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Synthesis and in Vitro Antimalarial Testing of Neocryptolepines: SAR Study for Improved Activity by Introduction and Modifications of Side Chains at C2 and C11 on Indolo[2,3-*b*]quinolines

Zhen-Wu Mei,[†] Li Wang,[†] Wen-Jie Lu,[†] Cui-Qing Pang,[†] Tsukasa Maeda,[†] Wei Peng,[†] Marcel Kaiser,[§,∥] Ibrahim El Sayed,[†,‡] and Tsutomu Inokuchi*[,†]

[†]Division of Chemistry and Biotechnology, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan

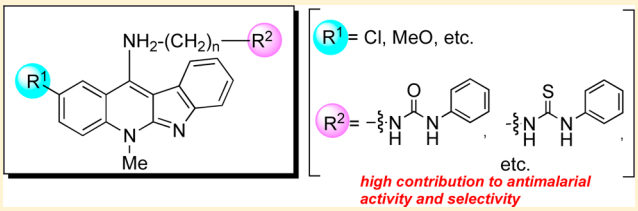
[‡]Chemistry Department, Faculty of Science, El Menoufeia University, Shebin El Koom, Egypt

[§]Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4002 Basel, Switzerland

[∥]University Basel, Petersplatz 1, CH-4003 Basel, Switzerland

Supporting Information

ABSTRACT: To obtain a high antimalarial activity with neocryptolepine derivatives, modifying and changing the side chains at the C11 position with varying the substituents of an electron-withdrawing or electron-donating nature at the C2 position for a SAR study were executed. Installation of alkylamino and ω-aminoalkylamino groups at the C11 position of the neocryptolepine core was successful. For further variation, the aminoalkylamino substituents were transformed into the corresponding acyclic or cyclic carbamides or thiocarbamides. These side chain modified neocryptolepine derivatives were tested for antimalarial activity against CQR (K1) and CQS (NF54) of *Plasmodium falciparum* in vitro and for cytotoxicity toward mammalian L6 cells. Among the tested compounds, the compound **17f** showed an IC_{50} of 2.2 nM for CQS (NF54) and a selectivity index of 1400, and **17i** showed an IC_{50} of 2.2 nM for CQR (K1), a selectivity index of 1243, and a resistance index of 0.5.



INTRODUCTION

Malaria is still one of the most frightening parasitic diseases in the tropical and subtropical regions where both developing and nonindustrialized nations are confronted with them. 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 nonavailability of proper treatment, involving mostly children under 5 years old.[1] These facts justify its classification as a dreaded infectious disease along with tuberculosis and AIDS.[2,3] Malaria is caused by five main species of malaria parasites, which are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*, and the most serious form is the *P. falciparum*. Fortunately, chloroquine (CQ), which was discovered in the 1940s, is used as the main antimalarial drug until today because of its remarkable therapeutic effect as an antimalarial drug and its low cost.[4] However, the spread of *P. falciparum* strains resistant to CQ is dramatically increasing over these years in the endemic areas. Despite the introduction of the artemisinin-based combination therapies (ACTs), the development of new chemotherapeutic agents for the treatment of malaria is still urgently needed to help to ensure the availability of new compounds to feed the preclinical pipeline.[5]

Plants are still an important resource for the discovery of new drugs. The potential of natural compounds as a new candidate of drugs is demonstrated by the introduction of artemisinin and its derivatives as antimalarial agents.[6] These important roles of natural products isolated from plants have stimulated us to evaluate natural resources in endemic areas. Many naturally occurring compounds, including the major alkaloid cryptolepine and minor alkaloid neocryptolepine, were isolated from the roots of the West African plants *Cryptolepis sanguinolenta* (Figure 1).[7−9] Both of the two tetracyclic heteroaromatic compounds are linearly fused indoloquinolines and exhibit a promising antiplasmodial activity both against chloroquine-sensitive (CQS) and chloroquine-resistant (CQR) *P. falcipa-*

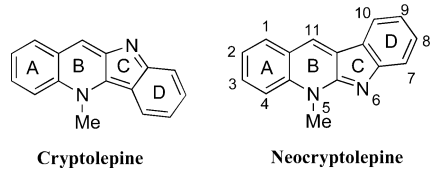


Figure 1. Structures of cryptolepine and neocryptolepine.

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rum.[10,11] Further experiments have also indicated that cryptolepine inhibits the β-hematin formation which is responsible for the treatment of malaria infections and also has a cytotoxicity due to a DNA intercalation activity.[12,13] Therefore, we selected neocryptolepine as the lead compound for the development of new antimalarial agents because of its lower affinity for DNA intercalation and topoisomerase II compared to cryptolepine.[14]

On the other hand, during the study of the structure−activity relationship (SAR) of CQ, the importance of the 4-amino-pyridine substructure for hematin binding and antimalarial activity was illustrated by both experimental and molecular modeling studies by Cheruku et al.[15] They also suggested that sufficiently large changes in the side chain alone could overcome the chloroquine resistance without having to make changes in the 4-amino-7-haloquinoline template responsible for the Fe(III)PPIX complexation and inhibition of β-hematin formation.[16] Because various methodical modifications of the CQ side chain have been reported to date (Figure 2),[17−19] a

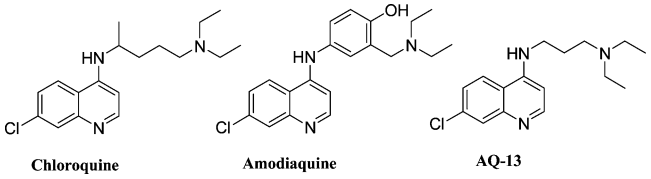

Figure 2. Structures of antimalarial 4-aminoquinolines.

systematic variation in the side chain structure and basicity seems to be more promising for the development of new antimalarial agents based on the indolequinoline derivatives.

In this study, we focused our attention on the side chain modification of the neocryptolepine core for development of antimalarial agents. For this, introduction of various amino-alkylamino groups into the C11 position and further selective modifications of the pendent amino group with different functional groups were examined (Figure 3). Our methods are based on the fact that the thiazolidin-4-one (Figure 3, compounds **9** and **11**) is a biologically privileged scaffold and

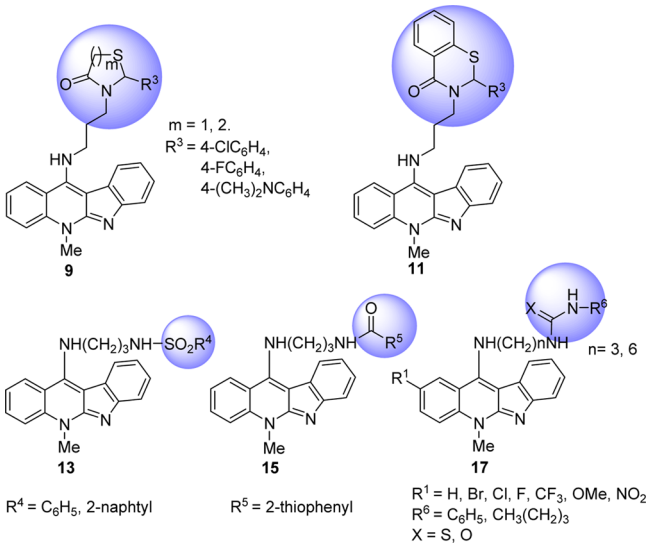

Figure 3. Structures of terminal amino group of the side chain modified neocrytolepine derivatives.

well tolerated in human subjects.[20] The sulfonamide (Figure 3, compound **13**), thiophene-2-carboxamide (Figure 3, compound **15**), and urea/thiourea (Figure 3, compound **16**) were incorporated with the idea of improving the solubility properties and in vitro antiparasitic activities according to literature data of related compounds with them.[21,22] On the basis of previous work, we were aware of that the substituents at the C2 position of neocryptolepine could reduce cytotoxicity as in 2-bromoneocryptolepine for decrease of the DNA intercalating properties.[23] A similar behavior was found with CQ whose substituents at the C7 position have important effects on the strength of association with hematin.[24] Therefore, we envisioned that the introduction of electron-withdrawing substituents, such as halogen and nitro groups, and electron-donating substituents, such as the methoxy group, at the C2 position of the neocryptolepine core would reduce the cytotoxicity of the parent compounds. We now report the synthesis and evaluation of the antimalarial activity of novel 11-amino-substituted neocryptolepine derivatives with further modifications of the amino group at the terminal side chain.

CHEMISTRY

Several synthetic strategies of the neocryptolepine core have been developed in recent years. Timaril et al. reported the synthesis of neocryptolepine from 3-bromoquinoline using the Suzuki procedure,[25] Molina et al. reported the synthesis of neocryptolepine via the Staudinger aza-Wittig and electrocyclization reactions,[26] Ho et al. synthesized neocryptolepine from the common intermediates 1,3-bis-(2-nitrophenyl)-propan-2-one by transition-metal-mediated reductive cyclization,[27] and the Perkin reaction and double reduction-double cyclization as the main steps were used for the synthesis of neocryptolepine by Parvatkar et al.,[28] the heteroatom-directed photoannulation technique reported by Dhanabal et al.,[29] the Graebe-Ullmann reaction by Peczynska-Czoch et al.,[30] and other miscellaneous methods.[31−34]

In a previous study, we developed an improved procedure for the syntheses of the 11-chloroneocryptolepine core and its 6-methyl congener with substitutes at the C-2−4 positions, which started from methyl 1*H*-indole-3-carboxylate (**1**) and *N*-methylanilines **2** or anilines (Scheme 1).[23,35] Thus, the intermediate, 2-*N*-(*N*-methylanilino)indole-3-carboxylate (**3**) was obtained from **1** by chlorination with *N*-chlorosuccinimide in the presence of 1,4-dimethylpiperzine followed by the addition of a mixture of **2** and trichloroacetic acid. The cyclization of **3** was carried out at 250 °C in diphenyl ether to form the tetracyclic ketone **4**, which was converted to 11-chloroneocrypotolepine **5** by dehydrative chlorination with $POCl_3$. Subsequently, the amination of **5** with appropriate amines by heating in DMF yielded the aminoalkylamino-substituted neocryptolepine derivatives **6**.

Further modifications of the 11-amino neocryptolepine derivatives **6** at the terminal amino group of the lateral attachment were carried out as outlined in Scheme 2. The amino group was transformed into the thiazolidin-4-one skeleton by the one-pot, three-component condensation with an aldehyde and mercapto acid.[36] Thus, the neocryptolepine derivatives bearing 2-substituted thiazolidin-4-ones **9a−d** were obtained from the *N*-(3-aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine **6a** by treatment with the substituted aldehydes **8a−c** and mercapto acids **7a−b** in the presence of DCC in dry THF at room temperature in good yields. On the other hand, due to the insolubility of the 2-mercaptobenzoic

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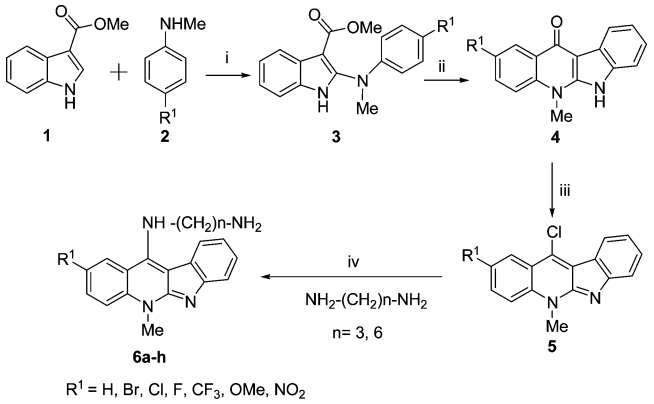
Scheme 1. Synthesis of Neocryptolepines with Substituents at the C-2 and C-11 Positions[a]



[a]Reagents and conditions: (i) (a) *N*-chlorosuccinimide, 1,4-dimethylpiperazine, CH_2Cl_2, 0 °C, 2 h; (b) trichloroacetic acid, RT, 2 h; (ii) diphenyl ether, reflux, 1−3 h; (iii) $POCl_3$, toluene, reflux, 6−12 h; (iv) appropriate amine, DMF, 135 °C.

acid **10** in THF, the synthesis of the neocryptolepine derivatives of the 2-substituted 2,3-dihydrobenzo[*e*][1,3]-thiazin-4-one **11** were carried out by heating a mixture of the appropriate amine **6a**, the substituted aldehydes **8a**, and **10** in the presence of DCC in dry toluene.

The neocryptolepine derivatives of the 2-substituted sulfonamides **13a−b** and 2-substituted thiophene-2-carboxamide **15** were easily synthesized by sulfonylation of the appropriate amine **6a** with sulfonyl chloride **12a−b** and with thiophene-2-carbonyl chloride **14** in DMF in the presence of Et_3N at room temperature, respectively. Furthermore, the neocryptolepine derivatives of the 2-substituted thiourea **17a** and 2-substituted urea **17b−j** could also be easily obtained by mixing of the appropriate amine **6a−h** with isothiocyanate **16a** and isocyanate **16b−c** in dry CH_2Cl_2 at room temperature, respectively.

All the synthesized neocryptolepine derivatives with high purity (HPLC purity ≥95%) for biological screening tests are listed in Tables 1 and 2, and some neocryptolepine derivatives with low purity, such as **6b**, **6h**, and **13a**, are listed in Table 3, attached tables 1 and 2.

RESULTS AND DISCUSSION

Antiplasmodial Activity and Cytotoxicity. The introduction of 3-amioalkylamino group, such as 3-aminoalkylamino- and *N*,*N*-diethyl-5-amino-pentyl-2-amino groups at the C11 on the 5-methylindole[2,3-*b*]quinoline (neocryptolepine) core, significantly increased the antiproliferative activities.[37] Recently, the aminoalkylamino-substituted neocryptolepine was shown to be 1500-fold more efficacious when compared to the natural product itself against the chloroquine-sensitive *P. falciparum* Ghana strain.[23] On the basis of these facts, we introduced the 3-aminopropylamino groups at the C11 position by the varying kinds of substituents at the C2 positions for the SAR study of the neocryptolepine core.

We first examined the antimalarial activity of the 2-substituted 11-(3-aminopropylamino)neocryptolepines against the CQS (NF54). As shown in Table 1, the compounds **6c**, **6d**, and **6f** substituted with halogens, such Br, Cl, and electron-withdrawing CF_3, increased the activity against the NF54 compared to the nonsubstituted **6a**. No remarkable increase in the activity was found using the F-substituted **6e**. Noteworthy is the remarkably increased activity of **6h** (Table 3) substituted with NO_2 at the C2 position. On the other hand, the longer chain-substituted **6b** (Table 3) with the 6-aminohexylamino group showed a slightly decreased activity compared to the shorter one, such as 1,3-diamino-tri(methylene) **6a**. Accordingly, the 3-aminopropylamino group was then further modified to produce a higher activity.

In the 7-chloroquinoline core of CQ, the 4-(*n*-aminoalkylamino) side chains were modified in order to explore the antiplasmodial activity of the derivatives having a thiazolidin-4-one nucleus at the terminal side chain amino group. Furthermore, this type of modification would prevent dealkylation without adversely affecting the lipophilicity and antimalarial activity of the molecule as described in the literature.[38] In our study, we have designed compounds wherein the 3-aminoalkylamino side chain of **6a** was further transformed into the thiazolidine-4-one, i.e., the [1,3]thiazinan-4-one groups, without varying the substituent at the C2 position. The thiazolidine-4-one substituted **9a−d** and **11** showed slightly increased activities compared to **6a**. Thus, it turned out that protected nonbasic nitrogen at the terminal of the 3-aminoalkylamino- side chain significantly affected the antimalarial activity. These derivatives with nonbasic substituents showed higher SI data compared to the free terminal amine derivative **6a**.

Similarly, the terminal nitrogen of the 3-aminoalkylamino-side chain of **6a** was simply and straightforwardly transferred into the corresponding sulfonamides **13a−b**, amide **15**, and urea derivatives **17a−c**. The naphthalenesulfonyl derivative **13b** showed a higher activity than its benzenesulfonyl derivative **13a** (Table 3) with a similar SI value. A decreasing antimalarial activity was obtained by the thiophenecarbonyl derivative **15**, giving the higher IC_{50} value of 258.1 nM for CQS (NF54) than **6a** (IC_{50} = 78.8 nM) but an improved SI value from 3.5 to 17.3. A significant antimalarial activity was observed with the urea derivatives **17a−c**, and among them, **17a** showed the highest antimalarial activity (IC_{50} = 9.1 nM) for CQS (NF54) and higher than CQ (IC_{50} = 9.4 nM) with the high SI value of 134.9. Dominguez et al.,[39] in which urea derivatives have been identified as inhibitors of *β*-hematin formation, which are digested by malaria parasites as they grow within their host red blood cells, also reported similar results during the study of the 4-aminoquinolines. The urea derivatives **17d** with a long chain (6-aminohexylamino group) showed a slightly decreased activity compared to the shorter one, such as 1,3-diamino-tri(methylene) **17c**. This result also agreed with the comparison of **6a** and **6b**.

In this study, we also investigated the effect of substituents at the C2 position of the urea derivatives **17c** by varying the substituents at the C2 position. Among these compounds **17e−j**, several compounds with 2-substituent showed much higher antimalarial activities than **17c** without a substituent at the C2 position. Interestingly, both the electron-withdrawing group, such as CF_3 (**17h**) and NO_2 (**17j**), and electron-donating group, such as MeO (**17i**), at the C2 position of the urea derivatives **17c** obviously showed an increased antimalarial activity against CQS (NF54) compared to **17c**. A similarly increased antimalarial activity was also obtained with **17e** and **17f** when substituted with halogens (Br, Cl). Especially, the 2-Cl substituted **17f** showed the highest SI value of 1400 and IC_{50} value of 2.2 nM for CQS (NF54) among all the synthesized compounds.

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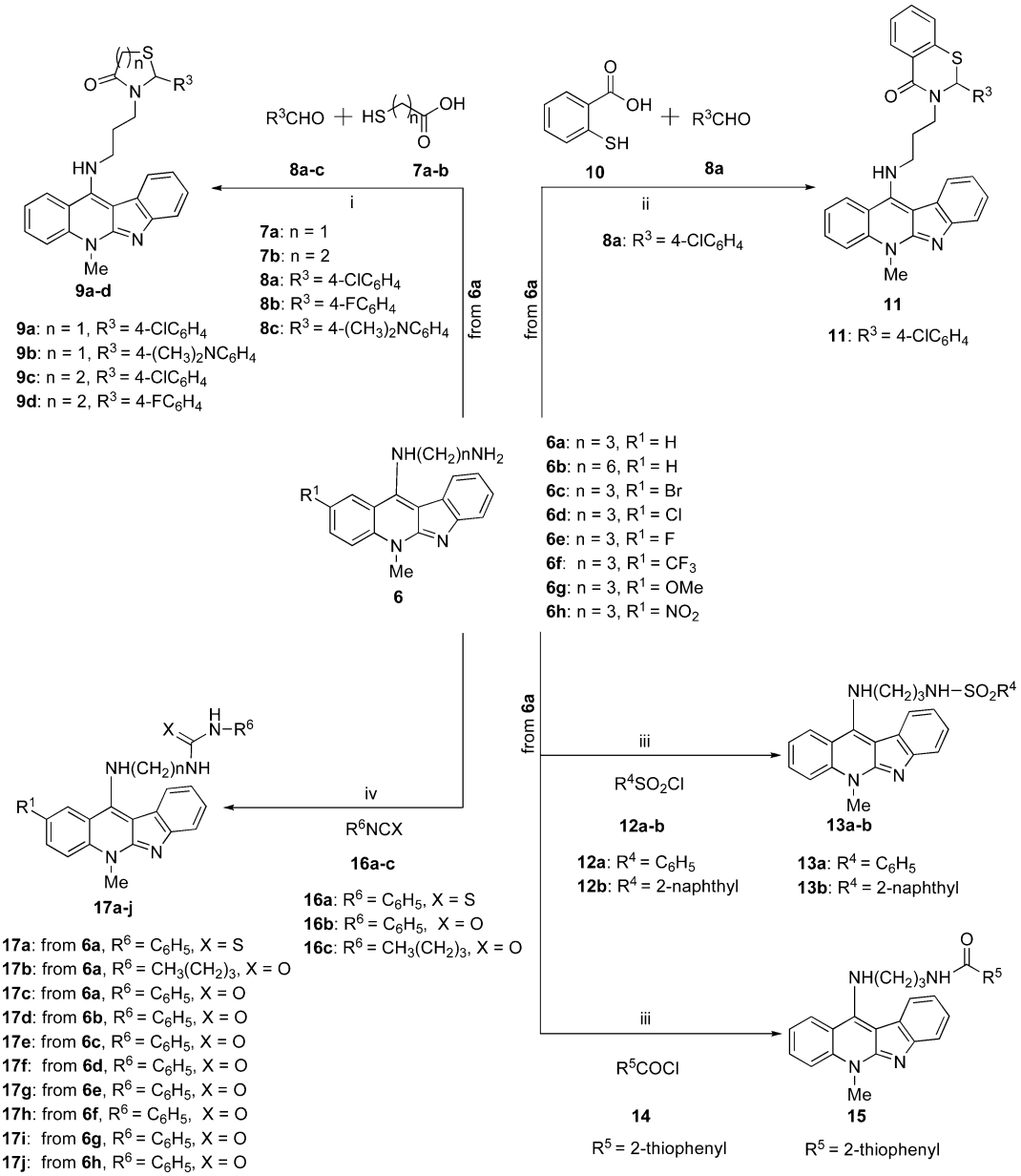
Scheme 2. Synthesis of Neocryptolepine Derivatives by Further Modifications of the Lateral Amino Group[a]



[a]Reagents and conditions: (i) DCC, THF, RT; (ii) DCC, toluene, reflux; (iii) DMF, Et_3N, RT; (iv) CH_2Cl_2, RT.

As shown in Table 1, some compounds with different substituents at the terminal amino group, **9a−d**, **11**, **13a** (Table 3), **17a**, **17c**, and **17e−j** were also submitted to measurement involving CQR (K1) strains. All compounds tested showed promising antimalarial activities against both strains and a low resistance index (RI). The RI provides a quantitative measurement of the antiplasmodial activity against the CQR strains relative to that against CQS strains and reveals promising drug discovery leads.[40] Gratifyingly, we found that almost all compounds (except compound **17g**) were significantly more active against the CQR (K1) than CQ, giving IC_{50} values between 2.2 and 143.7 nM for K1 (CQ, IC_{50} = 209.5 nM) and RI values ranging from 0.4 to 14.2 (CQ, RI = 22.3), especially, **17i** had an IC_{50} value of 2.2 nM for K1 and RI value of 0.5.

Six compounds with different substituents at the terminal amino group (**9b−c**, **11**,**13a** (Table 3), **17a**, and **17c**) were also selected for testing against three parasitic protozoa, *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*), *Trypanosoma cruzi* (*T. cruzi*), and *Leishmania donovani* (*L. donovani*) (Table 2). Several of these compounds showed antiprotozoal activities against *T. b. rhodesiense* and *T. cruzi*, but all these neocryptolepine derivative also showed cytotoxicity and thus no selectivity.

In Vivo Antimalarial Activity. Some compounds of high antimalarial activities against NF54 strain, i.e., **17a**, **17c**, and **17f**, were selected for testing in vivo drug screening model against *Plasmodium berghei* in Swiss mice. The in vivo study was carried out according to standard protocol following the "4 Day Test". After daily intraperitoneal dosing at 50 mg/kg for four consecutive days, neocryptolepine derivatives **17a** and **17c** showed some reduction in parasitaemia on day 4, activities of 15.4% and 22.1%, respectively, and neocryptolepine derivative **17f** showed no activity and all mice lost weight.

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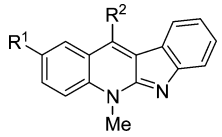
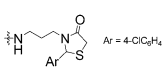
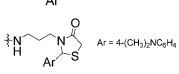
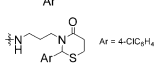
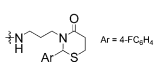
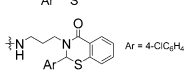
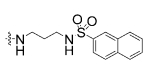
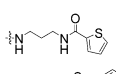
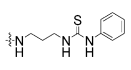
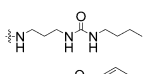
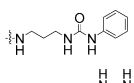
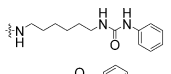
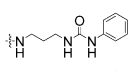
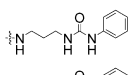
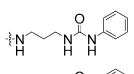
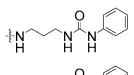
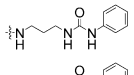
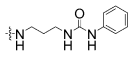
Table 1. Antiplasmodial Activity against *P. falciparum* (CQS,NF54; CQR, K1) and Cytotoxicity toward L6 Cells of Neocryptolepine Derivatives



NO	R^1	R^2	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	2.0	1696	1.9	1.1
5a	2-H	Cl	1459	2055	0.7	1998	0.7	1.0
6a	2-H		279.2	78.8	3.5	29.6	9.4	0.4
6c	2-Br		258.3	10.4	24.8	NT[d]		
6d	2-Cl		268.6	11.8	22.8	NT[d]		
6e	2-F		338.1	49.6	6.8	NT[d]		
6f	2-CF_3		923.8	10.7	86.3	NT[d]		
6g	2-OMe		382.8	74.8	5.1	NT[d]		
9a	2-H		3473	63.9	54.4	143.7	24.2	2.2
9b	2-H		3551	54.9	64.7	143.2	24.8	2.6
9c	2-H		3359	52.4	64.1	38.8	86.6	0.7
9d	2-H		2728	38.1	71.6	112.3	24.3	2.9
11	2-H		1099	26.6	41.3	21.3	51.6	0.8
13b	2-H		18.2	14.2	1.3	NT[d]		
15	2-H		4463	258.1	17.3	NT[d]		
17a	2-H		1228	9.1	134.9	111.5	11.0	12.3
17b	2-H		1740	104.1	16.7	NT[d]		
17c	2-H		1244	21.3	58.4	9.4	132.3	0.4
17d	2-H		397.3	25.8	15.4	NT[d]		
17e	2-Br		2826	4.0	706.5	11.9	237.5	3.0
17f	2-Cl		3079	2.2	1400	21.8	141.2	9.9
17g	2-F		3556	24.9	142.8	312.6	11.4	12.6
17h	2-CF_3		1282	4.1	312.7	48.8	26.3	11.9
17i	2-OMe		2734	4.4	621.4	2.2	1243	0.5
17j	2-NO_2		2732	2.1	1301	29.9	91.4	14.2
Podophylotoxin			14.5					
Chloroquine				9.4		209.5		22.3
Artemisinin				4.3		2.8		0.7

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

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Table 2. Antiprotozoal Activity and Cytotoxicity of Tested Compounds

no.	cytotoxicity (L6 cells) IC_{50} nM[a]	*T. b. rhodesiense* IC_{50} nM[a]	*T. cruzi* IC_{50} nM[a]	*L. donovani* (axenic amastigotes) IC_{50} nM[a]
9b	3551	1373	2727	26095
9c	3359	547.5	3184	12950
11	1099	868.4	2362	9749
17a	1228	589.2	6120	79167
17c	1244	606.8	10602	97518
podophylotoxin	14.5			
melarsoprol		5.0		
benznidazole			1606	
miltefosine				360.7

[a]The IC_{50} values are the means of two independent assays; the individual values vary less than a factor 2.

CONCLUSIONS

We have prepared a novel series of new neocryptolepine derivatives by systematically varying the 2-substituents of the neocryptolepine core and modifying the terminal amino group of the C11-aminoalkylamino side chain. All the synthesized neocryptolepine derivatives showed potent antiplasmodial activities against CQS (NF54) and CQR (K1) in vitro. A comparison with CQ clearly revealed that urea derivatives **17a**, **17e−f**, and **17h−j** had increased activities against CQS (NF54), **9a−d**, **11**, **13a**, **17a**, **17c**, **17e−f**, and **17h−j** were also clearly observed to afford superior activities against CQR (K1). In particular, among the tested compounds, **17f** showed 4 times more potent activity than CQ for CQS (NF54) with an IC_{50} of 2.2 nM and a selectivity index of 1400, and similarly high antimalarial activity was showed by **17j**. Furthermore, **17i** showed more potent activity 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, which is also even higher antimalarial activity than artemisinin (IC_{50} value of K1 = 2.8 nM, RI = 0.7). Three compounds were selected for in vivo evaluation in infected mice, but they were not sufficiently potent or toxic to the mice. These present findings show that the methodical variation of the side chain of the neocryptolepine core could also provide a promising entry point toward affordable heme-targeted antimalarial, even though further variations in substituents or their pattern may be necessary to obtain nontoxic compounds showing in vivo activity.

EXPERIMENTAL SECTION

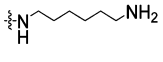
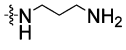
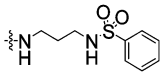
Chemistry. *Materials and Methods.* Column chromatographies were achieved on a silica gel column (230−400 mesh) using a gradient solvent system (*n*-hexane/ethyl acetate as the eluent unless otherwise specified). The ^{1}H, ^{13}C, and ^{19}F NMR spectra were taken on a Varian INOVA-600 spectrometer with $CDCl_3$ or 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. IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer. 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 separation module using Waters 2998 photodiode array (PDA) detector equipped with a Symmetry C18 column (4.6 mm × 150 mm, 5 μm). Water (A) and MeCN (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, and 254 nm was used as wavelength.

*General Procedure for the Synthesis of 11-Aminoneocryptolepines **6a−h**.* 11-Chloroindoloquinolines **1** (0.3 mmol) and an excess of the appropriate aminoalkylamine (3.0 mmol) were heated together at 135−155 °C for 1−4 h. TLC monitoring was used to ensure the completion of reaction. The resulting brown crude oil was purified by flash chromatography using AcOEt−2N ammonia in MeOH (9:1) as an eluent to yield pure **6a−h** as yellowish-orange solids.

*N-(3-Aminopropyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (**6a**).* Yield 96%; yellow solids; mp, 69−71 °C. IR (KBr) 3435, 2928, 2868, 2359, 2342, 1622, 1559, 1489, 1441, 1418, 1287, 1248, 1057, 750, 669 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.80 (quint, J = 6.0 Hz, 2H), 3.01 (t, J = 6.0 Hz, 2H), 4.01 (t, J = 6.0 Hz, 2H), 4.22 (s, 3H), 7.17 (t, J = 7.2 Hz, 1H), 7.18 (br s, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.41 (t, J = 7.2 Hz, 1H), 7.61 (d, J = 9.0 Hz, 1H), 7.67 (td, J = 7.2, 1.2 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.95 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 32.64, 32.66,

Table 3. Biological Activities of 6b, 6h, and 13a with Low Purity

Attached table 1.

NO	R^1	R^2	L6 cells	NF54	SI
			IC_{50} nM	IC_{50} nM	L6/NF54
6b	2-H		692.7	98.1	7.1
6h	2-NO_2		208.9	11.4	18.3
13a	2-H		65.2	63.0	1.0

Attached table 2.

NO	K1	SI	T.b.rhodesiense	T.cruzi.	L.donovani (axenic amastigotes)
	IC_{50} nM	L6/K1	IC_{50} nM	IC_{50} nM	IC_{50} nM
13a	123.7	0.5	164.2	1597	58036

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41.26, 49.25, 105.61, 114.40, 115.86, 116.94, 118.49, 120.35, 121.37, 124.06, 124.12, 125.16, 130.08, 137.79, 148.52, 151.94, 156.55. HPLC purity 95.1%. HRMS (ESI) calcd for $C_{19}H_{21}N_4$ $[M + H]^+$. Exact mass: 305.1761, found 305.1765.

N-(6-Aminohexyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (**6b**). Yield 96%; yellow solids; mp 54−56 °C. IR (KBr) 3350, 2928, 2855, 1622, 1593, 1568, 1489, 1441, 1422, 1281, 1246, 1200, 1142, 882, 752 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.19 (m, 5H), 1.36 (m, 1H), 1.68 (m, 2H), 2.38 (t, J = 6.6 Hz, 1H), 2.95 (t, J = 6.6 Hz, 1H), 3.83 (m, 2H), 4.16 (s, 3H), 6.99 (br s, 1H), 7.07 (t, J = 7.2 Hz, 1H), 7.28 (t, J = 7.2 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 8.53 (d, J = 7.2 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 22.58, 26.24, 26.27, 30.98, 32.66, 41.20, 48.13, 104.68, 115.41, 115.82, 116.79, 118.47, 121.12, 122.22, 124.20 (2C), 124.98, 131.13, 137.61, 148.78, 152.10, 156.59. HPLC purity 79.5%. HRMS (ESI) calcd for $C_{22}H_{27}N_4$ $[M + H]^+$. Exact mass: 347.2230, found 347.2235.

N-(3-Aminopropyl)-2-bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (**6c**). Yield 94%; yellow solids; mp 137−139 °C. IR (KBr) 3229, 3152, 3067, 2940, 2909, 2857, 1622, 1589, 1557, 1505, 1487, 1441, 1418, 1389, 1281, 1238, 1209, 1109, 1057, 876, 800, 762, 743 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 1.82 (quint, J = 6.0 Hz, 2H), 3.08 (t, J = 6.0 Hz, 2H), 4.03 (m, 2H), 4.21 (s, 3H), 7.17 (t, J = 7.2 Hz, 1H), 7.42 (t, J = 7.2 Hz, 1H), 7.46 (br s, 1H), 7.50 (d, J = 9.0 Hz, 1H), 7.72−7.75 (m, 2H), 7.97 (d, J = 7.8 Hz, 1H), 8.30 (d, J = 2.4 Hz, 1H). ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 32.23, 32.68, 41.51, 49.68, 106.28, 113.02, 116.07, 117.29, 117.52, 118.70, 121.67, 124.17, 125.56, 126.72, 132.57, 136.66, 147.17, 152.59, 156.65. HPLC purity 94.7%. HRMS (ESI) calcd for $C_{19}H_{18}BrN_4$ $[M - H]^-$. Exact mass: 381.0720, found 381.0710.

N-(3-Aminopropyl)-2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (**6d**). Yield 92%; yellow solids; mp 132−134 °C. IR (KBr) 3420, 3264, 3050, 2934, 2870, 1618, 1587, 1559, 1491, 1443, 1418, 1341, 1290, 1279, 1248, 1217, 1115, 1065, 876, 797, 756, 731 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.74 (quint, J = 6.6 Hz, 2H), 2.59 (t, J = 6.6 Hz, 2H), 3.91 (t, J = 6.6 Hz, 2H), 4.14 (s, 3H), 7.08 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.77−7.81 (m, 1H), 7.85 (m, 1H), 7.92 (d, J = 7.8 Hz, 1H), 8.59 (d, J = 2.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.40, 33.53, 39.46, 46.75, 104.57, 116.70, 116.80, 117.11, 118.18, 122.38, 123.09, 124.11, 124.79, 125.04, 130.24, 136.06, 147.10, 152.38, 156.34. HPLC purity 95.8%. HRMS (ESI) calcd for $C_{19}H_{20}ClN_4$ $[M + H]^+$. Exact mass: 339.1371, found 339.1382.

N-(3-Aminopropyl)-2-fluoro-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (**6e**). Yield 89%; yellow solids; mp 87−88 °C. IR (KBr) 3283, 3055, 2932, 2359, 1614, 1601, 1568, 1489, 1445, 1424, 1344, 1281, 1242, 1136, 856, 795, 760, 739 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.73 (quint, J = 6.6 Hz, 2H), 2.60 (t, J = 6.6 Hz, 2H), 3.92 (t, J = 6.6 Hz, 2H), 4.16 (s, 3H), 7.07 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.68−7.71 (m, 1H), 7.88 (m, 1H), 7.93 (d, J = 7.8 Hz, 1H), 8.36 (dd, J = 11.4, 2.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.48, 33.53, 39.46, 46.79, 104.78, 109.10 (d, J = 24.1 Hz), 116.31 (d, J = 7.8 Hz), 116.53, 117.16 (d, J = 8.4 Hz), 117.97, 118.49 (d, J = 24.1 Hz), 122.35, 123.86, 124.79, 134.20, 147.32 (d, J = 3.3 Hz), 152.52, 156.46, 156.58 (d, J = 236.8 Hz). ^{19}F NMR (564 MHz, DMSO-d_6) δ ppm −121.26. HPLC purity 96.1%. HRMS (ESI) calcd for $C_{19}H_{20}FN_4$ $[M + H]^+$. Exact mass: 323.1667, found 323.1670.

N-(3-Aminopropyl)-5-methyl-2-(trifluoromethyl)-5H-indolo[2,3-b]quinolin-11-amine (**6f**). Yield 87%; yellow solids; mp 64−65 °C. IR (KBr) 3430, 2930, 2351, 1634, 1595, 1568, 1505, 1443, 1429, 1402, 1333, 1279, 1246, 1146, 1117, 1088, 814, 762, 746 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.76 (quint, J = 6.0 Hz, 2H), 2.63 (t, J = 6.0 Hz, 2H), 3.97 (t, J = 6.6 Hz, 2H), 4.18 (s, 3H), 7.11 (t, J = 7.2 Hz, 1H), 7.30 (m, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 8.00 (t, J = 6.6 Hz, 1H), 8.04 (m, 1H), 8.86 (s, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.51, 33.24, 39.56, 46.98, 104.22, 115.25, 116.17, 116.93, 118.53, 123.21 (q, J = 268.1 Hz), 121.79, 124.12, 124.78, 124.89, 126.37, 139.37, 147.60, 152.10, 156.41. ^{19}F NMR (564 MHz, DMSO-d_6) δ ppm −58.64. HPLC purity 96.5%. HRMS (ESI) calcd for $C_{20}H_{20}F_3N_4$ $[M + H]^+$. Exact mass: 373.1635, found 373.1646.

N-(3-Aminopropyl)-2-methoxy-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (**6g**). Yield 99%; yellow solids; mp 105−106 °C. IR (KBr) 3381, 3268, 2934, 1614, 1593, 1568, 1489, 1445, 1424, 1348, 1288, 1246, 1184, 1140, 1038, 937, 810, 760 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.72 (quint, J = 6.6 Hz, 2H), 2.64 (t, J = 6.6 Hz, 2H), 3.91 (s, 3H), 3.94 (t, J = 6.6 Hz, 2H), 4.14 (s, 3H), 7.04 (t, J = 7.2 Hz, 1H), 7.26 (t, J = 7.2 Hz, 1H), 7.43−7.47 (m, 2H), 7.79 (d, J = 9.6 Hz, 1H), 7.92 (m, 2H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.23, 33.44, 39.82, 47.18, 55.80, 104.47, 105.88, 116.18, 116.28, 116.43, 117.47, 119.52, 122.15, 123.98, 124.43, 132.18, 147.73, 152.50, 153.55, 156.26. HPLC purity 94.6%. HRMS (ESI) calcd for $C_{20}H_{23}N_4O$ $[M + H]^+$. Exact mass: 335.1866, found 335.1864.

N-(3-Aminopropyl)-5-methyl-2-nitro-5H-indolo[2,3-b]quinolin-11-amine (**6h**). Yield 83%; red solids; mp 159−162 °C. IR (KBr) 3430, 3069, 2940, 2868, 1616, 1570, 1505, 1441, 1424, 1329, 1294, 1246, 1121, 941, 826, 760, 739 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.78 (quint, J = 6.6 Hz, 2H), 2.67 (t, J = 6.6 Hz, 2H), 4.00 (t, J = 6.6 Hz, 2H), 4.20 (s, 3H), 7.13 (t, J = 7.2 Hz, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.98 (t, J = 7.8 Hz, 2H), 8.52 (m, 1H), 9.45 (d, J = 2.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.85, 32.96, 39.90, 47.21, 103.96, 115.06, 116.11, 117.26, 119.04, 121.08, 122.41, 124.21, 124.56, 124.88, 140.18, 141.06, 147.60, 151.90, 156.17. HPLC purity 93.9%. HRMS (ESI) calcd for $C_{19}H_{18}N_5O_2$ $[M - H]^-$. Exact mass: 348.1466, found 348.1485.

General Procedure for the Synthesis of Compounds **9a−d**. *N*-(3-Aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a**, 100 mg) and 2.0 equiv of benzaldehyde (**8a−c**) was stirred in dry THF under ice-cold conditions for 5 min, and then mercaptopropionic acid (**7a−b**) (3.0 equiv) was added. After 5 min, DCC (1.2 equiv) was added to the reaction mixture at 0 °C, and the reaction mixture was stirred for 1−3 h at room temperature. DCU was removed by filtration and the filtrate was concentrated to dryness under reduced pressure and the residue was taken up in chloroform. The organic layer was successively washed with aq 5% $NaHCO_3$ and then finally with brine. The organic layer was dried over $MgSO_4$ and evaporated to give a crude product which was purified by flash chromatography using ethyl acetate AcOEt−2N ammonia in MeOH (9:1 v/v) as an eluent to yield pure products as yellowish-orange solids (**9a−d**).

2-(4-Chlorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)thiazolidin-4-one (**9a**). Yield 30.4%; yellowish-orange solids; mp 151−154 °C. IR (KBr) 3368, 3057, 2930, 2864, 1668, 1616, 1593, 1489, 1462, 1408, 1316, 1261, 1217, 1088, 1013, 856, 802, 756 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.86 (quint, J = 7.2 Hz, 2H), 2.60 (m, 1H), 3.37−3.46 (m, 2H), 3.77 (dd, J = 15.6, 1.8 Hz, 1H), 3.82−3.92 (m, 2H), 4.20 (s, 3H), 5.75 (d, J = 1.2 Hz, 1H), 7.27 (m, 2H), 7.34 (m, 3H), 7.49 (t, J = 7.2 Hz, 1H), 7.64 (m, 2H), 7.98 (t, J = 8.4 Hz, 2H), 8.08 (d, J = 9.0 Hz, 1H), 8.15 (br s, 1H), 8.61 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 28.26, 32.97, 36.41, 40.03, 44.55, 63.37, 100.58, 113.49, 115.67, 116.45, 119.81, 121.40, 122.16, 124.17, 125.16, 126.21, 128.82 (2C), 129.45 (2C), 132.56, 135.44, 136.70, 137.22, 137.80, 147.42, 151.77, 172.75. HPLC purity 95.5%. HRMS (ESI) calcd for $C_{28}H_{24}ClN_4OS$ $[M - H]^-$. Exact mass: 499.1365, found 499.1374.

2-(4-(Dimethylamino)phenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)thiazolidin-4-one (**9b**). Yield 65%; yellow solids; mp 102−104 °C. IR (KBr) 3356, 2928, 1661, 1614, 1593, 1564, 1524, 1493, 1441, 1418, 1352, 1281, 1246, 1184, 1167, 1063, 945, 799, 752 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 1.49 (m, 1H), 1.69 (m, 1H), 2.94 (s, 6H), 3.15 (m, 1H), 3.58 (m, 1H), 3.69 (m, 1H), 3.77−3.94 (m, 3H), 4.28 (s, 3H), 5.56 (s, 1H), 6.63 (d, J = 9.0 Hz, 2H), 6.91 (br s, 1H), 7.19 (m, 3H), 7.38 (t, J = 7.2 Hz, 1H), 7.46 (t, J = 7.2 Hz, 1H), 7.66 (d, J = 9.0 Hz, 1H), 7.74 (t, J = 7.8 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 8.41 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 28.36, 33.19, 33.96, 39.94, 40.23 (2C), 43.71, 64.58, 105.16, 112.13 (2C), 114.90, 116.10, 116.43, 119.80, 121.76, 121.96, 122.63, 124.01, 124.48, 125.87, 128.57 (2C), 131.01, 137.38, 147.94, 149.30, 151.15, 154.07, 172.54. HPLC

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purity 98.5%. HRMS (ESI) calcd for $C_{30}H_{30}N_5OS$ $[M - H]^-$. Exact mass: 508.2171, found 508.2197.

*2-(4-Chlorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-1,3-thiazinan-4-one (**9c**).* Yield 42%; yellow solids; mp 87−89 °C. IR (KBr) 3420, 2934, 1622, 1593, 1568, 1489, 1441, 1420, 1314, 1285, 1246, 1202, 1144, 1092, 1013, 829, 754 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.95 (m, 2H), 2.53−2.65 (m, 5H), 3.81 (m, 1H), 3.85−3.95 (m, 2H), 4.18 (s, 3H), 5.76 (s, 1H), 7.12 (d, J = 8.4 Hz, 2H), 7.21 (t, J = 7.2 Hz, 1H), 7.33−7.42 (m, 3H), 7.51 (t, J = 7.8 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.62 (br s, 1H), 7.88 (t, J = 7.8 Hz, 1H), 7.96 (d, J = 7.8 Hz, 2H), 8.56 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 20.83, 28.50, 33.48, 33.82, 44.37, 45.45, 59.97, 114.63, 115.73, 115.93, 119.81, 122.06, 122.38, 122.55, 124.12, 125.22, 128.12 (2C), 128.33 (2C), 129.39, 131.20, 131.53, 132.21, 137.01, 138.88, 149.75, 168.60, 171.47. HPLC purity 100%. HRMS (ESI) calcd for $C_{29}H_{26}ClN_4OS$ $[M - H]^-$. Exact mass: 513.1521, found 513.1535.

*2-(4-Fluorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-1,3-thiazinan-4-one (**9d**).* Yield 29.3%; yellowish-orange solids; mp 92−94 °C. IR (KBr) 3420, 3063, 2932, 1616, 1593, 1564, 1506, 1464, 1435, 1402, 1323, 1225, 1157, 1103, 1065, 835, 754 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 1.85 (m, 1H), 1.96 (m, 1H), 2.70 (m, 1H), 2.77−2.84 (m, 2H), 2.91 (m, 2H), 3.62−3.67 (m, 1H), 4.13−4.18 (m, 1H), 4.27 (s, 3H), 4.29−4.35 (m, 1H), 5.54 (s, 1H), 7.05 (t, J = 8.4 Hz, 2H), 7.16−7.23 (m, 3H), 7.33 (t, J = 7.2 Hz, 1H), 7.42 (br s, 1H), 7.51 (t, J = 7.2 Hz, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.77 (t, J = 7.8 Hz, 1H), 7.83 (t, J = 9.0 Hz, 2H), 8.48 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 21.62, 28.67, 29.66, 34.22, 34.76, 44.00, 61.11, 103.37, 115.17, 115.75 (d, J = 21.9 Hz, 2C), 116.56, 120.68, 121.74, 122.79, 124.31, 125.99, 128.16 (2C), 128.21 (2C), 131.53, 134.25, 134.27, 137.11, 150.23, 161.61, 163.26, 170.82. ^{19}F NMR (564 MHz, $CDCl_3$) δ ppm −113.09. HPLC purity 95.6%. HRMS (ESI) calcd for $C_{29}H_{26}FN_4OS$ $[M - H]^-$. Exact mass: 497.1817, found 497.1820.

*General Procedure for the Synthesis of Compound (**11**).* *N*-(3-Aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a**, 100 mg) and 2.0 equiv of benzaldehyde (**8a**) was stirred in dry toluene at 70−80 °C for 5 min, followed by addition of thiosalicylic acid (**10**) (3.0 equiv) and DCC (1.2 equiv). Then the reaction mixture was heated to reflux for 10−15 h. The reaction mixture was cooled to room temperature and concentrated to dryness under reduced pressure and the residue was taken up in chloroform and washed with aq 5% $NaHCO_3$ and then finally with brine. The organic layer was dried over and evaporated to get a crude product that was purified by flash chromatography using ethyl acetate (AcOEt)−2N ammonia in MeOH (9:1 v/v) as the eluent to yield pure products as yellowish-orange solids (**11**).

*2-(4-Chlorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-2,3-dihydrobenzo[e][1,3]thiazin-4-one (**11**).* Yield 49.7%; yellow solids; mp 124−126 °C. IR (KBr) 3349, 3055, 2932, 1622, 1591, 1564, 1489, 1456, 1441, 1422, 1310, 1277, 1246, 1204, 1146, 1092, 1013, 841, 748 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 2.06 (m, 1H), 2.13 (m, 1H), 3.23 (dt, J = 15.0, 5.4 Hz, 1H), 3.82 (m, 1H), 4.22 (m, 1H), 4.34 (s, 3H), 4.51 (m, 1H), 5.73 (s, 1H), 7.11 (d, J = 7.8 Hz, 1H), 7.15−7.22 (m, 5H), 7.29 (t, J = 7.8 Hz, 1H), 7.32−7.36 (m, 2H), 7.58 (t, J = 7.2 Hz, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.70 (br s, 1H), 7.81 (t, J = 7.8 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 8.16 (dd, J = 7.8, 1.2 Hz, 1H), 8.63 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 29.56, 35.64, 44.08, 45.23, 61.03, 102.48, 114.67, 115.41, 116.73, 120.94, 121.41, 121.77, 123.46, 124.67, 126.25, 126.71, 127.45 (2C), 127.79, 128.49, 128.82 (2C), 129.01, 129.95, 131.99, 132.53, 132.74, 134.51, 136.77, 136.97, 150.09, 151.09, 165.22. HPLC purity 98.6%. HRMS (ESI) calcd for $C_{33}H_{26}ClN_4OS$ $[M - H]^-$. Exact mass: 561.1516, found 561.1517.

*General Procedure for the Synthesis of Compounds **13a−b**.* *N*-(3-Aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a**, 50 mg) was completely dissolved in dry DMF (2 mL), and then a mixture of arenesulfonyl chloride (**12a−b**) (1.2 equiv) and dry DMF (1 mL) were added drop by drop with stirring, and finally 2.0 equiv of triethylamine was added and the reaction was carried out at room temperature for 2−4 h. TLC monitoring was used to ensure the completion of reaction. The crude product was purified by flash chromatography using AcOEt−2N ammonia in MeOH (9:1 v/v) as the eluent to yield pure products as yellowish-orange solids.

*N-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-benzenesulfonamide (**13a**).* Yield 68%; yellow solids; mp 189−192 °C. IR (KBr) 3389, 3055, 2932, 1626, 1568, 1489, 1445, 1420, 1308, 1281, 1246, 1153, 1092, 878, 860, 743, 691 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.79 (quin, J = 7.2 Hz, 2H), 2.70 (q, J = 6.6 Hz, 2H), 3.80 (q, J = 6.6 Hz, 2H), 4.16 (s, 3H), 6.88 (t, J = 5.4 Hz, 1H), 7.06 (t, J = 6.6 Hz, 1H), 7.29 (t, J = 7.8 Hz, 1H), 7.40 (t, J = 6.6 Hz, 1H), 7.45 (t, J = 7.8 Hz, 2H), 7.49 (d, J = 7.2 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.60 (m, 3H), 7.80 (t, J = 7.2 Hz, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 8.45 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 30.80, 32.24, 40.10, 45.42, 105.16, 115.04, 115.07, 115.62, 116.48, 118.04, 120.59, 122.07, 123.97, 124.73, 126.28 (2C), 129.06 (2C), 130.64, 132.25, 137.34, 140.12, 148.08, 152.20, 156.28. HPLC purity 87.7%. HRMS (ESI) calcd for $C_{25}H_{25}N_4O_2S$ $[M + H]^+$. Exact mass: 445.1693, found 445.1718.

*N-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-naphthalene-2-sulfonamide (**13b**).* Yield 76%; yellow solids; mp 198 °C. IR (KBr) 3393, 3055, 2924, 2874, 2359, 1738, 1626, 1574, 1489, 1443, 1424, 1314, 1283, 1242, 1144, 1103, 1080, 878, 862, 814, 745 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.81 (quin, J = 7.2 Hz, 2H), 2.75 (q, J = 6.0 Hz, 2H), 3.80 (q, J = 7.2 Hz, 2H), 4.13 (s, 3H), 6.86 (t, J = 6.0 Hz, 1H), 7.03 (t, J = 7.8 Hz, 1H), 7.27 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.62−7.71 (m, 4H), 7.75 (t, J = 7.2 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 7.2 Hz, 1H), 7.99 (d, J = 8.4 Hz, 2H), 8.05 (d, J = 8.4 Hz, 1H), 8.29 (s, 1H), 8.41 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 30.84, 32.19, 40.16, 45.44, 105.21, 114.98, 115.57, 116.52, 117.98, 120.48, 122.04, 122.06, 123.92, 123.98, 124.70, 127.25, 127.50, 127.79, 128.62, 129.10, 129.28, 130.58, 131.62, 134.04, 137.15, 137.31, 148.02, 152.35, 156.34. HPLC purity 100%. HRMS (ESI) calcd for $C_{29}H_{27}N_4O_2S$ $[M + H]^+$. Exact mass: 495.1849, found 495.1878.

*General Procedure for the Synthesis of Compound (**15**).* *N*-(3-Aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a**, 50 mg) was completely dissolved in dry DMF (2 mL), and then a mixture solution of acyl chloride (**14**) (1.2 equiv) and dry DMF (1 mL) were added drop by drop under stirring, and finally 2.0 equiv of triethylamine was added and the reaction was carried out at room temperature for 2−4 h. TLC monitoring was used to ensure the completion of reaction. The crude product was purified by flash chromatography using AcOEt−2N ammonia in MeOH (9:1 v/v) as an eluent to yield pure products as yellowish-orange solids.

*N-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-thiophene-2-carboxamide (**15**).* Yield 95%; yellow solids; mp 204−205 °C. IR (KBr) 3424, 3333, 3059, 2930, 1732, 1622, 1593, 1566, 1545, 1499, 1439, 1418, 1358, 1294, 1248, 1204, 1144, 1071, 862, 745, 719 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.92 (quint, J = 7.2 Hz, 2H), 3.26 (q, J = 6.6 Hz, 2H), 3.88 (q, J = 7.2 Hz, 2H), 4.16 (s, 3H), 7.02−7.07 (m, 2H), 7.10 (dd, J = 4.8, 2.4 Hz, 1H), 7.27 (t, J = 7.2 Hz, 1H), 7.41 (t, J = 7.2 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.64 (dd, J = 3.6, 1.2 Hz, 1H), 7.72 (dd, J = 5.4, 1.2 Hz, 1H), 7.79 (td, J = 7.2 1.2 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 8.52 (d, J = 6.6 Hz, 2H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 30.92, 32.22, 36.69, 45.39, 105.08, 115.06, 115.65, 116.55, 118.00, 120.58, 122.08, 123.97, 124.02, 124.68, 127.81, 127.93, 130.64 (2C), 137.39, 139.84, 148.14, 152.38, 156.40, 161.33. HPLC purity 98.3%. HRMS (ESI) calcd for $C_{24}H_{23}N_4OS$ $[M + H]^+$. Exact mass: 415.1587, found 415.1597.

*General Procedure for the Synthesis of Compounds **17a−j**.* 2-Substituted 5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a−h**, 50 mg) was completely dissolved in dry CH_2Cl_2 (1 mL), and then a solution of isocyanate (**16a−c**) (1.1 equiv) and dry CH_2Cl_2 (1 mL) were added drop by drop under stirring at room temperature for 2−4 h. TLC monitoring was used to ensure the completion of reaction. After reaction was finished, and the reaction mixture was evaporated to dryness under reduced pressure. The crude product was purified by

flash chromatography using AcOEt−2N ammonia in MeOH (9:1 v/v) as an eluent to yield pure products as yellowish-orange solids.

*1-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylthiourea (**17a**).* Yield 99%; yellow solids; mp 201−203 °C. IR (KBr) 3335, 3208, 3055, 2938, 1622, 1595, 1568, 1539, 1512, 1485, 1441, 1422, 1406, 1310, 1277, 1242, 1190, 1142, 1103, 1072, 955, 858, 748, 733 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.98 (quint, J = 6.6 Hz, 2H), 3.51 (m, 2H), 3.89 (q, J = 6.6 Hz, 2H), 4.16 (s, 3H), 7.06−7.11 (m, 3H), 7.24 (d, J = 4.2 Hz, 4H), 7.30 (t, J = 7.2 Hz, 1H), 7.43 (t, J = 7.8 Hz, 1H), 7.51 (d, J = 7.8 Hz, 1H), 7.73 (br s, 1H), 7.80 (t, J = 7.2 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 8.55 (d, J = 8.4 Hz, 1H), 9.46 (br s, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 30.36, 32.33, 41.39, 45.60, 104.58, 115.09, 115.68, 116.35, 118.22, 120.69, 122.17 (2C), 123.16, 123.93, 124.04 (2C), 124.21, 124.69, 128.66, 130.73, 137.33, 138.87, 148.28, 151.74, 156.11, 180.24. HPLC purity 100%. HRMS (ESI) calcd for $C_{26}H_{24}N_5S$ $[M - H]^-$. Exact mass: 438.1758, found 438.1751.

*1-Butyl-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)-propyl)urea (**17b**).* Yield 86%; yellow solids; mp 80−81 °C. IR (KBr) 3360, 3312, 2953, 2920, 2870, 1622, 1593, 1568, 1520, 1489, 1443, 1416, 1400, 1288, 1250, 1196, 1065, 1022, 841, 750 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 0.86 (t, J = 7.8 Hz, 3H), 1.31 (sext, J = 7.8 Hz, 2H), 1.45 (quint, J = 7.2 Hz, 2H), 1.68 (quint, J = 6.0 Hz, 2H), 3.19 (q, J = 6.6 Hz, 2H), 3.36 (q, J = 6.6 Hz, 2H), 3.85 (q, J = 6.0 Hz, 2H), 4.10 (s, 3H), 5.49 (br s, 1H), 5.79 (br s, 1H), 6.92 (m, 1H), 7.13 (t, J = 7.2 Hz, 1H), 7.31−7.38 (m, 2H), 7.51 (d, J = 7.8 Hz, 1H), 7.63−7.67 (m, 2H), 7.82 (d, J = 7.8 Hz, 1H), 8.39 (dd, J = 9.0, 1.2 Hz, 1H). ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 13.77, 20.04, 32.33, 32.38, 33.07, 36.60, 40.19, 44.25, 105.39, 114.51, 116.07, 116.41, 119.25, 121.44, 121.83, 123.48, 124.28, 125.44, 130.64, 137.45, 149.30, 152.45, 155.40, 159.77. HPLC purity 96.3%. HRMS (ESI) calcd for $C_{24}H_{28}N_5O$ $[M - H]^-$. Exact mass: 402.2299, found 402.2319.

*1-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (**17c**).* Yield 99%; yellow solids; mp 215 °C. IR (KBr) 3341, 3048, 3024, 2969, 2930, 1694, 1620, 1591, 1557, 1489, 1443, 1406, 1314, 1275, 1227, 1177, 1144, 891, 758, 718, 692 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.86 (quint, J = 6.6 Hz, 2H), 3.14 (q, J = 6.6 Hz, 2H), 3.86 (q, J = 6.6 Hz, 2H), 4.16 (s, 3H), 6.18 (t, J = 6.0 Hz, 1H), 6.87 (t, J = 7.2 Hz, 1H), 7.06 (m, 2H), 7.20 (t, J = 7.8 Hz, 2H), 7.27 (t, J = 7.2 Hz, 1H), 7.35 (d, J = 7.8 Hz, 2H), 7.42 (t, J = 7.2 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.79 (t, J = 7.2 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 8.42 (s, 1H), 8.54 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.62, 32.20, 36.48, 45.43, 105.02, 115.06, 115.62, 116.55, 117.72 (2C), 117.98, 120.55, 121.03, 122.05, 124.00, 124.07, 124.65, 128.59 (2C), 130.63, 137.40, 140.40, 148.23, 152.38, 155.52, 156.40. HPLC purity 98.8%. HRMS (ESI) calcd for $C_{26}H_{24}N_5O$ $[M - H]^-$. Exact mass: 422.1986, found 422.2003.

*1-(6-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)hexyl)-3-phenylurea (**17d**).* Yield 89%; yellow solids; mp 101−104 °C. IR (KBr) 3356, 3053, 2930, 2855, 1668, 1622, 1595, 1559, 1499, 1441, 1420, 1312, 1279, 1244, 1071, 750, 694 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.19−1.25 (m, 4H), 1.31 (quint, J = 6.6 Hz, 2H), 1.68 (quint, J = 6.6 Hz, 2H), 2.95 (q, J = 6.0 Hz, 2H), 3.83 (q, J = 6.6 Hz, 2H), 4.16 (s, 3H), 6.03 (t, J = 6.0 Hz, 1H), 6.86 (t, J = 7.2 Hz, 1H), 7.01 (t, J = 5.4 Hz, 1H), 7.08 (t, J = 7.2 Hz, 1H), 7.19 (t, J = 7.2 Hz, 2H), 7.28 (t, J = 7.2 Hz, 1H), 7.34 (d, J = 7.2 Hz, 2H), 7.42 (t, J = 7.8 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.78 (t, J = 7.8 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 8.34 (s, 1H), 8.53 (d, J = 8.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 25.90, 26.03, 29.60, 30.75, 32.22, 38.82, 48.00, 104.48, 115.06, 115.61, 116.50, 117.52 (2C), 117.96, 120.59, 120.86, 121.98, 123.98, 124.09, 124.59, 128.61 (2C), 130.66, 137.37, 140.58, 148.35, 152.10, 155.15, 156.34. HPLC purity 96.1%. HRMS (ESI) calcd for $C_{29}H_{30}N_5O$ $[M - H]^-$. Exact mass: 464.2456, found 464.2480.

*1-(3-(2-Bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)-propyl)-3-phenylurea (**17e**).* Yield 80%; yellow solids; mp 132−135 °C. IR (KBr) 3347, 3050, 2938, 1680, 1616, 1591, 1557, 1499, 1487, 1443, 1424, 1314, 1281, 1246, 1200, 1111, 1086, 885, 799, 760, 741, 694 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.88 (quint, J = 6.6 Hz, 2H), 3.14 (q, J = 6.0 Hz, 2H), 3.84 (q, J = 6.6 Hz, 2H), 4.13 (s, 3H), 6.18 (t, J = 6.0 Hz, 1H), 6.87 (t, J = 7.2 Hz, 1H), 7.07 (t, J = 7.2 Hz, 1H), 7.19 (t, J = 7.2 Hz, 3H), 7.28 (t, J = 7.2 Hz, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 7.8 Hz, 1H), 7.80 (m, 1H), 7.90 (d, J = 7.2 Hz, 2H), 8.41 (s, 1H), 8.78 (d, J = 1.8 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.46, 32.37, 36.50, 45.38, 105.23, 112.92, 116.74, 117.35, 117.38, 117.70 (2C), 118.28, 121.01, 122.37, 124.02, 124.94, 125.92, 128.59 (2C), 132.94, 136.32, 140.40, 146.99, 152.49, 155.48, 156.32. HPLC purity 96.6%. HRMS (ESI) calcd for $C_{26}H_{23}BrN_5O$ $[M - H]^-$. Exact Mass: 500.1091, found 500.1087.

*1-(3-(2-Chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)-propyl)-3-phenylurea (**17f**).* Yield 83%; yellow solids; mp 130−133 °C. IR (KBr) 3347, 3051, 2938, 1680, 1622, 1595, 1557, 1499, 1443, 1422, 1314, 1281, 1246, 1200, 1119, 991, 800, 760, 743, 694 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.89 (quint, J = 6.6 Hz, 2H), 3.14 (q, J = 6.0 Hz, 2H), 3.84 (q, J = 6.6 Hz, 2H), 4.14 (s, 3H), 6.17 (t, J = 6.0 Hz, 1H), 6.87 (t, J = 7.2 Hz, 1H), 7.07 (t, J = 7.2 Hz, 1H), 7.17 (m, 3H), 7.29 (t, J = 7.2 Hz, 1H), 7.34 (d, J = 7.8 Hz, 2H), 7.50 (d, J = 7.8 Hz, 1H), 7.80 (dd, J = 9.0, 2.4 Hz, 1H), 7.86 (d, J = 9.6 Hz, 1H), 7.91 (d, J = 7.2 Hz, 1H), 8.40 (s, 1H), 8.67 (d, J = 2.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.45, 32.41, 36.49, 45.41, 105.32, 116.70, 116.89, 117.10, 117.69 (2C), 118.27, 121.00, 122.37, 123.02, 123.96, 124.95, 125.14, 128.57 (2C), 130.25, 136.01, 140.39, 147.06, 152.45, 155.46, 156.32. HPLC purity 97.2%. HRMS (ESI) calcd for $C_{26}H_{25}ClN_5O$ $[M + H]^+$. Exact mass: 458.1742, found 458.1747.

*1-(3-(2-Fluoro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)-propyl)-3-phenylurea (**17g**).* Yield 87%; yellow solids; mp 218−220 °C. IR (KBr) 3339, 3053, 2926, 1690, 1613, 1599, 1566, 1499, 1487, 1445, 1400, 1317, 1281, 1234, 1144, 878, 795, 760, 692 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.84 (quint, J = 6.6 Hz, 2H), 3.11 (q, J = 6.0 Hz, 2H), 3.82 (q, J = 6.6 Hz, 2H), 4.14 (s, 3H), 6.13 (t, J = 6.0 Hz, 1H), 6.84 (t, J = 7.2 Hz, 1H), 6.99 (t, J = 6.0 Hz, 1H), 7.04 (t, J = 7.2 Hz, 1H), 7.16 (t, J = 7.8 Hz, 2H), 7.26 (t, J = 7.2 Hz, 1H), 7.31 (dd, J = 8.4, 1.2 Hz, 2H), 7.47 (d, J = 7.8 Hz, 1H), 7.67 (m, 1H), 7.86 (dd, J = 9.6, 4.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 8.37 (s, 1H), 8.42 (dd, J = 10.8, 2.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.49, 32.50, 36.49, 45.42, 105.66, 109.04 (d, J = 24.1 Hz), 116.44 (d, J = 7.8 Hz), 116.55, 117.16 (d, J = 8.4 Hz), 117.69 (2C), 118.08, 118.53 (d, J = 23.5 Hz), 121.00, 122.36, 123.73, 124.99, 128.57 (2C), 134.15, 140.39, 147.27 (d, J = 2.9 Hz), 152.65, 155.44, 156.45, 156.65 (d, J = 237.2 Hz). ^{19}F NMR (564 MHz, DMSO-d_6) δ ppm −121.25. HPLC purity 98.9%. HRMS (ESI) calcd for $C_{26}H_{23}FN_5O$ $[M - H]^-$. Exact mass: 440.1892, found 440.1890.

*1-(3-(5-Methyl-2-(trifluoromethyl)-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (**17h**).* Yield 77%; yellow solids; mp 204−205 °C. IR (KBr) 3335, 3055, 2938, 1688, 1614, 1597, 1553, 1499, 1443, 1435, 1333, 1317, 1277, 1242, 1196, 1148, 1119, 1090, 912, 816, 766, 752, 725 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.90 (quint, J = 6.6 Hz, 2H), 3.15 (q, J = 6.0 Hz, 2H), 3.88 (q, J = 6.6 Hz, 2H), 4.19 (s, 3 H), 6.17 (t, J = 6.0 Hz, 1H), 6.87 (t, J = 7.2 Hz, 1H), 7.10 (t, J = 7.2 Hz, 1H), 7.19 (t, J = 7.8 Hz, 2H), 7.29−7.34 (m, 3H), 7.46 (t, J = 6.0 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 9.0 Hz, 1H), 8.05 (dd, J = 9.0, 1.2 Hz, 1H), 8.39 (s, 1H), 8.94 (s, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.47, 32.55, 36.45, 45.34, 105.04, 115.37, 116.16, 116.97, 117.70 (2C), 118.66, 120.95, 121.03, 121.73, 121.75, 123.22 (q, J = 244.6 Hz), 123.80, 124.98, 126.38, 128.57 (2C), 139.33, 140.39, 147.61, 152.25, 155.52, 156.46. ^{19}F NMR (564 MHz, DMSO-d_6) δ ppm −58.57. HPLC purity 100%. RMS (ESI) calcd for $C_{27}H_{23}F_3N_5O$ $[M - H]^-$. Exact mass: 490.1860, found 490.1889.

*1-(3-(2-Methoxy-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (**17i**).* Yield 98%; yellow solids; mp 122−124 °C. IR (KBr) 3383, 3050, 2936, 1682, 1614, 1597, 1568, 1532, 1499, 1445, 1314, 1288, 1246, 1142, 1036, 804, 758, 694 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.84 (quint, J = 6.6 Hz, 2H), 3.15 (q, J = 6.6 Hz, 2H), 3.85 (q, J = 6.6 Hz, 2H), 3.94 (s, 3H), 4.15 (s, 3H), 6.19 (t, J = 6.0 Hz, 1H), 6.88 (t, J = 7.2 Hz, 1H), 7.02−7.06 (m, 2H), 7.20 (t, J = 7.8 Hz, 2H), 7.27 (t, J = 7.2 Hz, 1H), 7.35 (d, J = 7.2 Hz, 2H), 7.45−7.49 (m, 2H), 7.81 (d, J = 9.6 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 2.4 Hz, 1H), 8.43 (s, 1H). ^{13}C NMR (150.8

MHz, DMSO-d_6) δ ppm 31.74, 32.31, 36.45, 45.19, 55.87, 105.69, 105.86, 116.32, 116.35, 116.50, 117.65, 117.76 (2C), 119.65, 121.06, 122.19, 123.81, 124.75, 128.59 (2C), 132.20, 140.38, 147.72, 152.65, 153.66, 155.60, 156.21. HPLC purity 100%. HRMS (ESI) calcd for $C_{27}H_{26}N_5O_2$ $[M - H]^-$. Exact mass: 452.2092, found 452.2102.

*1-(3-(5-Methyl-2-nitro-5H-indolo[2,3-b]quinolin-11-ylamino)-propyl)-3-phenylurea (**17j**).* Yield 92%; orange solids; mp 222−225 °C. IR (KBr) 3360, 2930, 1649, 1616, 1568, 1501, 1439, 1424, 1323, 1290, 1240, 1121, 1071, 814, 754, 739, 694 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.92 (quint, J = 6.6 Hz, 2H), 3.15 (q, J = 6.0 Hz, 2H), 3.90 (q, J = 6.6 Hz, 2H), 4.17 (s, 3H), 6.15 (t, J = 6.0 Hz, 1H), 6.87 (t, J = 7.2 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 7.18 (t, J = 7.8 Hz, 2H), 7.30 (m, 3H), 7.54 (d, J = 7.8 Hz, 1H), 7.65 (t, J = 6.0 Hz, 1H), 7.94−7.97 (m, 2H), 8.36 (s, 1H), 8.50 (dd, J = 9.6, 2.4 Hz, 1H), 9.50 (d, J = 2.4 Hz, 1H). ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.31, 32.87, 36.48, 45.54, 104.83, 115.20, 116.10, 117.28, 117.66 (2C), 119.15, 121.00, 121.02, 122.41, 124.12, 124.55, 125.07, 128.56 (2C), 140.28, 140.37, 141.00, 147.68, 152.05, 155.42, 156.24. HPLC purity 95.7%. HRMS (ESI) calcd for $C_{26}H_{23}N_6O_3$ $[M - H]^-$. Exact mass: 467.1837, found 467.1835.

Biological Testing Assay. *Activity against Plasmodium falciparum.* In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^3H-hypoxanthine incorporation assay,[41,42] using the chloroquine and pyrimethamine resistant *P. falciparum* K1 strain that originated from Thailand (Thaitong et al. 1983)[43] and a strain susceptible to known antimalarial drugs (*P. falciparum* NF54) (Ponnudurai et al. 1981),[44] 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 (5 g/L), and washed human red cells A^+ at 2.5% hematocrit (0.3% parasitaemia). Serial drug dilutions of 11 three-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, and 93% N_2. After 48 h, 50 μL of ^3H-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 fiber 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)[45] using Microsoft Excel.

Activity against Trypanosoma brucei rhodesiense. Trypanosoma brucei rhodesiense, STIB900 strain, and the standard drug, melarsoprol, were used for the assay. This stock was isolated in 1982 from a human patient in Tanzania and after several mouse passages cloned and adapted to axenic culture conditions (Baltz et al 1985)[46] Minimum Essential Medium (50 μL) supplemented with 25 mM HEPES, 1g/L additional glucose, 1% MEM nonessential amino acids (100×), 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate, and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions of 11 three-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. Then 4000 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 μL was added to each well and the plate incubated at 37 °C under a 5% CO_2 atm for 70 h. Then 10 μL of Alamar Blue (resazurin, 12.5 mg in 100 mL of double-distilled water) was added to each well, and incubation continued for a further 2−4 h (Raz et al 1997).[47] Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated by linear regression (Huber 1993)[45] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

Activity against Trypanosoma cruzi. Rat skeletal myoblasts (L6 cells) were seeded in 96-well microtiter plates at 2000 cells/well in 100 μL of RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, the medium was removed and replaced by 100 μL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene (Buckner et al. 1996).[48] After 48 h, the medium was removed from the wells and replaced by 100 μL of fresh medium with or without a serial drug dilution of 11 three-fold dilution steps covering a range from 100 to 0.002 μg/mL. After 96 h of incubation, the plates were inspected under an inverted microscope to ensure growth of the controls and sterility. Then the substrate CPRG/Nonidet (50 μL) was added to all wells. A color reaction developed within 2−6 h and could be read photometrically at 540 nm. Data were analyzed with the graphic program Softmax Pro (Molecular Devices), which calculated IC_{50} values by linear regression (Huber 1993)[45] from the sigmoidal dose inhibition curves.

Activity against Leishmania donovani (Axenic Amastigotes). Amastigotes of *L. donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37 °C in SM medium (Cunnigham et al. 1977)[49] at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO_2 in air. Then 100 μL of culture medium with 10^5 amastigotes from axenic culture with or without a serial drug dilution were seeded in 96-well microtiter plates. Serial drug dilutions of 11 three-fold dilution steps covering a range from 90 to 0.002 μg/mL were prepared. After 70 h of incubation, the plates were inspected under an inverted microscope to ensure growth of the controls and sterile conditions. Then 10 μL of Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) (Mikus and Steverding. 2000)[50] were 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 wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (= inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. From the sigmoidal inhibition curves the IC_{50} values were calculated by linear regression (Huber 1993).[45]

Cytotoxicity against L6 Cells. 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).[51,52] Serial drug dilutions of 11 three-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 ensure growth of the controls and sterile conditions. Then 10 μL of Alamar Blue was added to each well and the plates incubated for another 2 h. The plates were then read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated by linear regression (Huber 1993)[45] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

■ ASSOCIATED CONTENT

Supporting Information

HPLC purities of target compounds, a detailed description of the in vivo drug screening model against *Plasmodium berghei* in Swiss mice, and spectroscopic details of the synthesized compounds **6a−h**, **9a−d**, **11**, **13a−b**, **15**, and **17a−j**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +81-86-251-8210; Fax: +81-86-251-8021. E-mail: inokuchi@cc.okayama-u.ac.jp.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CQ, chloroquine; CQS, chloroquine-sensitive; CQR, chloroquine-resistant; ACTs, artemisinin-based combination therapies; SAR, structure−activity relationship; DMF, dimethylformamide; DCC, *N*,*N*′-dicyclohexylcarbodiimide; THF, tetrahydrofuran; SI, selectivity index; RI, resistance index; DMSO, dimethyl sulfoxide; AcOEt, ethyl acetate; MeOH, methanol; TFA, trifluoroacetic acid; TLC, thin layer chromatography; NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; *L. donovani*, *Leishmania donovani*; L6, toxicity of the tested compounds assessed against a mammalian primary cell line derived from rat skeletal myoblasts; *T. b. rhodesiense*, *Trypanosoma brucei* rhodesiense; *T. cruzi*, *Trypanosoma cruzi*; *P. falciparum*, *Plasmodium falciparum*

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