 (m, 2H); ^{13}C NMR δ 152.8, 145.5, 140.3, 139.9, 129.9, 129.7, 126.9, 124.3, 121.9, 121.0, 120.8, 120.7, 114.9, 110.1, 103.4, 39.1, 33.8, 31.9, 22.9, 21.5. HPLC purity 99.1%.

5.1.2.30. N^1-(2-Methoxy-11-methyl-11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (10l). Yield: 53%, mp: 79–81 °C; ^{1}H NMR δ 8.43 (d, J = 7.9 Hz, 1H), 7.90 (s, 1H), 7.80 (m, 1H), 7.65 (d, J = 9.0 Hz, 1H), 7.47 (s, 1H), 7.31 (s, 1H), 7.20 (m, 1H), 6.87 (m, 1H), 4.36 (d, J = 4.3 Hz, 3H), 3.91 (s, 3H), 3.74 (s, 2H), 2.73 (t, J = 6.2 Hz, 2H), 1.881 (m, 2H); ^{13}C NMR δ 153.8, 152.1, 142.5, 140.2, 140.0, 128.5, 124.4, 121.1, 120.8, 120.7, 118.0, 115.0, 110.2, 103.9, 103.8, 55.8, 40.5, 39.7, 33.6, 32.9. HPLC purity 98.4%.

5.1.3. General procedure for the synthesis of compounds 6 and 11

11*H*-Indolo[3,2-*c*]quinolin-6-amine (**5**, 30 mg) and was completely dissolved in THF (1 mL), and then a solution of isocyanate (1.2 equiv) and THF (1 mL) were added drop by drop under stirring at room temperature for 2–6 h. TLC monitoring was used to ensure complete of the reaction. After reaction was finished, the reaction mixture was evaporated to dryness. The crude product was purified by flash chromatography using *n*-hexane-AcOEt (1:1) as rhe eluent to yield pure products.

5.1.3.1. 1-(6-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)hexyl)-3-phenylurea (6a). Yield: 61%; ^{1}H NMR δ 12.40 (s, 1H), 8.37 (m, 2H), 8.22 (m, 1H), 7.63(m, 2H), 7.45 (m, 1H), 7.41–7.30 (m, 3H), 7.25 (dt, *J* = 19.4, 7.4 Hz, 2H), 7.18 (dd, *J* = 10.3, 5.1 Hz, 2H), 6.84 (d, *J* = 6.9 Hz, 1H), 6.56 (s, 1H), 6.10 (s, 1H), 3.69 (d, *J* = 5.5 Hz, 2H), 3.08 (d, *J* = 5.7 Hz, 2H), 1.76 (d, *J* = 5.8 Hz, 2H), 1.48–1.35 (m, 6H); ^{13}C NMR δ 155.6, 153.4, 146.5, 141.1, 141.0, 138.5, 129.1 (3C), 128.4, 126.7, 124.3, 121.9, 121.3, 121.2, 121.1, 120.5, 118.0 (2C), 114.5, 111.8, 103.2, 40.9, 39.5, 30.3, 29.8, 26.9, 26.8. HPLC purity 94.5%. HRMS (ESI) calcd for $C_{28}H_{30}N_5O$ $[M+H]^+$ Exact Mass: 452.2450, found 452.2437.

5.1.3.2. 1-(3-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)propyl)-3-phenylthiourea (6b). Yield: 84%; ^{1}H NMR δ 12.50 (s, 1H), 9.64 (s, 1H), 8.43 (d, *J* = 8.0 Hz, 1H), 8.24 (d, *J* = 7.9 Hz, 1H), 8.00 (m, 1H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.50 (m, 1H), 7.48–7.38 (m, 4H), 7.29 (ddd, *J* = 22.0, 15.2, 7.5 Hz, 4H), 7.10 (t, *J* = 7.4 Hz, 1H), 6.79 (m, 1H), 3.79 (dd, *J* = 12.2, 6.1 Hz, 2H), 3.69 (s, 2H), 2.01 (m, 2H); ^{13}C NMR δ 180.7, 153.3, 141.2, 139.5, 138.5 (2C), 129.2, 128.5, 126.4, 124.8, 124.4, 123.7, 122.0, 121.8 (2C), 121.4, 121.2, 120.6, 114.5, 111.9, 103.0, 42.2, 38.33, 29.9. HPLC purity 99.4%. HRMS (ESI) calcd for $C_{25}H_{24}N_5S$ $[M+H]^+$ Exact Mass: 426.1752, found 426.1731.

5.1.3.3. 1-(3-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)propyl)-3-phenylurea (6c). Yield: 98%; ^{1}H NMR δ 12.47 (s, 1H), 8.61 (s, 1H), 8.49 (d, *J* = 7.9 Hz, 1H), 8.28 (d, *J* = 7.9 Hz, 1H), 7.73 (d, *J* = 8.3 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.48 (m, 3H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.29 (dt, *J* = 19.3, 7.5 Hz, 2H), 7.24 (t, *J* = 7.1 Hz, 2H), 6.90 (dd, *J* = 10.7, 4.0 Hz, 1H), 6.79 (t, *J* = 5.0 Hz, 1H), 6.39 (t, *J* = 5.4 Hz, 1H), 3.82 (dd, *J* = 11.8, 5.7 Hz, 2H), 3.32 (dd, *J* = 11.9, 5.8 Hz, 2H), 1.93 (dd, *J* = 12.5, 6.2 Hz, 2H); ^{13}C NMR δ 156.2, 153.4, 146.5, 141.2, 141.1, 138.6, 129.1 (2C), 128.4, 126.7, 124.4, 122.0, 121.9, 121.5, 121.2, 121.1, 120.6, 118.2 (2C), 114.6, 111.9, 103.2, 38.1, 37.3, 30.9. HPLC purity 98.1%. HRMS (ESI) calcd for $C_{25}H_{24}N_5O$ $[M+H]^+$ Exact Mass: 410.1981, found 410.1963.

5.1.3.4. 1-(3-(2-Fluoro-11*H*-indolo[3,2-*c*]quinolin-6-ylamino) propyl)-3-phenylurea (6d). Yield: 83%, mp: 207–209 °C; ^{1}H NMR δ 12.46 (s, 1H), 8.57 (s, 1H), 8.47 (d, *J* = 8.0 Hz, 1H), 8.02 (dd, *J* = 9.6, 2.9 Hz, 1H), 7.72 (dd, *J* = 9.1, 5.4 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.43 (d, *J* = 7.6 Hz, 3H), 7.34 (dd, *J* = 10.3, 7.3 Hz, 1H), 7.32–7.29 (m, 1H), 7.22 (q, *J* = 5.8 Hz, 2H), 6.88 (dd, *J* = 11.6, 4.2 Hz, 1H), 6.76 (s, 1H), 6.34 (s, 1H), 3.76 (q, *J* = 6.4 Hz, 2H), 3.27 (q, *J* = 6.4 Hz, 2H), 1.90–1.86 (m, 2H); ^{13}C NMR δ 156.2 (*J* = 237.1 Hz), 156.11, 153.0, 143.3, 141.0, 140.6 (*J* = 3.6 Hz), 138.6, 129.1(2C), 128.5 (*J* = 8.4 Hz), 124.6, 121.7, 121.5, 121.3, 120.7, 118.2(2C), 116.8 (*J* = 24.1 Hz), 114.4 (*J* = 9.6 Hz), 111.9, 106.2 (*J* = 23.1 Hz), 103.6, 38.1, 37.3, 30.7. HPLC purity 98.4%. HRMS (ESI) calcd for $C_{25}H_{23}FN_5O$ $[M+H]^+$ Exact Mass: 428.1887, found 428.1873.

5.1.3.5. 1-(3-(1-Bromo-11*H*-indolo[3,2-*c*]quinolin-6-ylamino) propyl)-3-phenylurea (6e). Yield: 86%, mp: >230 °C decompose; ^{1}H NMR δ 11.77 (s, 1H), 8.57 (s, 1H), 8.51 (d, *J* = 8.0 Hz, 1H), 7.99 (d, *J* = 8.2 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.48–7.41 (m, 3H), 7.37 (m, 2H), 7.22 (t, *J* = 7.5 Hz, 2H), 6.96 (s, 1H), 6.88 (m, 1H), 6.32 (s, 1H), 3.78 (d, *J* = 6.2 Hz, 2H), 3.27 (d, *J* = 6.2 Hz, 2H), 1.89 (m, 2H); ^{13}C NMR δ 156.2, 153.5, 148.5, 141.1, 138.7, 138.5, 129.1 (2C), 128.7, 127.0, 126.3, 124.9, 121.5, 121.2, 1209, 120.7, 118.2 (2C), 115.9, 114.1, 113.5, 104.8, 38.2, 37.3, 30.8. HPLC purity 99.8%. HRMS (ESI) calcd for $C_{25}H_{23}BrN_5O$ $[M+H]^+$ Exact Mass: 488.1086, found 488.1064.

5.1.3.6. 1-(3-(2-Bromo-11*H*-indolo[3,2-*c*]quinolin-6-ylamino) propyl)-3-phenylurea (6f). Yield: 79%, mp: 126–128 °C; ^{1}H NMR δ 12.52 (s, 1H), 8.57 (s, 1H), 8.48 (m, 2H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.56 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 3H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.22 (t, *J* = 7.9 Hz, 2H), 6.88 (t, *J* = 7.3 Hz, 2H), 6.33 (s, 1H), 3.76 (d, *J* = 6.1 Hz, 2H), 3.26 (d, *J* = 6.2 Hz, 2H), 1.88 (m, 2H); ^{13}C NMR δ 156.1, 153.7, 145.2, 141.0, 140.0, 138.6, 131.0, 129.1 (2C), 128.8, 124.8, 124.2, 121.6, 121.5, 121.3, 120.8, 118.2 (2C), 116.1, 112.9, 112.0, 103.6, 38.1, 37.2, 30.7. HPLC purity 91.3%. HRMS (ESI) calcd for $C_{25}H_{23}BrN_5O$ $[M+H]^+$ Exact Mass: 488.1086, found 488.1057.

5.1.3.7. 1-(3-(2-Methyl-11*H*-indolo[3,2-*c*]quinolin-6-ylamino) propyl)-3-phenylurea (6g). Yield: 76%, mp: 140–142 °C; ^{1}H NMR δ 12.37 (s, 1H), 8.57 (s, 1H), 8.43 (d, *J* = 7.8 Hz, 1H), 8.04 (s, 1H), 7.62 (dd, *J* = 17.8, 8.2 Hz, 2H), 7.43 (d, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.22 (t, *J* = 7.8 Hz, 2H), 6.88 (t, *J* = 7.3 Hz, 1H), 6.64 (d, *J* = 5.0 Hz, 1H), 6.35 (d, *J* = 5.1 Hz, 1H), 3.76 (d, *J* = 5.9 Hz, 2H), 3.27 (d, *J* = 6.0 Hz, 2H), 2.47 (s, 3H), 1.91–1.84 (m, 2H); ^{13}C NMR δ 156.1, 153.0, 144.7, 141.0, 140.9, 138.5, 130.1, 123.0, 129.1(2C), 126.6, 124.2, 121.9, 121.4, 121.3, 121.1, 120.4, 118.2(2C), 114.3, 111.8, 103.3, 38.1, 37.3, 30.8, 215. HPLC purity 96.3%. HRMS (ESI) calcd for $C_{26}H_{26}N_5O$ $[M+H]^+$ Exact Mass: 424.2137, found 424.2115.

5.1.3.8. 1-(3-(2-Methoxy-11*H*-indolo[3,2-*c*]quinolin-6-ylamino) propyl)-3-phenylurea (6h). Yield: 85%, mp: 129–131 °C; ^{1}H NMR δ 12.38 (s, 1H), 8.58 (d, *J* = 3.7 Hz, 1H), 8.44 (d, *J* = 7.6 Hz, 1H), 7.78 (s, 1H), 7.65 (d, *J* = 7.9 Hz, 2H), 7.46–7.38 (m, 3H), 7.28 (s, 1H), 7.22 (t, *J* = 7.8 Hz, 2H), 7.14 (d, *J* = 9.0 Hz, 1H), 6.89 (d, *J* = 7.4 Hz, 1H), 6.59 (m, 1H), 6.36 (d, *J* = 5.0 Hz, 1H), 3.89 (s, 3H), 3.74 (d, *J* = 5.0 Hz, 2H), 3.27 (m, 2H), 1.88 (dd, *J* = 5.9, 3.9 Hz, 2H); ^{13}C NMR δ 156.2, 154.3, 152.1, 141.1, 141.0, 138.6, 129.1 (2C), 128.0, 124.5, 122.0, 121.5, 121.3, 120.5, 118.9, 118.2 (2C), 118.1, 114.6, 111.8, 103.5, 102.6, 55.9, 38.2, 37.3, 30.6. HPLC purity 95.7%. HRMS (ESI) calcd for $C_{26}H_{26}N_5O_2$ $[M+H]^+$ Exact Mass: 440.2087, found 440.2047.

5.1.3.9. 1-(3-(2-Nitro-11*H*-indolo[3,2-*c*]quinolin-6-ylamino)propyl)-3-phenylurea (6i). Yield: 60%, mp: >300 °C decompose; ^{1}H NMR δ 12.92 (s, 1H), 9.33 (d, *J* = 2.6 Hz, 1H), 8.58 (s, 1H), 8.54 (d, *J* = 8.0 Hz, 1H), 8.23 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.71 (dd, *J* = 17.8, 8.6 Hz, 2H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.41 (dd, *J* = 13.1, 6.8 Hz, 3H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.22 (t, *J* = 7.9 Hz, 2H), 6.88 (t, *J* = 7.3 Hz, 1H), 6.32 (t, *J* = 5.8 Hz, 1H), 3.83 (q, *J* = 6.4 Hz, 2H), 3.26 (q, *J* = 6.4 Hz, 2H), 1.92–1.85 (m, 2H); ^{13}C NMR δ 156.1, 155.5, 150.6, 141.2, 141.0, 140.4, 138.6, 129.1 (2C), 1271, 125.2, 122.6, 121.5, 121.4, 121.3, 121.2, 119.5, 118.2 (2C), 113.4, 112.3, 103.6, 38.3, 37.2, 30.7. HPLC purity 95.6%. HRMS (ESI) calcd for $C_{25}H_{23}N_6O_2$ $[M+H]^+$ Exact Mass: 455.1832, found 455.1799.

5.1.3.10. 1-(3-(2-Chloro-11*H*-indolo[3,2-*c*]quinolin-6-ylamino) propyl)-3-phenylurea (6j). Yield: 93%; ^{1}H NMR δ 12.51 (s, 1H), 8.58 (s, 1H), 8.49 (d, *J* = 7.9 Hz, 1H), 8.34 (d, *J* = 2.3 Hz, 1H), 7.66 (m, 2H), 7.44 (dd, *J* = 14.9, 8.1 Hz, 4H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.22 (t, *J* = 7.7 Hz, 2H), 6.88 (t, *J* = 7.0 Hz, 2H), 6.34 (t, *J* = 5.4 Hz, 1H), 3.78 (m, 2H), 3.328 (m, 2H), 1.88 (m, 2H); ^{13}C NMR δ 156.1, 153.6, 145.0, 141.0, 140.0, 138.6, 129.0 (2C), 128.5, 128.4, 125.0, 124.8, 121.6, 121.4, 121.3, 121.1, 120.8, 118.2 (2C), 115.4, 112.0, 103.7, 38.1, 37.2, 30.7. HPLC purity 99.8%. HRMS (ESI) calcd for $C_{25}H_{23}ClN_5O$ $[M+H]^+$ Exact Mass: 444.1591, found 444.1573.

5.1.3.11. 1-(3-(2-Chloro-11*H*-indolo[3,2-*c*]quinolin-6-ylamino)-2-methylpropyl)-3-phenylurea (6k). Yield: 78%, mp: 143–145 °C; ^{1}H NMR δ 12.50 (s, 1H), 8.58 (s, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 8.32 (d, *J* = 2.5 Hz, 1H), 7.67–7.63 (m, 2H), 7.45–7.40 (m, 4H), 7.33–7.27 (m, 1H), 7.24–7.17 (m, 2H), 6.93 (t, *J* = 6.1 Hz, 1H),

6.89–6.85 (m, 1H), 6.42 (t, J = 6.1 Hz, 1H), 3.69 (dd, J = 12.6, 6.6 Hz, 1H), 3.62–3.55 (m, 1H), 3.18 (qd, J = 13.7, 7.8 Hz, 2H), 2.10 (dd, J = 12.7, 6.8 Hz, 1H), 0.96 (d, J = 6.8 Hz, 3H); ^{13}C NMR δ 156.4, 153.7, 145.0, 141.0, 140.2, 138.6, 129.2(2C), 128.5, 128.4, 124.9, 124.8, 121.6, 121.5, 121.2, 121.1, 120.8, 118.2(2C), 115.4, 112.0, 103.6, 43.6, 42.8, 34.5, 16.3. HPLC purity 100%. HRMS (ESI) calcd for $C_{26}H_{25}ClN_5O$ $[M+H]^+$ Exact Mass: 458.1748, found 458.1726.

5.1.3.12. 1-(3-(2-Chloro-11*H*-indolo[3,2-*c*]quinolin-6-ylamino)-2,2-dimethylpropyl)-3-phenylurea (6l). Yield: 84%, mp: 138–140 °C; ^{1}H NMR δ 12.52 (s, 1H), 8.71 (s, 1H), 8.65 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 2.0 Hz, 1H), 7.70–7.64 (m, 2H), 7.46 (dd, J = 17.4, 8.2 Hz, 4H), 7.34 (t, J = 7.5 Hz, 1H), 7.24 (t, J = 7.9 Hz, 2H), 7.06 (s, 1H), 6.90 (t, J = 7.3 Hz, 1H), 6.68 (t, J = 6.0 Hz, 1H), 3.64 (d, J = 6.3 Hz, 2H), 3.10 (d, J = 6.4 Hz, 2H), 0.95 (s, 6H); ^{13}C NMR δ 156.8, 154.0, 144.9, 140.9, 140.2, 138.6, 129.1(2C), 128.4, 128.3, 124.9, 124.8, 121.7, 121.6, 121.0(2C), 120.9, 118.3(2C), 115.4, 112.0, 103.5, 46.8, 46.1, 37.2, 24.0(2C). HPLC purity 98.0%. HRMS (ESI) calcd for $C_{27}H_{27}ClN_5O$ $[M+H]^+$ Exact Mass: 472.1904, found 472.1863.

5.1.3.13. 1-(3-(11-Methyl-11*H*-indolo[3,2-*c*]quinolin-6-ylamino) propyl)-3-phenylurea (11). Yield: 72%, mp: 218–220 °C; ^{1}H NMR δ 8.56 (s, 1H), 8.54 (d, J = 8.3 Hz, 1H), 8.49 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.50 (dd, J = 16.2, 8.2 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.34 (t, J = 7.5 Hz, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.7 Hz, 2H), 6.88 (t, J = 7.3 Hz, 1H), 6.74 (d, J = 5.6 Hz, 1H), 6.33 (d, J = 5.7 Hz, 1H), 4.36 (s, 3H), 3.77 (q, J = 6.3 Hz, 2H), 3.27 (dd, J = 12.5, 6.3 Hz, 2H), 1.88 (m, 2H); ^{13}C NMR δ 1561, 153.2, 147.4, 141.1, 140.6, 134.0, 129.1(2C), 128.1, 127.3, 124.4, 122.8, 121.4, 121.2, 121.0, 120.9, 120.7, 118.2(2C), 115.1, 110.3, 103.4, 38.1, 37.3, 33.9, 30.8. HPLC purity 100%.

5.1.4. General procedure for the synthesis of compound 7

N^1-(11*H*-indolo[3,2-*c*]quinolin-6-yl)propane-1,3-diamine (**5a**, 30 mg) and was completely dissolved in THF (1 mL), and then a solution of arenesulfonyl chloride (1.2 equiv) and THF (1 mL) were added drop by drop with stirring, and finally 2.0 equiv of triethylamine was added, the reaction was carried out at room temperature for 2–6 h. TLC monitoring was used to ensure complete of the reaction. After reaction was finished, the crude product was purified by flash chromatography using hexane/AcOEt (1:1) as the eluent to yield pure products.

5.1.4.1. *N*-(3-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)propyl) methanesulfonamide (7a). Yield: 66%, mp: 193–2195 °C; ^{1}H NMR δ 12.51 (s, 1H), 8.41 (d, J = 7.9 Hz, 1H), 8.26 (d, J = 7.9 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.66 (d, J = 8.1 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.44 (dt, J = 15.2, 6.8 Hz, 2H), 7.30 (dd, J = 15.4, 7.8 Hz, 2H), 6.68 (m, 1H), 3.80 (dd, J = 12.0, 5.9 Hz, 2H), 3.10 (q, J = 6.2 Hz, 2H), 2.85 (d, J = 1.2 Hz, 3H), 1.97 (m, 2H); ^{13}C NMR δ 153.5, 146.2, 141.3, 138.6, 128.7, 126.3, 124.5, 122.1, 121.9, 121.5, 121.2, 120.7, 114.6, 112.0, 103.1, 40.7, 39.8, 38.2, 30.4. HPLC purity 98.8%. HRMS (ESI) calcd for $C_{19}H_{21}N_4O_2S$ $[M+H]^+$ Exact Mass: 369.1385, found 369.1359.

5.1.4.2. *N*-(3-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)propyl)benzenesulfonamide (7b). Yield: 91%, mp: 88–90 °C; ^{1}H NMR δ 12.46 (s, 1H), 8.34 (d, J = 8.0 Hz, 1H), 8.24 (dd, J = 8.0, 1.2 Hz, 1H), 7.96 (t, J = 6.1 Hz, 1H), 7.71 (dd, J = 5.2, 3.3 Hz, 2H), 7.65 (dd, J = 16.0, 8.1 Hz, 2H), 7.54–7.49 (m, 2H), 7.44 (dd, J = 10.7, 4.8 Hz, 2H), 7.42–7.38 (m, 1H), 7.28 (dd, J = 7.9, 7.1 Hz, 2H), 6.59 (d, J = 5.9 Hz, 1H), 3.69 (q, J = 6.3 Hz, 2H), 2.89 (q, J = 6.7 Hz, 2H), 1.87–1.80 (m, 2H); ^{13}C NMR δ 153.4, 146.2, 141.2, 140.9, 138.5, 132.7, 129.5(2C), 128.6, 126.8(2C), 126.5, 124.4, 122.0, 121.8, 121.4, 121.0, 120.6, 114.5, 111.9, 103.0, 40.9, 38.0, 30.0. HPLC purity 100%. HRMS (ESI) calcd for $C_{24}H_{23}N_4O_2S$ $[M+H]^+$ Exact Mass: 431.1542, found 431.1519.

5.1.5. General procedure for the synthesis of compound 8

11*H*-Indolo[3,2-*c*]quinolin-6-amine (**5a**, 30 mg) and was completely dissolved in THF (1 mL), and then a solution of acyl chloride (1.2 equiv) and THF (1 mL) were added drop by drop with stirring, and finally 2.0 equiv of triethylamine was added, the reaction was carried out at room temperature for 2–6 h. TLC monitoring was used to ensure complete of the reaction. After reaction was finished, the crude product was purified by flash chromatography using hexane-AcOEt (1:1) as the eluent to yield pure products.

5.1.5.1. 3-(3-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)propyl)-1,1-diethylurea (8a). Yield: 78%, mp: 83–85 °C; ^{1}H NMR δ 12.44 (s, 1H), 8.49 (d, J = 7.9 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.48 (t, J = 7.6 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.32–7.16 (m, 2H), 6.88 (s, 1H), 6.32 (t, J = 4.9 Hz, 1H), 3.73 (dd, J = 11.2, 5.5 Hz, 2H), 3.28–3.17 (m, 6H), 1.82 (dd, J = 11.9, 5.8 Hz, 2H), 1.08–0.94 (m, 6H); ^{13}C NMR δ 157.7, 153.3, 146.5, 141.2, 138.5, 128.4, 126.5, 124.3, 121.9(2C), 121.1(2C), 120.6, 114.5, 111.8, 103.2, 40.6(2C), 37.9, 37.8, 31.0, 14.3(2C). HPLC purity 97.1%.

5.1.5.2. *N*-(3-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)propyl)thiophene-2-carboxamide (8b). Yield: 90%, mp: 200–202 °C; ^{1}H NMR δ 12.47 (s, 1H), 8.64 (t, J = 5.4 Hz, 1H), 8.45 (d, J = 7.9 Hz, 1H), 8.26 (d, J = 7.9 Hz, 1H), 7.80–7.70 (m, 2H), 7.66 (t, J = 7.8 Hz, 2H), 7.48 (dd, J = 8.2, 7.0 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.29 (dt, J = 17.9, 7.5 Hz, 2H), 7.14 (ddd, J = 8.8, 5.7, 3.2 Hz, 1H), 6.75 (s, 1H), 3.79 (dd, J = 12.1, 6.1 Hz, 2H), 3.46–3.43 (m, 2H), 1.99 (dt, J = 6.1, 3.9 Hz, 2H); ^{13}C NMR δ 161.8, 153.3, 146.4, 141.2, 140.6, 138.5, 131.0, 128.5, 128.3(2C), 126.6, 124.4, 122.0, 121.9, 121.2, 121.4, 120.6, 114.5, 111.9, 103.2, 38.3, 37.5, 30.2. HPLC purity 96.5%.

5.2. Biological testing assay

5.2.1. Activity against *Plasmodium falciparum*

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay,[58,59] using the chloroquine and pyrimethamine resistant *P. falciparum* K1 strain that originate from Thailand (Thaitong et al. 1983)[60] and strain susceptible to known antimalarial drugs (*P. falciparum* NF54) (Ponnudurai et al. 1981),[61] 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, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression (Huber 1993)[62] using Microsoft Excel.

5.2.2. 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).[63,64] 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 IC50 values were calculated by linear regression (Huber 1993)[62] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

5.2.3. Detergent mediated assay for β-haematin inhibition

The β-haematin formation inhibition assay method described by Carter et al.[60,64] 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 0–1000 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 1M 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.[65] 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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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.03.030.

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