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Synthesis and in vitro antiproliferative activity of new 11-aminoalkylamino-substituted 5*H*- and 6*H*-indolo[2,3-*b*]quinolines; structure–activity relationships of neocryptolepines and 6-methyl congeners

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ABSTRACT

The present report describes the synthesis and antiproliferative evaluation of certain 11-aminoalkylamino-substituted 5*H*- and 6*H*-indolo[2,3-*b*]quinolines and their methylated derivatives. These 5-Me- and 6-Me-indolo[2,3-*b*]quinoline derivatives **10–14**, **20** were prepared by amination at the C-11 position of the 11-chloro-5-methyl-5*H*- and 11-chloro-6-methyl-6*H*-indolo[2,3-*b*]quinolines with different substituents on the quinoline ring. The 11-aminoalkylaminomethylated **23**, the homologue of **11**, was prepared from the same intermediate for a further SAR study. These intermediates are accessible from 4-substituted anilines or their N-methylated analogues and methyl indole-3-carboxylate as a counterpart. The in vitro antiproliferative assay indicated that the 5-methylated derivatives **10–14** are more cytotoxic than their respective 6-methylated 6*H*-indolo[2,3-*b*]quinoline derivatives **20**. Among them, *N*-(3-aminopropyl)-2-bromo-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine **12f** was the most cytotoxic with a mean IC_{50} value of 0.12 μM against human leukemia MV4-11 cell line, and also exhibited selective cytotoxicities against A549 (lung cancer), HCT116 (colon cancer) cell lines and normal fibroblast BALB/3T3 with IC_{50} values of 0.543, 0.274 and 0.869 μM, respectively. The binding constant of products **12f** and **20f** to salmon fish sperm DNA were also evaluated using UV–vis absorption spectroscopy, indicating intercalation binding with a constant of 2.93×10^5 and 3.28×10^5 L mol^{-1}, respectively.

1. Introduction

Nature is a treasured source to find and develop new therapeutic agents.[1] Indoloquinolines are unique natural alkaloids found almost exclusively in the West African climbing shrub *Cryptolepis sanguinolenta* which are used in traditional medicine for the treatment of infectious diseases, including malaria.[2] Among the various isolated products with indoloquinoline structures,[3] cryptolepine **i** (5-methylindolo[3,2-*b*]quinoline), and neocryptolepine **ii** (5-methylindolo[2,3-*b*]quinoline) (Fig. 1), a minor alkaloid, have been intensively studied for their biological activities, namely antibacterial,[4a–c] antifungal,[4a–c] and antimalarial.[4d–f]

Due to the linearly arranged tetracyclic plane structure, cryptolepine **i** is a DNA intercalating agent and inhibits topoisomerase II, showing a high level of cytotoxicity.[5] On the other hand, neocryptolepine **ii**, a regioisomer of **i** in the orientation of indole moiety to the quinoline ring, can also intercalate into DNA, but its affinity

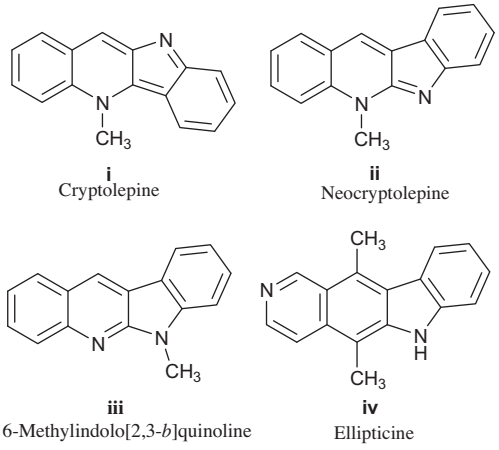

Figure 1. Structures of cryptolepine **i**, neocryptolepine **ii**, 6-methylindolo[2,3-*b*]quinoline **iii**, and ellipticine **iv**.

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is slightly weaker compared with **i**, and its capability to inhibit topoisomerase II is reduced.[5c]

Regarding the structurally related anticancer drug ellipticine **iv** (Fig. 1),[6] a tetracyclic 2-aza analogue with the same indole core, a series of 5,11-dimethylindolo[2,3-*b*]quinolines derivatives have been tested for their chemotherapeutic activities,[7] showing a cytotoxicity against several human cancer cell lines, with IC_{50} value ranging from 0.6 to 1.4 μM.[7c] Furthermore, the 6,11-dimethylindolo[2,3-*b*]quinolines,[8] the 6*H* congener of neocryptolepine, showed a cytotoxicity against KB cell lines with IC_{50} value ranging from 2.1 to 9 μM.[8b] In these studies, the substituents, such as the Me and MeO groups, introduced into the indolo[2,3-*b*]quinoline core were shown to have highly infectious activities. Later, the 11-anilinoindolo[2,3-*b*]quinolines were synthesized and their anticancer activity as bioisosteric compounds of acridine derivatives were evaluated,[9] which have been extensively studied as potential chemotherapeutic agents due to their capability of intercalating DNA that leading to the inhibition of mammalian topoisomerase II.

However, until now, the types of examined substituents on the 11-aminoindolo[2,3-*b*]quinolines have been limited, and the effect of halogens, such as Cl and Br, were scarcely discussed. Recently, aminoalkylamino-substituted neocryptolepine was shown to be 1500-fold efficacious in comparison to the natural product itself against the chloroquine-sensitive *Plasmodium falciparum* Ghana strain.[10] Based on these facts, we examined the introduction of the alkylamino and aminoalkylamino groups at the C-11 positions of indolo[2,3-*b*]quinoline skeleton with varying the kinds of substituents at the C-2 positions. In this study, we investigated the effects of substituents types at the C-2 and C-11 positions on antiproliferative activity in vitro in the 5-methyl-**ii** and 6-methylindolo[2,3-*b*]quinoline series **iii**.

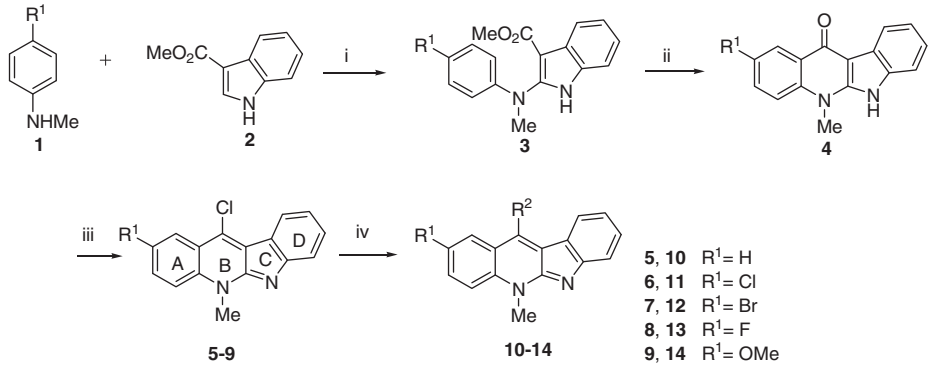
2. Chemistry

The preparations of the 11-chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolines **5**–**9**, the scaffold for the synthesis of the 11-amino derivatives **10**–**14** are shown in Scheme 1.[11] Thus, the *N*-methylanilines **1** and methyl indole-3-carboxylate **2** were oxidatively combined by the action of *N*-chlorosuccinimide (NCS), giving **3**, which was converted into the tetracyclic ketones **4** upon heating at 250 °C in diphenyl ether. The treatment of **4** with $POCl_3$ afforded the desired chlorides **5**–**9**.[11c]

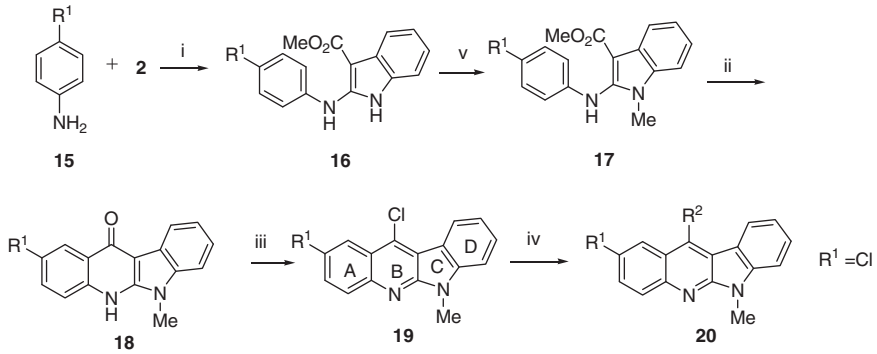
On the other hand, the 11-chloro-6-methyl congeners **19** were prepared starting from anilines **15**. Thus, the treatment of **16**, prepared from **15** and **2** by oxidation with NCS, with NaH–MeI in THF afforded the 2-arylamino-1-methylindoles-3-carboxylates **17** as a result of methylation at the nitrogen of the indole ring.[11c] The cyclization of **17** to **18** was smoothly achieved by heating at reflux in diphenyl ether, and the subsequent dehydroxy chlorination of the resulting **18** with $POCl_3$ afforded the desired **19**.

As shown in Schemes 1 and 2, these 11-chloro-5-methyl- and 11-chloro-6-methylindolo[2,3-*b*]quinolines **5–9** and **19** were converted into the corresponding 11-amino derivatives **10–14** and **20**, respectively. The results shown in Table 1 and 3 indicate that, in general, the 11-chloroneocryptolepines **5–9** are prone to undergo addition-elimination reactions of the amine nucleophiles of the linear and cyclic primary amines at 70–130 °C, giving **10**–**14** in good to excellent yields. On the other hand, the amination of the 6-methyl congeners **19** with linear and cyclic primary amines were performed in DMF at 120 °C for a longer reaction time, giving the corresponding amine adducts **20** in slightly lower yields.

In addition to these 11-aminoalkylamino derivatives **10–14** and **20**, we prepared the 11-aminoalkylaminomethylated **23**, the



Scheme 1. Preparation of 11-chloroneocryptolepines **5**–**9** and their 11-amino derivatives **10**–**14**. Reagents and conditions: (i) (a) *N*-chlorosuccinimide, 1, 4-dimethylpiperazine. (b) trichloroacetic acid; (ii) diphenyl ether, reflux; (iii) $POCl_3$, toluene, reflux; (iv) appropriate amines.



Scheme 2. Preparation of 11-chloro-6-methylindolo[2,3-*b*]quinolines **19** and their 11-amino derivatives **20**. Reagents and conditions: (i) (a) *N*-chlorosuccinimide, 1, 4-dimethylpiperazine; (b) trichloroacetic acid; (ii) diphenyl ether, reflux; (iii) $POCl_3$, toluene, reflux; (iv) appropriate amines; (v) NaH, MeI, THF.

Table 1
Yields of 11-alkylaminated 5-methyl-indolo[2,3-*b*]quinolines and their antiproliferative activity against human leukemia MV4-11 cell line

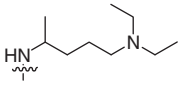
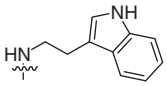
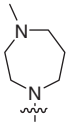
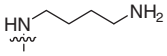
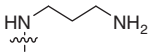
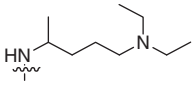
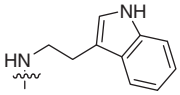
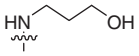
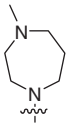
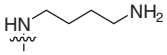
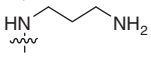
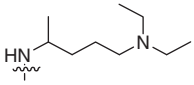
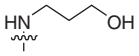
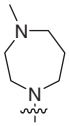
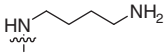
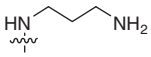
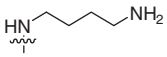
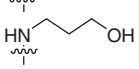
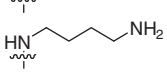
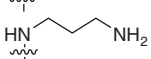
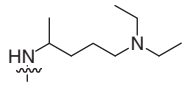
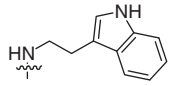
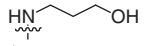
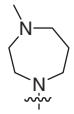
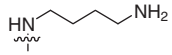
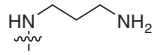
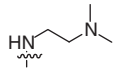
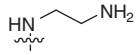
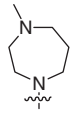
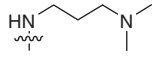
Compound	R^1	R^2	Yield (%)	MV4-11 IC_{50} (μM)
Cisplatin				2.820 ± 0.450
Doksorubicin HCl				0.006 ± 0.002
5	H	Cl		1.312 ± 0.262
7	Br	Cl		0.810 ± 0.145
9	OMe	Cl		1.206 ± 0.182
10a	H		98	0.721 ± 0.154
10b	H		90	0.871 ± 0.256
10c	H		94	0.170 ± 0.010
10d	H		94	7.171 ± 0.987
10e	H		96	0.100 ± 0.035
10f	H		45	0.066 ± 0.023
10g	H		92	0.164 ± 0.047
11a	Cl		55	0.780 ± 0.142
11b	Cl		67	0.447 ± 0.212
11c	Cl		27	0.530 ± 0.118
11d	Cl		48	0.106 ± 0.053
11e	Cl		86	0.680 ± 0.028
11f	Cl		97	0.068 ± 0.018
12a	Br		64	0.578 ± 0.043
12c	Br		70	0.703 ± 0.088
12d	Br		82	0.737 ± 0.208
12e	Br		96	0.091 ± 0.015
12f	Br		63	0.012 ± 0.002
13e	F		94	0.095 ± 0.012
14c	OMe		66	0.135 ± 0.052
14e	OMe		79	0.132 ± 0.017
14f	OMe		80	0.102 ± 0.021

Table 2
Antiproliferative activity of 11-alkylaminated 5-methyl-indolo[2,3-*b*]quinolines against normal mice fibroblast BALB/3T3 and against cancer cell lines A549 and HCT116

Compound	BALB/3T3 IC_{50} (μM)	A549 IC_{50} (μM)	HCT116 IC_{50} (μM)
Cisplatin	8.700 ± 0.970	9.870 ± 2.400	8.500 ± 0.540
Doksorubicin HCl	1.078 ± 0.033	0.329 ± 0.097	0.390 ± 0.098
10c	0.635 ± 0.190	0.973 ± 0.239	0.714 ± 0.085
10e	0.936 ± 0.069	0.911 ± 0.113	0.669 ± 0.229
10f	0.884 ± 0.115	0.205 ± 0.079	0.302 ± 0.056
10g	1.607 ± 0.624	1.509 ± 0.410	1.795 ± 0.277
11d	6.413 ± 2.278	7.390 ± 3.880	1.750 ± 0.335
11f	0.401 ± 0.015	0.761 ± 0.169	0.195 ± 0.044
12c	1.197 ± 0.807	1.587 ± 0.729	0.989 ± 0.234
12d	7.600 ± 0.260	4.535 ± 2.031	1.160 ± 0.097
12e	0.742 ± 0.091	0.579 ± 0.204	0.209 ± 0.111
12f	0.869 ± 0.018	0.543 ± 0.256	0.274 ± 0.050
13e	0.853 ± 0.104	0.785 ± 0.169	0.178 ± 0.048
14c	0.807 ± 0.054	1.010 ± 0.069	0.278 ± 0.160
14e	1.622 ± 0.537	0.674 ± 0.189	0.657 ± 0.382
14f	0.978 ± 0.021	0.407 ± 0.117	0.155 ± 0.042

Table 3
Yields of 11-alkylaminated 6-methyl-indolo[2,3-*b*]quinolines and their antiproliferative activity against human leukemia MV4-11 cell line

Compound	R^1	R^2	Yield (%)	MV4-11 IC_{50} (μM)
Cisplatin				2.820 ± 0.450
Doksorubicin HCl				0.006 ± 0.002
20a	Cl		27	5.248 ± 0.804
20b	Cl		28	18.827 ± 3.106
20c	Cl		70	6.209 ± 0.824
20d	Cl		35	0.773 ± 0.346
20e	Cl		26	0.669 ± 0.312
20f	Cl		61	0.800 ± 0.159
20g	Cl		27	>25
20h	Cl		44	2.834 ± 0.876
20i	Cl		50	0.456 ± 0.123
23d	Cl		36	3.436 ± 0.585
23j	Cl		56	3.465 ± 0.814

homologue of **11**, from **6** for a SAR study (Scheme 3). Thus, the reaction of **6** with sodium nitromethylate afforded the adduct of nitromethane, **21**, which was treated with $KMnO_4$ to give the C-11 formylated **22**. The reductive amination of **22** with the appropriate amines using $NaBH_4$ as a hydride donor afforded **23**.

3. Biological results and discussion

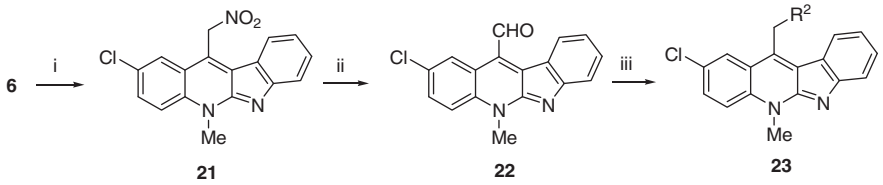
The results of the cytotoxic activity in vitro were expressed as IC_{50}-the concentration of the compound (in μM) that inhibits proliferation of the cells by 50% as compared to the untreated control cells. The IC values were separately calculated for each experiment and the mean values ± SD were calculated from at least 3–5 independent experiments.

The results of the studies on the antiproliferative activity of the neocryptolepine analogues against human leukemia MV4-11 cells are summarized in Table 1, along with the results of anticancer drugs, cisplatin and doksorubicin HCl. All tested compounds, except the **10d**, were cytotoxic against the MV4-11 leukemia cells (IC_{50} below 0.9 μM), and their antiproliferative activity is higher than the activity of the known anticancer drug, cisplatin (IC_{50} 2.82 μM). The most potent of all the tested compounds was **12f** (IC_{50} 0.012 μM) with a 3-aminopropylamino group at the 11-position. A very high antiproliferative activity was also revealed for compounds **10c**, **10e**–**g**, **11b**, **11d**, **11f**, **12e**, **13** and **14** of IC_{50} between 0.06–0.45 μM. A halogen substituent at the 2-position can influence the antiproliferative activity. In some cases, the halogen substitution can increase the antiproliferative activity. Thus, The 11-aminopropylamino-2-bromo-indolo[2,3-*b*]quinoline **12f** was about 4 times more active than the corresponding 11-aminopropylamino-2*H*-indolo[2,3-*b*]quinoline **10f** against MV4-11 cells. The same trend was also observed for compounds **10b** and **11b**. On the contrary, for the 3-hydroxypropylamino-substituted agents **10c**, **11c**, **12c** and **14c,** the antiproliferative activity decreased about 4 times when the H at the C2 was changed by Cl or Br. Good antiproliferative activity against MV4-11 cells was also observed when an electron-donating group –MeO was introduced as a substituent at the C-2 position.

Accordingly, these compounds, **10c**, **10e**–**g**, **11d**, **11f**, **12c**–**f**, **13** and **14**, were then chosen for next study: the antiproliferative activity against human lung (A549) and colon (HCT116) cancer cell lines and normal murine fibroblasts (BALB/3T3). These results are summarized in Table 2. All the tested compounds were cytotoxic against the A549 and HCT116 cancer cells, and their antiproliferative activities against the cancer cells were much higher than the activity of cisplatin used as control agent (Table 2). Compound **10f**, **12e**, **12f** and **14f** have high antiproliferative activities against cancer cell lines A549 and also HCT116, but a lower cytotoxicity. Against the normal fibroblast BALB/3T3, their antiproliferative activities were 3–4 times lower than that against cancer cells. Compound **11d**, **11f**, **13e** and **14c** have a selective antiproliferative activity, mostly against the HCT116 cell line. Against the A549 cancer cell line and normal fibroblast BALB/3T3 cell line, the activities were about 4 times lower. It is quite obvious that the introduction of proper alkylamino substituents into biologically active derivatives can favorably influence their activities and selectivities in DNA binding.

An SAR study of indolo[2,3-*b*]quinoline from series 5*H*- and 6*H*-series showed that the 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline is an inactive isomer of 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline (DIMIQ).[7c] Therefore, we hoped that the 6*H*-series might acquire an activity when proper substituent was introduced.

Similarly, the antiproliferative activity of the 6-methylated congeners **20** were tested and summarized in Tables 3 and 4. Generally, the 6-methylated derivatives were still less cytotoxic than their respective 5-methylated analogues. The activities of 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline series were improved when proper amino groups were introduced at the C-11. Only compounds **20b** and **20g** were not cytotoxic against MV4-11 leukemia cells. Compounds **20d-f**, and **20i** showed the IC_{50} value between 0.46–0.80 μM against MV4-11 leukemia cells, which were higher than the known anticancer drug cisplatin.



Scheme 3. Preparation of 11-aminoalkylaminomethylated neocryptlepine **23**. Reagents and conditions: (i) CH_3NO_2, NaH; (ii) $KMnO_4$; (iii) appropriate amines, $NaBH_3CN$.

Table 4
Antiproliferative activity of 11-alkylaminated 6-methylindolo[2,3-*b*]quinolines against normal mice fibroblast BALB/3T3 and against cancer cell lines A549 and HCT116

Compound	BALB/3T3 IC_{50} (μM)	A549 IC_{50} (μM)	HCT116 IC_{50} (μM)
Cisplatin	8.700 ± 0.970	9.870 ± 2.400	8.500 ± 0.540
Doksorubicin HCl	1.078 ± 0.033	0.329 ± 0.097	0.390 ± 0.098
20d	>25	11.24 ± 0.950	8.000 ± 0.369
20e	9.012 ± 1.304	6.093 ± 0.595	5.498 ± 2.182
20f	9.798 ± 0.354	7.378 ± 0.974	4.486 ± 1.417
20i	10.437 ± 0.400	8.282 ± 0.585	6.989 ± 1.416

From the results summarized in Table 4, we can see that the antiproliferative activities of these four compounds against human lung (A549) and colon (HCT116) cancer cell lines are comparable with the cisplatin. Against normal fibroblast BALB/3T3, compound **20d** showed selectivity, which was not cytotoxic (IC_{50} higher than 25 μM).

The 11-aminoalkylaminomethylated neocryptolepine derivatives **23**, the homologs of **20**, were also tested, the results of which are listed in Table 3. Though the 11-aminoalkylaminomethylated neocryptolepine derivatives **23** were also cytotoxic against MV4-11 leukemia cells, the activity was significantly decreased when compared to the 11-aminated neocryptolepines or their congeners (**11d**, **20d** and **23d**).

Indeed, according to literature data, the inactive isomer of 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline (DIMIQ) can acquire activity after introduction of substituents.[9] On the other hand, introduction of proper aminoalkyl substituents into biologically active derivatives can favor its activity and selectivity in DNA binding.[8b] However, the cytotoxicity against normal in relation to cancer cells of these compounds was not described. Therefore, obtained new derivatives of indolo[2,3-*b*]quinoline possessing a high antiproliferative activity against cancer cells accompanied by a low cytotoxicity against normal fibroblasts can offer a new valuable group of potential anticancer compounds.

Further studies are needed to explain the mechanism of action of the obtained derivatives with selective activity against cancer cells.

4. Spectroscopic characterization of neocryptolepine derivative 12f and 20f interacting with salmon fish sperm DNA

The DNA binding studies of compounds **12f** or **20f** was performed using UV–vis absorption spectroscopy with salmon fish sperm DNA in phosphate buffer of pH 7.0 at 20 °C. The red shift and hypochromic effect were observed in the absorption spectra while the DNA solution was gradually added to the solution of

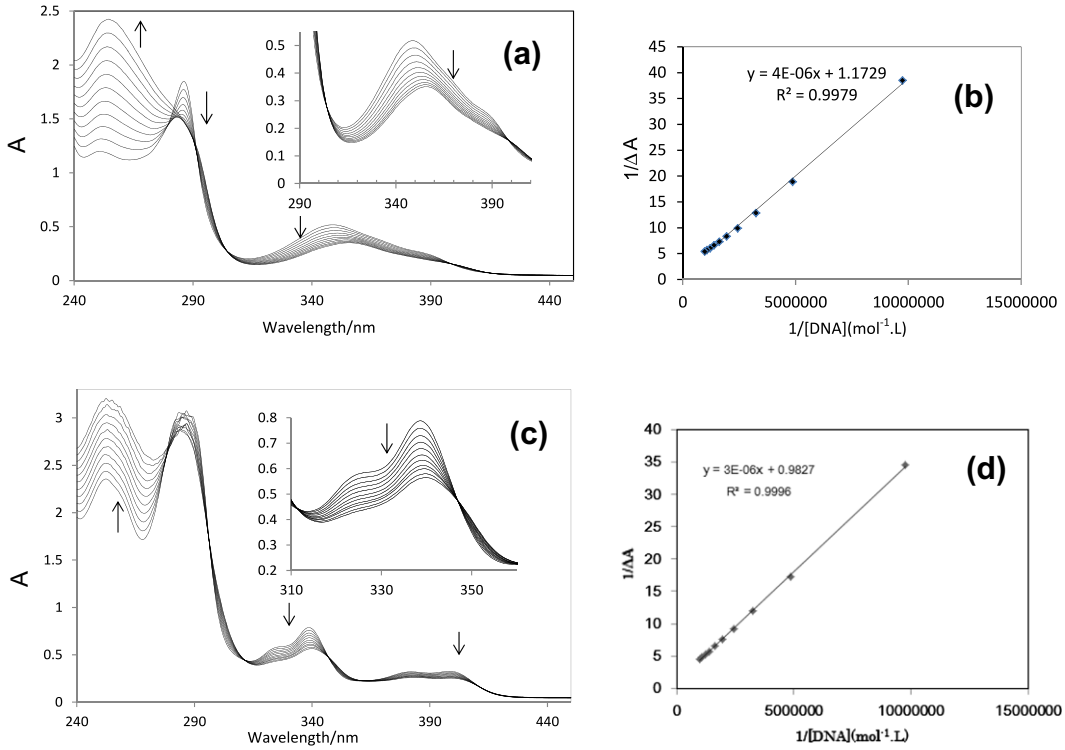


Figure 2. (a) UV–vis absorption spectra of compound **12f** and **12f**-DNA at 20 °C. C_{12f} = 50 μmol/L, C_{DNA} = 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 μmol/L for curve 1–11 in pH 7.0 phosphate buffer solution; (b) the plot of 1/ΔA vis 1/[DNA] for **12f**-DNA; (c) UV–vis absorption spectra of compound **20f** and **20f**-DNA at room temperature. C_{20f} = 50 μmol/L, C_{DNA} = 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 μmol/L for curve 1–11 in pH 7.0 phosphate buffer solution; (d) the plot of 1/ΔA vis 1/[DNA] for **20f**-DNA.

the compound **12f** or **20f**. The results in Figure 2 showed that the absorption band at 349 nm for the **12f** (a) decreased while increasing the DNA concentration. The maximum absorption shifted from 349 nm to a longer wavelength at 356 nm. It illustrated that the mode of **12f** binding to DNA was intercalation. Then the binding constant of **12f**-DNA and **20f**-DNA was calculated as 2.93×10^5 and 3.28×10^5 L mol^{-1}, respectively, according to double-reciprocal equation.[13]

5. Conclusion

We have described the synthesis of a series of 11-amino-substituted 5*H*- and 6*H*-indolo[2,3-*b*]quinolines, whose antiproliferative activities were evaluated using the MV4-11 (human leukemia), A549 (human lung cancer), HCT116 (human colon cancer), and normal mice fibroblast (BALB/3T3) cell lines. A few of the tested compounds showed very high antiproliferative activities against the MV4-11 leukemia cell line (IC_{50}: 0.012–0.450 μM). Some drug candidate compounds showed selective antiproliferative activity against cancer cell lines A549 or HCT116, but have lower cytotoxicities against the normal fibroblast BALB/3T3. These results indicated that the 11-amino group is important for their activity, especially the 3-aminopropylamino group, which could increase the activity against MV4-11 about 67 times compared to its precursor **7**. The antiproliferaive test indicated that the 5-methylated derivatives are usually more cytotoxic than their respective 6-methylated congeners.

6. Experimental

6.1. Chemistry

6.1.1. General

The commercially obtained reagents were used without further purification. The ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were measured on the Varian INOVA-600 or Varian INOVA-400 spectrometer. High resolution mass spectra were obtained on a Bruker micrOTOF II-SKA spectrometer. Melting points were determined on a J-Science RFS-10 hot stage microscope. The salmon fish sperm DNA was received as a gift from Maruha-nichiro company, and it was used directly without further purification. The UV spectra were recorded with Hitachi U-2910 spectrophotometer in a 1 cm path length quartz cuvette at indicated wavelength, and the sample was dissolved in phosphate buffer with 2.5% DMSO. The scaffolds **5**–**9** and **19** were prepared by the method we previously mentioned.[11c]

6.1.2. General procedure for the synthesis of 11-amino-2-chloro-5-methyl-5*H*-indolo[2,3-*b*]quinoline (10–14) and 11-amino-2-chloro-6-methyl-6*H*-indolo[2,3-*b*]quinoline 20

11-Chloro-indolo[2,3-*b*]quinoline and a large excess of the appropriate amine were heated together at 80−120 °C for 1–8 h. The reaction was monitored by TLC. Then the mixture was washed with water and extracted with AcOEt. The organic phase was dried over $MgSO_4$ and concentrated under vacuo. The crude product was purified by chromatography using eluent changed from AcOEt to AcOEt-2 N ammonia in MeOH (20: 1) to give final product.

6.1.2.1. *N*-(5-(Diethylamino)pentan-2-yl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (10a). Yield: 98%, yellow solid, Mp: 89–91 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 0.70 (t, J = 7.19 Hz, 6H), 1.13–1.27 (m, 2H), 1.44 (d, J = 6.46 Hz, 3H), 1.57–1.65 (m, 1H), 1.65–1.74 (m, 1H), 2.01–2.12 (m, 2H), 2.16 (q, J = 5.87 Hz, 4H), 4.18 (s, 3H), 4.29–4.39 (m, 1H), 6.57 (d, J = 10.27 Hz, 1H), 7.09 (m, 1H), 7.30 (m Hz, 1H), 7.43 (t, J = 1.17 Hz, 1H), 7.51 (d, J = 7.92 Hz, 1H), 7.80 (m, 1H), 7.86 (t, J = 6.90 Hz, 2H), 8.58 (dd, J = 8.36, 1.03 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 11.5, 21.6, 23.4, 32.2, 36.2, 46.0, 51.9, 52.6, 106.1, 115.0, 116.0, 116.6, 117.9, 120.6, 121.7, 124.1, 124.2, 124.9, 130.6, 137.4, 148.3, 152.5, 156.4; HRMS (ESI) Calcd for $C_{25}H_{31}N_4$ $[M-H]^-$ 387.2554. Found 387.2522.

6.1.2.2. *N*-(2-(1*H*-Indol-3-yl)ethyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (10b). Yield: 90%, yellow solid, Mp: 201–203 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 3.12 (t, J = 7.63 Hz, 2H), 4.09–4.15 (m, 2H), 4.16 (s, 3H), 6.91 (ddd, J = 7.92, 7.04, 0.88 Hz, 1H), 6.98–7.05 (m, 2H), 7.08 (d, J = 2.35 Hz, 1H), 7.12 (t, J = 5.87 Hz, 1H), 7.25–7.28 (m, 1H), 7.28–7.31 (m, 1H), 7.41 (ddd, J = 8.22, 7.04, 1.17 Hz, 1H), 7.47 (d, J = 7.92 Hz, 1H), 7.50 (d, J = 7.92 Hz, 1H), 7.77–7.81 (m, 1H), 7.82–7.85 (m, 1H), 7.87 (d, J = 7.63 Hz, 1H), 8.54 (dd, J = 8.36, 1.03 Hz, 1H), 10.80 (br s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 26.7, 32.2, 48.6, 104.7, 110.9, 111.4, 115.0, 115.6, 116.5, 117.9, 118.2, 118.3, 120.5, 121.0, 121.9, 122.9, 124.0, 124.1, 124.6, 127.0, 130.6, 136.1, 137.4, 148.1, 152.3, 156.4; HRMS (ESI) Calcd for $C_{26}H_{21}N_4$ $[M-H]^-$ 389.1772. Found 389.1760.

6.1.2.3. 3-(5-Methyl-5*H*-indolo[2,3-*b*]quinolin-11-ylamino)propan-1-ol (10c). Yield: 94%, yellow solid, Mp: 183–186 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.85 (quin, J = 6.38 Hz, 2H), 3.46 (t, J = 5.43 Hz, 2H), 3.90–3.98 (m, 2H), 4.16 (s, 3H), 4.60 (br s, 1H), 7.04 (t, J = 5.58 Hz, 1H), 7.06–7.10 (m, 1H), 7.26–7.31 (m, 1H), 7.41 (ddd, J = 8.14, 6.97, 1.03 Hz, 1H), 7.50 (d, J = 7.63 Hz, 1H), 7.76–7.81 (m, 1H), 7.82–7.87 (m, 1H), 7.97 (d, J = 7.63 Hz, 1H), 8.48 (dd, J = 8.22, 1.17 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 32.2, 33.5, 46.0, 58.5, 104.5, 115.0, 115.5, 116.4, 117.9, 120.5, 121.9, 124.0, 124.1, 124.5, 130.6, 137.4, 148.3, 152.0, 156.2; HRMS (ESI) Calcd for $C_{19}H_{18}N_3O$ $[M-H]^-$ 304.1455. Found 304.1461.

6.1.2.4. 5-Methyl-11-(4-methyl-1,4-diazepan-1-yl)-5*H*-indolo[2,3-*b*]quinoline (10d). Yield: 94%, orange solid, Mp: 106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.12–2.20 (m, 2H), 2.53 (s, 3H), 2.82–2.88 (m, 2H), 2.92–2.97 (t, J = 5.60 Hz, 2H), 3.70 (t, J = 6.06 Hz, 2H), 3.74–3.79 (m, 2H), 4.31 (s, 3H), 7.21–7.27 (m, 1H), 7.42 (ddd, J = 8.17, 5.14, 2.93 Hz, 1H), 7.48–7.55 (m, 1H), 7.65–7.80 (m, 3H), 8.19 (dd, J = 7.63, 0.59 Hz, 1H), 8.45–8.53 (d, J = 8.40 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 30.0, 32.9, 47.5, 52.0, 53.0, 58.3, 60.9, 114.3, 117.3, 119.3, 120.8, 121.2, 122.3, 122.9, 123.8, 126.6, 128.0, 130.1, 138.2, 151.4, 154.1, 157.8.

6.1.2.5. *N*-(4-Aminobutyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (10e). Yield: 96%, yellow solid, Mp: 69–70 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.44–1.49 (m, 2H), 1.70 (dt, J = 14.75, 7.15 Hz, 2H), 2.66 (t, J = 6.75 Hz, 2H), 3.73 (t, J = 6.90 Hz, 2H), 4.15 (s, 3H), 5.53 (br s, 1H), 7.15–7.19 (m, 1H), 7.24–7.28 (m, 1H), 7.40–7.44 (m, 1H), 7.53 (d, J = 8.22 Hz, 1H), 7.61 (ddd, J = 8.44, 6.97, 1.32 Hz, 1H), 7.76 (d, J = 7.92 Hz, 1H), 7.83 (d, J = 7.63 Hz, 1H), 8.04 (dd, J = 8.22, 1.17 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 29.1, 30.5, 32.5, 41.4, 48.9, 107.3, 114.5, 115.6, 117.1, 118.6, 120.3, 120.8, 123.9, 124.1, 125.6, 130.1, 137.8, 148.4, 152.4, 156.4; HRMS (ESI) Calcd for $C_{20}H_{21}N_4$ $[M-H]^-$ 317.1772. Found 317.1769.

6.1.2.6. *N*-(3-Aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (10f). Yield: 45%, yellow solid, Mp: 95–97 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.77–1.80 (m, 2H), 2.97–3.01 (t, J = 6.16 Hz, 2H), 3.99 (t, J = 6.16 Hz, 2H), 4.20 (s, 3H), 7.16 (td, J = 7.48, 1.17 Hz, 1H), 7.29 (ddd, J = 8.22, 7.04, 1.17 Hz, 1H), 7.41 (td, J = 7.48, 1.17 Hz, 1H), 7.56–7.61 (m, 1H), 7.65 (ddd, J = 8.51, 7.04, 1.47 Hz, 1H), 7.75 (d, J = 7.63 Hz, 1H), 7.95 (d, J = 7.63 Hz,

1H), 8.11 (dd, J = 8.36, 1.32 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 32.6, 32.7, 41.3, 49.3, 105.8, 114.4, 115.9, 117.0, 118.5, 120.3, 121.4, 124.1, 124.2, 125.2, 130.1, 137.9, 148.5, 152.2, 156.7.

6.1.2.7. 5-Methyl-11-morpholino-5*H*-indolo[2,3-*b*]quinoline (10g). Yield: 92%, red solid, Mp: 212–215 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 3.50–3.55 (m, 4H) 4.03–4.08 (m, 4H) 4.30 (s, 3H) 7.23–7.28 (m, 1H) 7.43 (ddd, J = 8.16, 6.84, 1.10 Hz, 1H) 7.52 (td, J = 7.61, 1.10 Hz, 1H) 7.68–7.76 (m, 3H) 8.33 (d, J = 7.94 Hz, 1H) 8.53 (dd, J = 8.27, 1.21 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 33.0, 49.2, 67.6, 114.3, 117.5, 119.4, 120.6, 121.2, 122.7, 123.2, 124.8, 126.1, 128.3, 130.3, 137.9, 149.8, 154.5, 157.6.

6.1.2.8. 2-Chloro-*N*-(5-(diethylamino)pentan-2-yl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (11a). Yield: 55%, brown solid, Mp: 76–78 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 0.71 (t, J = 6.60 Hz, 6H), 1.11–1.26 (m, 2H), 1.44 (d, J = 6.46 Hz, 3H), 1.55–1.64 (m, 1H), 1.64–1.73 (m, 1H), 2.01–2.13 (m, 2H), 2.13–2.23 (m, 4H), 4.16 (s, 3H), 4.29–4.38 (m, 1H), 6.73 (d, J = 10.27 Hz, 1H), 7.11 (t, J = 7.48 Hz, 1H), 7.32 (t, J = 7.80 Hz, 1H), 7.52 (d, J = 7.92 Hz, 1H), 7.77–7.83 (m, 2H), 7.88 (dd, J = 9.10, 0.88 Hz, 1H), 8.74 (d, J = 2.05 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 11.4, 21.4, 23.4, 32.5, 36.1, 46.0, 51.8, 52.4, 106.5, 116.8, 117.1, 117.3, 118.2, 122.0, 123.2, 124.0, 125.2, 125.3, 130.3, 136.0, 147.1, 152.6, 156.4; HRMS (ESI) Calcd for $C_{25}H_{30}ClN_4$ $[M-H]^-$ 421.2164. Found 421.2153.

6.1.2.9. *N*-(2-(1*H*-Indol-3-yl)ethyl)-2-chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (11b). Yield: 67%, yellow solid, Mp: 173–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.19 (t, J = 6.40 Hz, 2H), 4.18 (t, J = 6.40 Hz, 2H), 4.22 (s, 3H), 5.25 (brs, 3H), 7.03–7.07 (m, 2H), 7.12 (t, J = 7.20 Hz, 6H), 7.22–7.26 (m, 2H), 7.38–7.43 (m, 2H), 7.46 (d, J = 12.00 Hz, 1H), 7.55–7.64 (m, 3H), 7.75 (d, J = 7.60 Hz, 1H), 7.96 (d, J = 2.00 Hz, 1H), 8.29 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 26.5, 32.50, 48.6, 104.9, 110.9, 111.4, 116.5, 116.9, 117.0, 118.2, 118.3, 118.3, 120.9, 122.4, 122.9, 123.1, 123.8, 125.0, 125.2, 127.0, 130.3, 135.9, 136.1, 147.1, 151.7, 155.9; HRMS (ESI) Calcd for $C_{26}H_{20}ClN_4$ $[M-H]^-$ 423.1382. Found 423.1393.

6.1.2.10. 3-(2-Chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-ylamino)propan-1-ol (11c). Yield: 27%, yellowish green solid, Mp: 223–226 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.87–1.89 (m, 2H), 3.45–3.48 (q, J = 4.80 Hz, 2H), 3.95 (q, J = 6.00 Hz, 2H), 4.19 (s, 3H), 4.58 (t, J = 4.80 Hz, 1H), 7.11–7.16 (m, 2H), 7.33 (t, J = 7.80 Hz, 1H), 7.55 (d, J = 7.80 Hz, 1H), 7.84–7.86 (m, 1H), 7.92 (d, J = 9.00 Hz, 1H), 7.97 (d, J = 7.80 Hz, 1H), 8.66 (d, J = 2.40 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 32.4, 33.5, 45.7, 58.3, 105.0, 116.7, 116.8, 117.1, 118.2, 122.3, 123.0, 124.1, 124.8, 125.0, 130.2, 136.0, 147.1, 152.4, 156.3; HRMS (ESI) Calcd for $C_{19}H_{18}ClN_3O$ $[M-H]^-$ 338.1066. Found 338.1036.

6.1.2.11. 2-Chloro-5-methyl-11-(4-methyl-1,4-diazepan-1-yl)-5*H*-indolo[2,3-*b*]quinoline (11d). Yield: 48%, orange solid, Mp: 103–105 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.13–2.16 (m, 2H), 2.57 (s, 3H), 2.89 (t, J = 5.10 Hz, 2H), 2.95 (t, J = 5.40 Hz, 2H), 3.69–3.77 (m, 4H), 4.31 (s, 3H), 7.23 (t, J = 9.90 Hz, 1H), 7.53 (t, J = 7.20 Hz, 1H), 7.66 (d, J = 1.20 Hz, 2H), 7.75 (d, J = 8.10 Hz, 1H), 8.15 (d, J = 7.20 Hz, 1H), 8.61 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 30.3, 33.2, 47.7, 52.1, 53.0, 58.6, 60.7, 115.9, 117.6, 119,8, 122,2, 122.9, 123.2, 124.1, 126.1, 127.0, 128.6, 130.2, 136.8, 150.1, 154.5, 157.7.

6.1.2.12. *N*-(4-Aminobutyl)-2-chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (11e). Yield: 86%, yellow solid, Mp: 208–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.40–1.50 (m, 2H), 1.66 (dt, J = 14.23, 7.07 Hz, 2H), 2.66 (t, J = 6.65 Hz, 2H), 3.64 (t, J = 6.75 Hz, 2H), 4.00–4.07 (s, 3H), 5.59–5.91 (br s, 1H), 7.09–7.17 (m, 1H), 7.32–7.42 (m, 2H), 7.43–7.52 (m, 1H), 7.68 (d, J = 7.83 Hz, 1H), 7.76 (d, J = 7.63 Hz, 1H), 7.99 (d, J = 2.15 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 28.9, 30.3, 32.6, 41.3, 48.8, 107.4, 115.7, 116.6, 117.1, 118.8, 121.1, 123.5, 123.8 125.6, 125.8, 129.9, 136.1, 147.2, 152.4, 156.2.

6.1.2.13. *N*-(3-aminopropyl)-2-chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (11f). Yield: 97%, yellow solid, Mp: 123–125 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.73–1.77 (m, 2H), 2.61 (t, J = 6.46 Hz, 2H), 3.91 (t, J = 6.75 Hz, 2H), 4.13–4.16 (s, 3H), 7.06–7.10 (m, 1H), 7.27–7.31 (m, 1H), 7.50 (d, J = 7.63 Hz, 1H), 7.78–7.82 (m, 1H), 7.85–7.88 (m, 1H), 7.92 (d, J = 7.92 Hz, 1H), 8.60 (d, J = 2.35 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 32.4, 33.1, 40.0, 46.6, 104.7, 116.7, 116.8, 117.1, 118.2, 122.4, 123.1, 124.1, 124.8, 125.0, 130.2, 136.0, 147.0, 152.4, 156.3.

6.1.2.14. 2-Bromo-*N*-(5-(diethylamino)pentan-2-yl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (12a). Yield: 64%, yellow solid, Mp: 77–79 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 0.72 (t, J = 6.90 Hz, 6H), 1.12–1.27 (m, 2H), 1.44 (d, J = 6.46 Hz, 3H), 1.55–1.64 (m, 1H), 1.64–1.74 (m, 1H), 2.08–2.19 (m, 6H), 4.16 (s, 3H), 4.27–4.39 (m, 1H), 6.72–6.79 (m, 1H), 7.11 (t, J = 7.20 Hz, 1H), 7.32 (t, J = 7.80 Hz, 1H), 7.52 (d, J = 7.92 Hz, 1H), 7.79 (d, J = 7.63 Hz, 1H), 7.82 (d, J = 9.10 Hz, 1H), 7.92 (dd, J = 9.10, 2.05 Hz, 1H), 8.86 (d, J = 2.35 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 11.4, 21.4, 23.3, 32.4, 36.0, 46.0, 51.8, 52.4, 106.4, 113.1, 116.8, 117.4, 117.8, 118.2, 122.0, 124.0, 125.2, 126.1, 133.0, 136.3, 147.0, 152.6, 156.3; HRMS (ESI) Calcd for $C_{25}H_{30}BrN_4$ $[M-H]^-$ 465.1659. Found 465.1667.

6.1.2.15. 3-(2-Bromo-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-ylamino)propan-1-ol (12c). Yield: 70%, yellow solid, Mp: 234–236 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.84 (s, 2H), 3.42 (d, J = 4.99 Hz, 2H), 3.90 (d, J = 6.16 Hz, 2H), 4.14 (s, 3H), 4.54 (s, 1H), 7.05–7.10 (m, 1H), 7.11–7.16 (m, 1H), 7.29 (s, 1H), 7.50 (s, 1H), 7.80 (d, J = 9.10 Hz, 1H), 7.88–7.94 (m, 2H), 8.73 (d, J = 2.05 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 32.3, 33.5, 45.7, 58.3, 104.9, 112.8, 116.7, 117.3, 117.3, 118.2, 122.4, 124.1, 124.8, 125.9, 132.9, 136.3, 147.0, 152.4, 156.3; HRMS (ESI) Calcd for $C_{19}H_{17}BrN_3O$ $[M-H]^-$ 382.0560. Found 382.0574.

6.1.2.16. 2-Bromo-5-methyl-11-(4-methyl-1,4-diazepan-1-yl)-5*H*-indolo[2,3-*b*]quinoline (12d). Yield: 82%, yellow solid, Mp: 134–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.10–2.19 (m, 2H), 2.55–2.61 (s, 3H), 2.86–2.92 (t, J = 4.80 Hz, 2H), 2.93–2.99 (t, J = 5.20 Hz, 2H), 3.70 (t, J = 5.97 Hz, 2H), 3.73–3.78 (t, J = 4.80 Hz, 2H), 4.29 (s, 3H), 7.22–7.28 (m, 1H), 7.49–7.55 (t, J = 7.20 Hz, 1H), 7.58 (d, J = 9.00 Hz, 1H), 7.71–7.75 (d, J = 8.00 Hz, 1H), 7.75–7.81 (dd, J = 8.80, 2.00 Hz, 1H), 8.12 (d, J = 7.63 Hz, 1H), 8.78 (d, J = 2.15 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 30.0, 33.2, 47.5, 51.9, 52.8, 58.4, 60.6, 114.4, 116.1, 117.5, 119.8, 122.5, 122.7, 122.9, 123.9, 128.5, 129.2, 132.8, 137.2, 149.9, 154.0, 157.4.

6.1.2.17. *N*-(4-Aminobutyl)-2-bromo-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (12e). Yield: 96%, yellow solid, Mp: 81–82 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.45–1.50 (m, 2H), 1.65–1.69 (m, 2H), 2.68 (t, J = 6.60 Hz, 2H), 3.64 (d, J = 4.11 Hz, 2H), 4.05 (s, 3H), 5.72 (br., 1H), 7.17 (t, J = 7.34 Hz, 1H), 7.29 (d, J = 8.80 Hz, 1H), 7.39–7.44 (m, 1H), 7.58 (dd, J = 9.10, 1.17 Hz, 1H), 7.73 (d, J = 7.92 Hz, 1H), 7.78 (d, J = 7.92 Hz, 1H), 8.09 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 29.0, 30.5, 32.6, 41.4, 48.9, 107.6, 112.9, 116.0, 117.1, 117.3, 118.8, 121.1, 123.9, 125.9, 126.6, 132.5, 136.5, 147.0, 152.7, 156.2; HRMS (ESI) Calcd for $C_{20}H_{20}BrN_4$ $[M-H]^-$ 395.0877. Found 395.0862.

6.1.2.18. 2-Bromo-5-methyl-11-(4-methyl-1,4-diazepan-1-yl)-5*H*-indolo[2,3-*b*]quinoline (12f). Yield: 63%, yellow solid, Mp: 143–145 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.71–1.77 (m, 2H), 2.59 (t, J = 6.46 Hz, 2H), 3.91 (t, J = 6.75 Hz, 2H), 4.11–4.16 (m, 3H), 7.08 (ddd, J = 7.92, 7.04, 0.88 Hz, 1H), 7.29 (td, J = 7.63, 1.17 Hz, 1H), 7.50 (dd, J = 7.92, 0.59 Hz, 1H), 7.79 (d, J = 9.10 Hz, 1H), 7.90 (dd, J = 9.10, 2.05 Hz, 1H), 7.92 (d, J = 7.63 Hz, 1H), 8.71 (d, J = 2.35 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 32.3, 33.5, 39.5, 46.7, 104.5, 112.8, 116.7, 117.3, 117.3, 118.1, 122.4, 124.1, 124.7, 126.0, 132.9, 136.3, 147.0, 152.4, 156.3; HRMS (ESI) Calcd for $C_{19}H_{18}BrN_4$ [M−H]$^-$ 381.0720. Found 381.0714.

6.1.2.19. *N*-(4-Aminobutyl)-2-fluoro-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (13e). Yield: 94%, yellow solid, Mp: 78–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47–1.53 (m, 2H), 1.65–1.75 (m, 2H), 2.69 (t, J = 6.65 Hz, 2H), 3.62–3.72 (m, 2H), 4.12 (s, 3H), 5.57 (br s, 1H), 7.12–7.20 (t, J = 7.60 Hz, 1H), 7.31–7.38 (m, 1H), 7.39–7.50 (m, 2H), 7.70–7.77 (m, 2H), 7.80 (d, J = 7.83 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 29.1, 30.5, 32.8, 41.4, 48.8, 108.2, 109.3, 109.6, 115.9, 116.0, 116.4, 116.4, 117.2, 118.0, 118.2, 118.7, 121.0, 123.6, 126.0, 134.5, 147.5, 152.8, 155.4, 156.4, 157.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ ppm 121.33; HRMS (ESI) Calcd for $C_{20}H_{20}FN_4$ [M−H]$^-$ 335.1677. Found 335.1650.

6.1.2.20. 3-(2-Methoxy-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-ylamino)propan-1-ol (14c). Yield: 66%, yellow solid, Mp: 206–208 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.82 (m, 2H), 3.42–3.47 (q, J = 4.20 Hz, 2H), 3.89–3.91 (m, 2H), 3.92 (s, 3H), 4.15 (s, 3H), 4.60 (t, J = 4.55 Hz, 1H), 7.00 (t, J = 5.43 Hz, 1H), 7.03–7.07 (m, 1H), 7.28 (td, J = 7.56, 1.03 Hz, 1H), 7.44–7.49 (m, 2H), 7.81 (d, J = 9.10 Hz, 1H), 7.91–7.95 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 32.3, 33.7, 45.9, 55.9, 58.6, 105.2, 106.0, 116.2, 116.3, 116.5, 117.6, 119.5, 122.1, 123.9, 124.6, 132.2, 147.8, 152.3, 153.6, 156.1; HRMS (ESI) Calcd for $C_{20}H_{20}N_3O_2$ [M−H]$^-$ 334.1556. Found 334.1537.

6.1.2.21. *N*-(4-Aminobutyl)-2-methoxy-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (14e). Yield: 79%, gel; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.41–1.49 (m, 2H), 1.67–1.72 (m, 2H), 2.62–2.67 (m, 2H), 3.64–3.72 (m, 2H), 3.85 (s, 3H), 4.11 (s, 3H), 5.49 (br s, 1H), 7.13 (t, J = 7.20 Hz, 1H), 7.23 (dd, J = 6.00, 3.00 Hz, 1H), 7.38 (t, J = 8.40 Hz, H), 7.42–7.47 (m, 2H), 7.70 (d, J = 7.92 Hz, 1H), 7.81 (d, J = 7.63 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 29.1, 30.5, 32.6, 41.4, 48.8, 55.6, 106.4, 108.2, 115.7, 116.4, 116.8, 118.3, 118.9, 120.9, 123.7, 125.7, 132.7, 148.0, 152.6, 153.4, 156.1; HRMS (ESI) Calcd for $C_{21}H_{23}N_4O$ [M−H]$^-$ 347.1877. Found 347.1857.

6.1.2.22. *N*-(3-Aminopropyl)-2-methoxy-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (14f). Yield: 80%, yellow solid, Mp: 73–74 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.68–1.71 (m, 2H), 2.92 (t, J = 5.87 Hz, 2H), 3.82 (s, 3H), 3.90 (t, J = 5.40 Hz, 2H), 4.12 (s, 3H), 7.05 (br s, 1H), 7.14 (t, J = 7.19 Hz, 1H), 7.21 (dd, J = 9.24, 2.79 Hz, 1H), 7.35–7.44 (m, 3H), 7.72 (d, J = 7.92 Hz, 1H), 7.93 (d, J = 7.63 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 32.5, 32.5, 41.4, 49.2, 55.6, 106.0, 106.4, 115.5, 116.4, 116.8 118.0, 118.8, 121.5, 124.0, 125.2, 132.6, 147.8, 152.7, 153.4, 156.6; HRMS (ESI) Calcd for $C_{20}H_{21}N_4O$ [M−H]$^-$ 333.1721. Found 333.1684.

6.1.2.23. 2-Chloro-*N*-(5-(diethylamino)pentan-2-yl)-6-methyl-6*H*-indolo[2,3-*b*]quinolin-11-amine (20a). Yield: 27%, brown gel; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.95 (t, J = 7.14 Hz, 6H), 1.31 (d, J = 6.26 Hz, 3H), 1.52–1.66 (m, 3H), 1.66–1.76 (m, 1H), 2.39 (t, J = 7.34 Hz, 2H), 2.46 (q, J = 7.11 Hz, 4H), 3.92 (s, 3H), 4.13 (m, 1H), 4.52 (d, J = 10.76 Hz, 2H), 7.30 (td, J = 7.63, 0.98 Hz, 2H), 7.38 (d, J = 8.02 Hz, 2H), 7.49–7.55 (m, 2H), 7.57 (dd, J = 9.00, 2.35 Hz, 1H), 7.97 (d, J = 9.00 Hz, 1H), 8.00 (d, J = 7.63 Hz, 2H), 8.09 (d, J = 2.15 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 11.1, 22.1, 23.3, 27.6, 36.9, 46.6, 52.5, 54.1, 106.3, 108.4, 119.4, 119.8, 120.3, 121.9, 121.9, 126.4, 126.7, 129.3, 129.5, 141.6, 146.3, 147.4, 153.8; HRMS (ESI) Calcd for $C_{25}H_{30}ClN_4$ [M−H]$^-$ 421.2164. Found 421.2155.

6.1.2.24. *N*-(2-(1*H*-Indol-3-yl)ethyl)-2-chloro-6-methyl-6*H*-indolo[2,3-*b*]quinolin-11-amine (20b). Yield: 28%, grey solid, Mp: 207–209 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 3.15 (t, J = 6.46 Hz, 2H), 3.87 (s, 3H), 4.07 (d, J = 5.58 Hz, 2H), 4.85 (br s, 1H), 7.04 (s, 1H), 7.05–7.09 (t, J = 7.80 Hz, 1H), 7.16 (t, J = 7.48 Hz, 1H), 7.26 (t, J = 9.60 Hz, 1H), 7.30 (d, J = 7.92 Hz, 1H), 7.40–7.46 (m, 2H), 7.49–7.56 (m, 2H), 7.65 (d, J = 7.92 Hz, 1H), 7.94–8.00 (m, 2H), 8.43 (br s, 1H); 13C NMR (150 MHz, $CDCl_3$) δ ppm 27.1, 27.7, 48.8, 104.9, 108.2, 111.4, 111.8, 118.5, 118.7, 119.7, 119.7, 120.0, 121.9, 122.0, 122.4, 122.9, 126.1, 126.4, 127.1, 129.1, 129.2, 136.5, 141.2, 146.1, 147.8, 153.7; HRMS (ESI) Calcd for $C_{26}H_{20}ClN_4$ [M−H]$^-$ 423.1441. Found 423.1422.

6.1.2.25. 3-(2-Chloro-6-methyl-6*H*-indolo[2,3-*b*]quinolin-11-ylamino)propan-1-ol (20c). Yield: 74%, grey solid, Mp: 133–135 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.81 (m, 2H), 3.39–3.49 (m, 2H), 3.76 (q, J = 6.52 Hz, 2H), 3.86 (s, 3H), 4.52 (br s, 1H), 6.58 (br s, 1H), 7.24–7.32 (m, 1H), 7.49 (t, J = 8.00 Hz, 1H), 7.57 (d, J = 8.00 Hz, 1H), 7.64 (dd, J = 9.00, 2.15 Hz, 1H), 7.88 (d, J = 9.00 Hz, 1H), 8.09 (d, J = 7.63 Hz, 1H), 8.58 (d, J = 2.15 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm (ppm) 27.6, 33.8, 46.0, 58.6, 102.7, 108.6, 118.5, 119.8, 119.9, 122.2, 122.9, 125.4, 125.8, 129.0, 140.7, 145.3, 148.2, 153.7; HRMS (ESI) Calcd for $C_{19}H_{17}ClN_3O$ [M−H]$^-$ 338.1066. Found 338.1062.

6.1.2.26. 2-Chloro-6-methyl-11-(4-methyl-1,4-diazepan-1-yl)-6*H*-indolo[2,3-*b*]quinoline (20d). Yield: 35%, off white solid, Mp: 183–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.21 (m, 2H), 2.58 (s, 3H), 2.86–2.94 (t, J = 2.40 Hz, 2H), 3.00 (t, J = 5.20 Hz, 2H), 3.68 (t, J = 6.06 Hz, 2H), 3.75 (t, J = 4.80 Hz, 2H), 3.94 (s, 3H), 7.34 (t, J = 7.53 Hz, 1H), 7.39 (d, J = 8.02 Hz, 1H), 7.55–7.62 (m, 2H), 8.02 (d, J = 9.00 Hz, 1H), 8.38 (d, J = 7.83 Hz, 1H), 8.43 (d, J = 2.35 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 27.7, 29.9, 47.4, 52.1, 52.9, 58.3, 61.1, 108.3, 114.9, 119.3, 120.0, 123.7, 124.0, 124.5, 127.6, 127.7, 129.1, 129.5, 142.3, 146.7, 150.6, 154.4.

6.1.2.27. *N*-(4-Aminobutyl)-2-chloro-6-methyl-6*H*-indolo[2,3-*b*]quinolin-11-amine (20e). Yield: 26%, light grey solid, Mp: 222–224 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.53 (m, 2H), 1.77 (m, 2H), 2.71 (t, J = 7.20 Hz, 2H), 3.68 (t, J = 7.20 Hz, 2H), 3.88 (s, 3H), 4.92 (br s, 1H), 7.25–7.29 (m, 1H), 7.35 (d, J = 8.40 Hz, 1H), 7.46–7.51 (m, 1H), 7.55 (dd, J = 8.80, 2.40 Hz, 1H), 7.91–7.98 (m, 2H), 8.09 (d., J = 2.40 Hz, 1H); ^{13}C NMR (150 MHz, CDCl3) δ ppm 27.6, 29.3, 30.9, 41.7, 49.2, 104.6, 108.3, 118.4, 119.7, 120.2, 121.8, 121.8, 126.1, 126.4, 129.2, 129.4, 141.3, 146.3, 147.9, 153.9; HRMS (ESI) Calcd for $C_{20}H_{20}ClN_4$ [M−H]$^-$ 351.1382. Found 351.1366.

6.1.2.28. *N*-(3-Aminopropyl)-2-chloro-6-methyl-6H-indolo[2,3-*b*]quinolin-11-amine (20f). Yield: 61%, light grey solid, Mp: 78–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.84–1.90 (m, 2H), 2.99 (t, J = 6.40 Hz, 2H), 3.85–3.86 (m, 2H), 3.92 (s, 3H), 6.03 (br s, 1H), 7.28 (t, J = 8.00 Hz, 1H), 7.38 (d, J = 7.60 Hz, 1H), 7.50 (t, J = 7.20 Hz, 1H), 7.57 (dd, J = 8.80, 2.40 Hz, 1H), 7.97 (s, J = 9.20 Hz, 1H), 8.08 (d, J = 8.00 Hz, 1H), 8.19 (d, J = 2.40 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 27.7, 33.7, 41.0, 48.9, 104.1, 108.2, 118.5, 119.7, 120.7, 122.1, 122.2, 126.0, 126.3, 129.2,

129.4, 141.3, 146.4, 148.2, 154.2; HRMS (ESI) Calcd for $C_{19}H_{18}ClN_4$ $[M-H]^-$ 337.122. Found 337.1205.

6.1.2.29. 2-Chloro-6-methyl-11-morpholino-6*H*-indolo[2,3-*b*]quinoline (20g). Yield: 27%, yellow solid, Mp: 245–247 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 3.54 (m, 4H), 3.95 (s, 3H), 4.09–4.14 (t, J = 4.80 Hz, 4H), 7.36 (t, J = 7.63 Hz, 1H), 7.41 (d, J = 8.22 Hz, 1H), 7.58–7.63 (m, 2H), 8.04 (d, J = 8.80 Hz, 1H), 8.50 (d, J = 2.35 Hz, 1H), 8.54 (d, J = 7.63 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm (ppm) 27.8,, 49.4, 67.8, 108.6, 115.6, 119.2, 20.0, 123.4, 124.0, 125.5, 127.9, 128.0, 129.4, 129.5, 142.6, 146.5, 149.1, 154.1.

6.1.2.30. 2-Chloro-*N*-(2-(dimethylamino)ethyl)-6-methyl-6*H*-indolo[2,3-*b*]quinolin-11-amine (20h). Yield: 44%, brown solid, Mp: 152–153°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.44 (s, 6H), 2.61 (t, J = 5.58 Hz, 2H), 3.82 (q, J = 5.22 Hz, 2H), 3.95 (s, 3H), 6.19 (br s, 1H), 7.28–7.34 (t, J = 7.20 Hz, 1H), 7.39 (d, J = 8.02 Hz, 1H), 7.49–7.54 (m, 1H), 7.57 (dd, J = 9.00, 2.35 Hz, 1H), 7.98 (d, J = 9.00 Hz, 1H), 8.22 (d, J = 7.83 Hz, 1H), 8.28 (d, J = 2.15 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 27.7, 44.9, 46.0, 58.9, 104.6, 108.3, 118.6, 119.9, 120.4, 121.8, 122.8, 126.0, 126.2, 129.2, 129.3, 141.2, 146.5, 148.5, 153.7; HRMS (ESI) Calcd for $C_{20}H_{20}ClN_4$ $[M-H]^-$ 351.1382. Found 351.1380.

6.1.2.31. *N*-(2-Aminoethyl)-2-chloro-6-methyl-6*H*-indolo[2,3-*b*]quinolin-11-amine (20i). Yield: 50%, light grey solid, Mp: 138–141 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.92 (t, J = 5.40 Hz, 2H), 3.65 (q, J = 5.28 Hz, 2H), 3.84 (s, 3H), 5.83 (t, J = 5.28 Hz, 1H), 7.24 (t, J = 7.48 Hz, 1H), 7.29 (d, J = 7.92 Hz, 1H), 7.45 (t, J = 7.80 Hz, 1H), 7.51 (dd, J = 9.10, 2.35 Hz, 1H), 7.90 (d, J = 9.10 Hz, 1H), 8.12 (d, J = 7.63 Hz, 1H), 8.15 (d, J = 2.35 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 27.5, 42.0, 50.4, 104.5, 108.0, 118.4, 119.6, 120.1, 121.8, 122.2, 125.9, 126.0, 129.0, 129.1, 141.0, 146.3148.0, 153.6; HRMS (ESI) Calcd for $C_{18}H_{16}ClN_4$ $[M-H]^-$ 323.1069. Found 323.1039.

6.1.3. Preparation of 2-chloro-5-methyl-11-(nitromethyl)-5*H*-indolo[2,3-*b*]quinoline 21

The solution of nitromethane (4 mmol) in DMSO (1 mL) was added to the suspension of NaH in dry DMSO (5 mL) with stirring. When the babbling subsided, the **6** (1 mmol) in DMSO was added. The mixture was heated at 80 °C for 4 h. After cooled down to room temperature, 20 mL water was added, then the mixture was washed with ethyl acetate for two times to remove the byproducts. The aquous phase was acidified with saturated NH_4Cl, and the precipitation was filtrated. The filtration **21** was washed with water and dried. Yield: 96%, brown solid; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 4.36 (s, 3H), 6.89 (s, 2H), 7.23 (t, J = 7.48 Hz, 1H), 7.54–7.59 (m, 1H), 7.61–7.65 (m, 1H), 7.94 (dd, J = 9.10, 2.35 Hz, 1H), 8.11 (d, J = 9.10 Hz, 1H), 8.18 (d, J = 7.92 Hz, 1H), 8.60 (d, J = 2.35 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ ppm 33.5, 72.4, 117.8, 117.8, 120.0, 120.5, 122.6, 123.9, 124.7, 126.8, 128.4, 129.0, 130.2, 130.7, 135.3, 154.9, 156.0; HRMS (ESI) Calcd for $C_{17}H_{11}ClN_3O_2$ $[M-H]^-$ 324.0545. Found 324.0539.

6.1.4. Preparation of 2-chloro-5-methyl-5*H*-indolo[2,3-*b*]quinoline-11-carbaldehyde 22

To the stirred suspension of **21** (0.5 mmol) in methanol (5 mL) at 0 °C, a freshly prepared solution of KOH (1.5 mmol) in methanol (10 mL) was added dropwise. After being stirred for 30 min, a solution of $KMnO_4$ (0.335 mmol) and $MgSO_4$ (1.5 mmol) in water (30 mL) was added dropwise with vigorous stirring. When the reaction was complete, the mixture was filtered over a thin layer of celit. The filtrate was extracted with CH_2Cl_2 and the organic phase was dried over anhydrous $MgSO_4$. Further purification was achieved by flash chromatography on silica gel. Yield: 27%, red solid, Mp: 261–263 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 4.15 (s, 3H), 7.08 (ddd, J = 7.78, 6.90, 1.17 Hz, 1H), 7.45–7.49 (m, 2H), 7.50–7.52 (m, 1H), 7.57 (dd, J = 9.10, 2.35 Hz, 1H), 7.84 (d, J = 7.63 Hz, 1H), 8.72 (d, J = 2.35 Hz, 1H), 11.03 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 33.7, 115.4, 117.1, 118.3, 120.9, 121.9, 125.9, 126.0, 128.6, 130.1, 130.6, 131.7, 131.8, 134.6, 155.8, 156.7, 190.6.

6.1.5. Preparation of 11-aminoalkylaminomethylated neocryptolepine derivatives 23

The aldehyde **22** (0.1 mmol) and appropriate amine (0.5 mmol) was dissolved in MeOH. After stirring overnight at room temperature, $NaBH_4$ (0.5 mmol) was added. Then the mixture was stirred for an additional 2 h. The reaction was quenched with saturated NH_4Cl, extracted with CH_2Cl_2, and the organic phase was dried over anhydrous $MgSO_4$. Further purification was achieved with chromatography on silica gel.

6.1.5.1. 2-Chloro-5-methyl-11-((4-methyl-1,4-diazepan-1-yl)methyl)-5*H*-indolo[2,3-*b*]quinoline (23d). Yield: 36%, orange solid, Mp: 164–166 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.83–1.90 (m, 2H), 2.34 (s, 3H), 2.54 (dt, J = 4.92, 2.38 Hz, 2H), 2.63–2.72 (m, 2H), 2.88–2.93 (m, 2H), 2.96 (t, J = 6.16 Hz, 2H), 4.32 (s, 3H), 4.49 (s, 2H), 7.20–7.25 (m, 1H), 7.54 (td, J = 7.63, 1.17 Hz, 1H), 7.63–7.70 (m, 2H), 7.72 (d, J = 7.92 Hz, 1H), 8.28 (d, J = 7.63 Hz, 1H), 8.60 (d, J = 2.05 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 27.3, 33.2, 46.6, 54.1, 55.1, 56.3, 58.1, 115.4, 117.9, 119.9, 122.1, 124.1, 124.3, 126.9, 126.9, 128.3, 129.3, 130.1, 135.2, 139.1, 155.7, 155.9; HRMS (ESI) Calcd for $C_{23}H_{24}ClN_4$ $[M-H]^-$ 391.1689. Found 391.1750.

6.1.5.2. N^1-((2-Chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-yl)methyl)-N^3,N^3-dimethylpropane-1,3-diamine (23j). Yield: 56%, red solid, Mp: 150–153 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.68–1.74 (m, 3H), 2.15–2.19 (m, 10H), 2.29–2.34 (m, 4H), 2.90 (t, J = 6.75 Hz, 3H), 4.25 (s, 5H), 4.53 (s, 3H), 7.21 (td, J = 7.48, 1.17 Hz, 2H), 7.50–7.54 (m, 2H), 7.57–7.60 (m, 2H), 7.62–7.65 (m, 2H), 7.69 (d, J = 7.92 Hz, 2H), 8.05 (d, J = 7.63 Hz, 2H), 8.26 (d, J = 2.05 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 27.9, 33.3, 45.6, 45.7, 46.6, 49.1, 58.1, 115.8, 118.1, 120.3, 121.6, 123.3, 123.8, 125.5, 127.3, 127.5, 129.4, 130.2, 135.4, 139.6, 155.7, 155.7.

6.2. Bioactivity study

6.2.1. Cell lines

Established in vitro, human cancer cell lines: MV4-11 (biphenotypic B myelomonocytic leukemia), A549 (non-small cell lung carcinoma), HCT116 (colorectal carcinoma) and normal mice fibroblasts BALB/3T3 was used.

All lines were obtained from American Type Culture Collection (Rockville, Maryland, USA). All the cell lines were maintained in the Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

MV4-11 cells were cultured in RPMI 1640 medium (Gibco, Scotland, UK) supplemented with 2 mM L-glutamine and 1.0 mM sodium pyruvate, 10% fetal bovine serum (all from Sigma–Aldrich Chemie GmbH, Steinheim, Germany), 100 units/mL penicillin, and 100 μg/mL streptomycin (both from Polfa, Tarchomin S.A., Poland). The cell line was grown at 37 °C with 5% CO_2 humidified atmosphere.

HCT116 and A549 cells were cultured in RPMI 1640+OptiMEM (50:50) medium (Gibco, Scotland, UK) supplemented with 2 mM L-glutamine and 5% fetal bovine serum (all from Sigma–Aldrich Chemie GmbH, Steinheim, Germany). BALB/3T3 cells were cultured in Dulbecco's medium (IIET, Wroclaw, Poland) supplemented with 2 mM L-glutamine and 1.0 mM sodium pyruvate, 10% fetal bovine serum (all from Sigma–Aldrich Chemie GmbH, Steinheim, Germany). All culture mediums were supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin (both from Polfa, Tarchomin S.A., Poland). The cells were grown at 37 °C with 5% CO_2 humidified atmosphere.

6.2.2. Anti-proliferative assay in vitro

Test solutions of the compounds tested (1 mg/mL) were prepared by dissolving the substances in 100 μl DMSO (Sigma–Aldrich, Germany) completed with 900 μl of tissue culture medium. Afterwards, the tested compounds were diluted in culture medium to reach the final concentrations of 10, 1, 0.1, 0.01 and 0.001 μg/mL.

Twenty-four hours before addition of the tested agents, the cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1×10^4 cells per well. The assay was performed after 72 h of exposure to varying concentrations (from 0.01 to 10 μg/mL) of the tested agents. The in vitro cytotoxic effect of all agents against HCT116, A549 and BALB/3T3 were examined using the SRB assay and the in vitro cytotoxic effect of all agents against MV4-11 was examined using the MTT assay to avoid rinsing out of the cells during washing. Both assays were recommended by NCI as giving similar results.[12] Each compound in given concentration was tested in triplicates in each experiment, which was repeated 3–5 times.

6.2.3. MTT assay

For the last 3–4 h of incubation 20 μl of MTT solution (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; stock solution: 5 mg/mL, Sigma–Aldrich, Germany) were added to each well. The mitochondria of viable cells reduce the pale yellow MTT to a navy blue formazan: the more viable cells are present in well, the more MTT will be reduced to formazan. When incubation time was completed, 80 μl of the lysing mixture were added to each well (lysing mixture: 225 mL dimethylformamide, POCh, Gliwice, Poland, 67.5 g sodium dodecyl sulphate, Sigma–Aldrich, Germany, and 275 mL of distilled water). After 24 h, when formazan crystals had been dissolved, the optical densities of the samples were read on a Multiskan RC photometer (Labsystems, Helsinki, Finland) at 570 nm wavelength. The background optical density was measured in the wells filled with culture medium, without the cells.

6.2.4. SRB assay

The cells attached to the plastic were fixed by gently layering cold 50% TCA (trichloroacetic acid, Sigma–Aldrich, Germany) on the top of the culture medium in each well. The plates were incubated at 4 °C for 1 h and then washed five times with tap water. The cellular material fixed with TCA was stained with 0.4% SRB sulforhodamine B (Sulforhodamine B, Sigma–Aldrich, Germany) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 min. Unbound dye was removed by rinsing (4×) with 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (Sigma–Aldrich, Germany) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Multiskan RC photometer (Labsystems, Helsinki, Finland). The background optical density was measured in the wells filled with culture medium, without the cells.

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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.2012.05.054.

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