Bioorganic & Medicinal Chemistry 18 (2010) 1948–1957

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and antiproliferative evaluation of certain indolo[3,2-*c*]quinoline derivatives

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ARTICLE INFO

Article history:
Received 27 November 2009
Revised 13 January 2010
Accepted 14 January 2010
Available online 28 January 2010

Keywords:
Indolo[3,2-*c*]quinoline derivatives
Antiproliferative activity
Anticancer agents

ABSTRACT

The present report describes the synthesis and antiproliferative evaluation of certain indolo[3,2-*c*]quinoline derivatives. For the C_6 anilino-substituted derivatives, (11*H*-indolo[3,2-*c*]quinolin-6-yl)phenylamine (**6a**) was inactive. Structural optimization of **6a** by the introduction of a hydroxyl group at the anilino-moiety resulted in the enhancement of antiproliferative activity in which the activity decreased in an order of *para*-OH, **7a** > *meta*-OH, **8a** > *ortho*-OH, **9a**. For the C_6 alkylamino-substituted derivatives, **11a**, **12a**, **13a**, **14a**, and **15a** exhibited comparable antiproliferative activities against all cancer cells tested and the skin Detroit 551 normal fibroblast cells. Three cancer cells, HeLa, A549, and SKHep, are very susceptible with IC_{50} of less than 2.17 μM while PC-3 is relatively resistant to this group of indolo[3,2-*c*]quinolines. For the 2-phenylethylamino derivatives, compound **20a** is active against the growth of HeLa with an IC_{50} of 0.52 μM, but is less effective against the growth of Detroit 551 with an IC_{50} of 19.32 μM. For the bis-indolo[3,2-*c*]quinolines, *N,N*-bis-[3-(11*H*-indolo[3,2-*c*]quinolin-6-yl)aminopropyl]amine hydrochloride (**25**) is more active than its *N*-methyl derivative **26** and the positive Doxorubicin. Mechanism studies indicated **25** can induce caspase-3 activation, γ-H2AX phosphorylation, cleavage of poly (ADP-ribose)polymerase and DNA fragmentation. These results provide evidence that DNA, topo I, and topo II are the primary targets of indolo[3,2-*c*]quinoline derivatives and that consequently inhibits proliferation and causes apoptosis in cancer cells.

1. Introduction

The tetracyclic indoloquinoline ring system constitutes an important structural moiety in natural products exhibiting numerous biological activities. For example, cryptolepine (an indolo[3,2-*b*]quinoline derivative) and isocryptolepine (an indolo[3,2-*c*]quinoline derivative) are two indoloquinolines isolated from the roots of the african plant *Cryptolepis sanguinolenta* which had been used in traditional medicine against malaria.[1–5] These two alkaloids, which only differ by the respective orientation of their indole and quinoline rings, display potent antiparasitic properties. Extensive investigations on the in vitro and in vivo biological activities of cryptolepine revealed its wide applications as anti-malarial,[6] anti-microbial,[7,8] anti-imflammatory,[9] and anticancer agents.[10–13] Mechanism studies indicated that cryptolepine binds tightly to DNA and behave as a typical intercalating agent. It stabilizes the topoisomerase II-DNA covalent complex and stimulates the cutting of DNA by topoisomerase II (topo II). The isomeric isocryptolepine (also referred to as cryptosanguinolentine), however, attracted only limited attention.[14–22] For the past few years, we have synthesized certain indolo[3,2-*c*]quinoline derivatives for anticancer evaluation on the ground that these tetracyclic heterocycles may function in a similar way with cryptolepine to intercalate into the DNA double helix resulting in the inhibition of DNA replication and transcription.[23–26] Among them, (*E*)-1-[3-(11*H*-indolo[3,2-*c*]quinolin-6-ylamino)phenyl]ethanone oxime (**1**) demonstrated a mean GI_{50} value of 1.70 μM in the NCI's full panel of 60 human cancer cells.[23] In continuation of our study to explore more potent anticancer drug candidates, we described herein the preparation and antiproliferative evaluation of indolo[3,2-*c*]quinoline and the bis-indolo[3,2-*c*]quinolines derivatives. To elucidate their mechanism of action, we have also studied their interactions with DNA and their effects on topo I and topo II.

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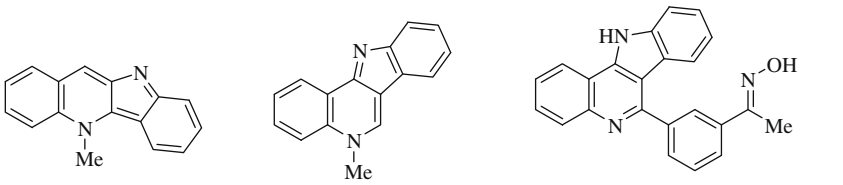
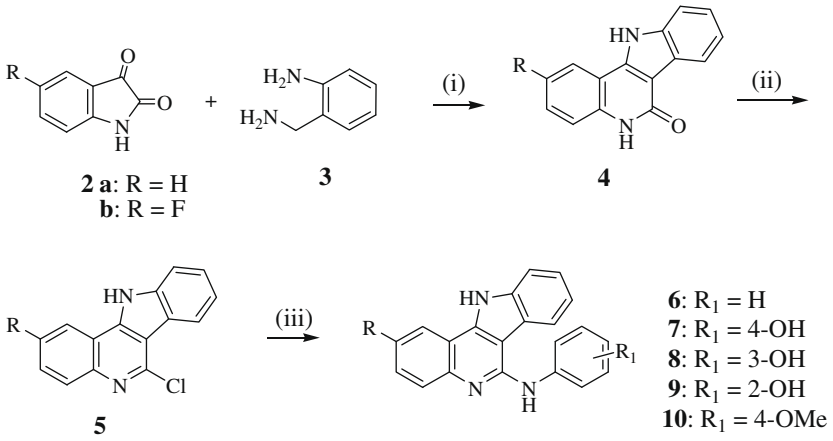
doi:10.1016/j.bmc.2010.01.033



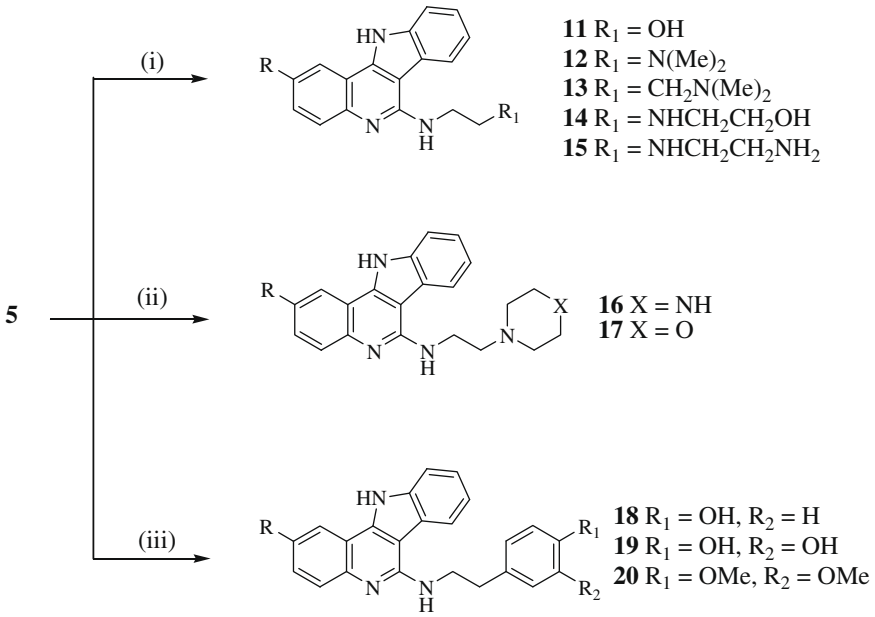
2. Chemistry

Reaction of 2-fluoroisatin (**2b**) and 2-aminobenzylamine (**3**) gave 5,11-dihydro-2-fluoroindolo[3,2-*c*]quinolin-6-one (**4b**) as described in Scheme 1. Treatment of **4b** with $POCl_3$ afforded 6-chloro-2-fluoro-11*H*-indolo[3,2-*c*]quinoline (**5b**) in a good overall yield. Preparation of 6-chloro-11*H*-indolo[3,2-*c*]quinoline (**5a**) had been previously reported.[23] Treatment of **5a** with substituted anilines gave (11*H*-indolo[3,2-*c*]quinolin-6-yl)phenylamine (**6a**) and its derivatives **7a–10a** respectively in a fairly good yield.

Treatment of **5a** with ethanolamine afforded 2-(11*H*-indolo[3,2-*c*]quinolin-6-ylamino)ethanol (**11**) as depicted in Scheme 2. Preparation of its C_6 alkylamino-substituted analogs **12–17** were achieved by the reaction of **5** and the respective alkylamines. 6-[2-(4-Hydroxyphenyl)ethylamino]-11*H*-indolo[3,2-*c*]quinoline hydrochloride (**18a**) was obtained by the treatment of **5a** with 4-(2-aminoethyl)phenol. Accordingly, compounds **19a** and **20a** were prepared by the treatment of **5a** with 2-(3,4-dihydroxyphenyl)ethylamine and 2-(3,4-dimethoxyphenyl)ethylamine, respectively. Compound **18b** was prepared from **5b** by the same reaction conditions.



Scheme 1. Reagents and conditions: (i) AcOH, reflux for 8 h; (ii) $POCl_3$, 150 °C, 48 h; (iii) aniline or substituted-aniline, *s*-BuOH, reflux for 18 h.



Scheme 2. Reagents and conditions: (i) aliphatic-amine, ethoxyethanol, 140 °C, 24 h; (ii) 2-(piperazin-1-yl)ethanamine or 2-morpholinoethanamine, ethoxyethanol, 140 °C, 24 h; (iii) substituted-phenylethylamine, ethoxyethanol, 140 °C, 24 h.

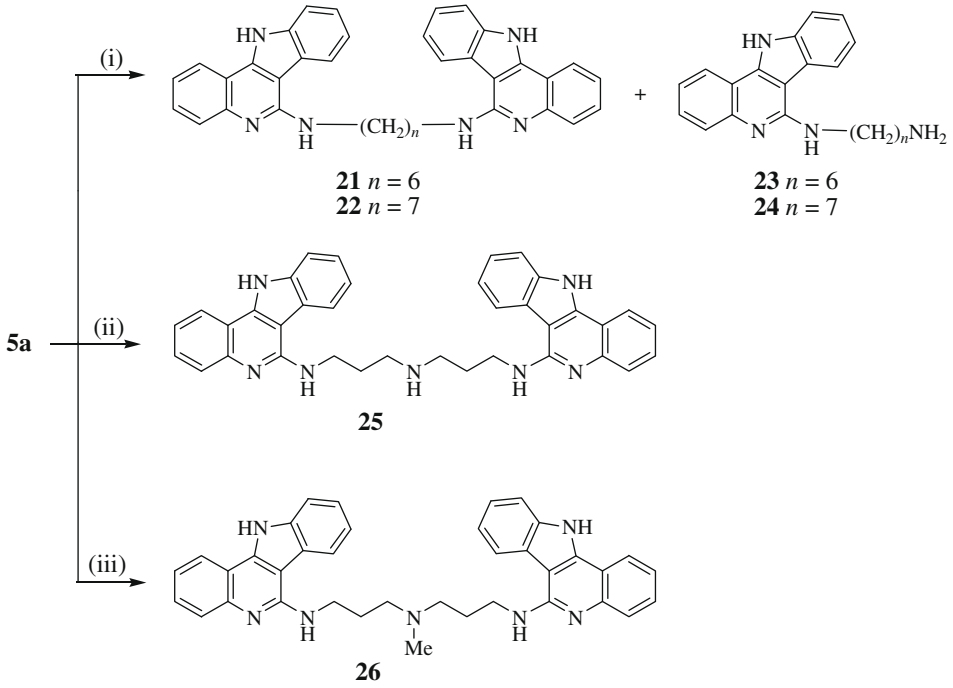
Preparation of bis-indolo[3,2-*c*]quinolines derivatives is depicted in Scheme 3. Reaction of **5a** and 1,6-diaminohexane in 2-ethoxyethanol gave a mixture of *N*-[6-(11*H*-indolo[3,2-*c*]quinolin-6-ylamino) hexyl]-11*H*-indolo[3,2-*c*]quinolin-6-amine (**21**) and *N*-(6-aminohexyl)-11*H*-indolo[3,2-*c*]quinolin-6-amine (**23**). Accordingly, a mixture of the bis-indolo[3,2-*c*]quinoline **22** and the aminoalkylamine derivative **24** was obtained by the treatment of **5a** with 1,7-diaminoheptane. *N*,*N*-Bis-[3-(11*H*-indolo[3,2-*c*]quinolin-6-yl)aminopropyl]amine hydrochloride (**25**) was synthesized by the reaction of **5a** and dipropylenetriamine. Under similar reaction conditions, the *N*-methyl derivative **26** was prepared from **5a** and *N*,*N*-bis(3-aminopropyl)methylamine.

3. Pharmacological results and discussion

All the synthesized indolo[3,2-*c*]quinoline derivatives were evaluated in vitro against a panel of four cancer cell lines including human cervical epithelioid carcinoma (HeLa), non-small cell lung cancer (A549), hepatocelluar carcinoma (SKHep), and prostate cancer (PC-3) using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The skin Detroit 551 normal fibroblast cells were also evaluated since a potential anticancer drug candidate should selectively affect only tumor cells and not somatic cells. The concentration that inhibited the growth of 50% of cells (IC_{50}) was determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells, compared to control cells, by 50%. The IC_{50} results of indolo[3,2-*c*]quinoline derivatives are summarized in Table 1. For the C_6 anilino-substituted indolo[3,2-*c*]quinoline derivatives, (11*H*-Indolo[3,2-*c*]quinolin-6-yl)phenylamine (**6a**) was inactive. Structural optimization of **6a** by the introduction of a hydroxyl group at the anilino-moiety resulted in the enhancement of antiproliferative activity in which the cytotoxicity decreased in an order of *para*-OH, **7a** > *meta*-OH, **8a** > *ortho*-OH, **9a**. The only exception was HeLa cell in which **9a** was more active than **8a**. Compound **7a** was more active than **10a** indicated a substituent of *para*-OH (an H-bonding donor) is more favorable than a *para*-OMe (an H-bonding acceptor). Compounds **7a** and **8a** exhibited selective cytotoxicity in which both compounds are inactive against Detroit 551 with IC_{50} of 25.43 and 42.54 μM, respectively.

For the C_6 alkylamino-substituted indolo[3,2-*c*]quinoline derivatives, **11a**, **12a**, **13a**, **14a**, and **15a** exhibited comparable antiproliferative activities against all cancer cells tested and Detroit 551 as shown in Table 1. Three cancer cells, HeLa, A549, and SKHep, are very susceptible with IC_{50} of less than 2.17 μM while PC-3 is relatively resistant to this group of indolo[3,2-*c*]quinolines. It is interesting that these compounds are more active than the positive Doxorubicin against the growth of A549. Compound **14a** was more active than its C_2 methoxy derivative **14b** implied the substitution of an electron-donating group at C_2 position is unfavorable. The same trends were observed in which **16a** > **16b** and **17a** > **17b**. Comparison of **15a** and **16a** indicated the cyclic 2-piperazinylethylamino substituent **16a** is more cytotoxic than acyclic polyaminoalkylamino derivative **15a**. The piperazinyl derivative **16a** is more active than its morpholino isomer **17a**. For the 2-phenylethylamino derivatives, *para*-hydroxy **18a** and *meta, para*-dimethoxy **20a** are equally active against the growth of cancer cells while *meta, para*-dihydroxy **19a** was much less active. It is worth to mention that compound **20a** is active against the growth of HeLa with an IC_{50} of 0.52 μM, but is inactive against the growth of Detroit 551 with an IC_{50} of 19.32 μM. For the bis-indolo[3,2-*c*]quinolines **21**–**26**, compound **23** is more active than Doxorubicin against the growth of HeLa and A549 with IC_{50} values of 0.072 and 0.084 μM, respectively. *N*,*N*-Bis-[3-(11*H*-indolo[3,2-*c*]quinolin-6-yl)aminopropyl]amine hydrochloride (**25**) is more active than its *N*-methyl derivative **26** and the positive Doxorubicin against the growth of all cancer cells tested.

To elucidate their mechanism of actions, compound **25** was selected for evaluation of its effect on the topoisomerase I (topo I) inhibitory activities and the unwinding of supercoiled plasmid DNA as shown in Figure 1A. Compound **25** at 10 μM can almost completely inhibit topo I activity in vitro. However, Figure 1B shows that compound **25** did not inhibit topo I activity at the low concentration (form 0.1 to 1.0 μM). Our results also show that



Scheme 3. Reagents and conditions: (i) Polymethylenediamine (n = 6 and n = 7), ethoxyethanol in a sealed tube, 130–140 °C, 4 days; (ii) dipropylenetriamine, pyridine, and ethoxyethanol in a sealed tube, 100–120 °C, 4 days; (iii) *N*,*N*-bis(3-aminopropyl)methylamine, pyridine, and ethoxyethanol in a sealed tube, 100–120 °C, 4 days.

Table 1
Antiproliferative activity (IC_{50}, μM)[a] of indolo[3,2-*c*]quinoline derivatives

Compd	HeLa	A549	SKHep	PC-3	Detroit 551
DOX	0.33 ± 0.05	2.23 ± 0.30	0.09 ± 0.03	1.76 ± 0.71	ND
6a	>30	>30	>30	>30	ND
7a	1.54 ± 0.27	3.87 ± 1.57	5.70 ± 1.17	5.43 ± 0.90	25.43 ± 1.99
8a	10.34 ± 2.83	10.35 ± 1.51	7.23 ± 1.62	18.40 ± 3.95	42.54 ± 4.37
9a	4.69 ± 1.63	17.69 ± 6.40	21.89 ± 7.78	>30	ND
10a	11.40 ± 0.59	10.94 ± 1.80	7.78 ± 0.84	20.98 ± 7.78	ND
11a	0.62 ± 0.14	0.99 ± 0.07	1.02 ± 0.18	5.65 ± 0.24	3.15 ± 0.46
12a	0.61 ± 0.16	0.83 ± 0.04	1.60 ± 0.62	5.79 ± 1.21	2.21 ± 0.14
13a	0.85 ± 0.28	0.50 ± 0.03	0.79 ± 0.08	3.65 ± 0.38	2.18 ± 0.35
14a	0.74 ± 0.06	0.70 ± 0.08	0.72 ± 0.11	5.81 ± 0.87	1.74 ± 0.14
14b	1.49 ± 0.12	0.93 ± 0.05	1.31 ± 0.27	6.51 ± 0.28	ND
15a	0.65 ± 0.15	1.03 ± 0.09	2.17 ± 0.37	7.98 ± 0.11	3.88 ± 0.22
16a	0.68 ± 0.27	0.53 ± 0.02	0.38 ± 0.04	1.77 ± 0.12	2.60 ± 0.18
16b	5.35 ± 0.48	6.20 ± 0.12	2.67 ± 0.20	12.26 ± 1.00	ND
17a	0.87 ± 0.10	1.13 ± 0.08	1.29 ± 0.02	2.84 ± 0.60	ND
17b	4.32 ± 0.77	6.73 ± 0.17	5.29 ± 0.38	6.84 ± 1.12	ND
18a	0.59 ± 0.09	5.74 ± 0.21	5.24 ± 0.56	8.94 ± 0.95	4.30 ± 0.65
18b	1.33 ± 0.43	9.19 ± 0.76	7.78 ± 0.07	6.32 ± 0.38	ND
19a	3.77 ± 0.49	10.28 ± 0.89	6.66 ± 0.54	15.20 ± 6.68	ND
20a	0.52 ± 0.34	5.39 ± 0.60	5.83 ± 1.42	8.98 ± 1.05	19.32 ± 3.13
21	0.31 ± 0.05	0.50 ± 0.07	1.47 ± 0.13	0.59 ± 0.02	ND
22	1.19 ± 0.17	1.19 ± 0.27	1.61 ± 0.41	6.56 ± 0.75	ND
23	0.072 ± 0.01	0.084 ± 0.01	2.51 ± 0.68	2.55 ± 1.17	ND
24	2.67 ± 0.33	2.23 ± 0.39	2.54 ± 0.50	6.25 ± 0.82	ND
25	0.068 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.08 ± 0.05	1.98 ± 0.04
26	0.89 ± 0.18	0.43 ± 0.09	0.28 ± 0.03	2.01 ± 0.09	2.14 ± 0.18

[a] Data are presented as the mean ± sem (standard error of the mean) from four to six separated experiments. Statistical analyses were performed using Bonferroni *t*-test method after ANOVA for multigroup comparison and Student's *t*-test method for two-group comparison. $P = 0.05$ was considered significant. Analysis of linear regression (at least three data within 20–80% inhibition) was used to calculate IC_{50} values.

compound **25** can bind tightly to DNA and may behave as a typical intercalating agent in a concentration of 10 μM. In Figure 1C, our results indicated that at a concentration of 10 μM, compound **25** can inhibit topoisomerase II activity effectively when compared with the positive Doxorubicin at the same concentration. An interesting feature was that compound **25** inhibits topo II in a dose dependent manner (1.0, 0.3, and 0.1 μM; lanes 3–5 in Fig. 1D). These results suggested that compound **25** has preferentially inhibited DNA topo II relaxation activity.

To investigate the potential cell proliferative inhibition activity of **25** in cancer cells, we first examined the effect of **25** on cell proliferation and clonogenic survival in HeLa cells. As shown in Figure 2A, compound **25** inhibited in HeLa cell proliferation in both concentration- and time-dependent manners. The maximal proliferation inhibition of **25** at 0.3 μM (or 1.0 μM) after 48 h treatment was 95–100%. Accordingly, the IC_{50} value for HeLa cells was 0.068 μM. Moreover, we performed in vitro clonogenic assays to determine the antitumor activities of **25**. As shown in Figure 2B, our results show that clonogenicity (colony size and number) of HeLa cells was reduced in a dose-dependent manner after exposure to **25** at 0.025, 0.05, 0.1, and 0.3 μM, the number of colonies was 48.2 ± 5.6 (S.D.), 10.5 ± 1.2, 6.0 ± 1.0, and 4.2 ± 0.6, respectively, while in control the number of colonies was 235.0 ± 13.8 (S.D.). This represents significantly decrease in the number of colonies formed from cells treated with **25**.

To examine the mechanism responsible for compound **25**-mediated cell proliferation inhibition, cell cycle distribution was evaluated using flow cytometric analysis. Results from Figure 3A indicated that treating cells with **25** caused a significant inhibition of cell cycle progression in PC-3 cells at 48 h, resulting in a significant increase of the sub-G1 phase population compared with that of the DMSO control. The observation of cells in sub-G1 phase indicates DNA fragmentation and programmed cell death. Apoptosis was also evident upon examination of common molecular markers of apoptosis, including the cleavage of the caspase substrate PARP from 116 kDa intact form into 85 kDa fragment product and caspase-3 activation. Treatment of **25** induced both of these markers, which was consistent with the results of DNA fragmentation. The double-strand breaks of DNA were measured by the following histone H2AX phosphorylation. Western blot analysis confirmed that at a concentration of 0.1 μM, compound **25** induced significant H2AX phosphorylation as shown in Figure 3B. These results indicate that compound **25** exerts its cytotoxicity through the inhibition of cell cycle progression and caused apoptosis of cancer cells followed by DNA fragmentation, and consequentially cell death.

4. Conclusion

Certain indolo[3,2-*c*]quinoline derivatives have been synthesized and evaluated for their antiproliferative activities. Among them, *N,N*-bis-[3-(11*H*-indolo[3,2-*c*]quinolin-6-yl)-aminopropyl]amine hydrochloride (**25**) is more active than its *N*-methyl derivative **26** and the positive Doxorubicin against the growth of all cancer cells tested. Mechanism studies indicated **25** inhibit topo I activity only at a high concentration of 10 μM. For the topo II inhibitory activity, compound **25** inhibits topo II in a dose dependent manner and is more active than Doxorubicin at the same concentration of 10 μM. The clonogenicity (colony size and number) of HeLa cells was reduced in a dose-dependent manner after exposure to compound **25**. Compound **25** caused inhibition of cell cycle progression in PC-3 cells at 48 h and resulted in a significant increase of the sub-G1 phase population. Western blot analysis confirmed that compound **25** induced significant H2AX phosphorylation. These results show that compound **25** exerts its cytotoxicity through the inhibition of cell cycle progression and caused apoptosis of cancer cells followed by DNA fragmentation, and consequentially cell death.

5. Experimental

5.1. General

Melting points were determined on an Electrothermal IA9100 melting point apparatus and are uncorrected. Nuclear magnetic

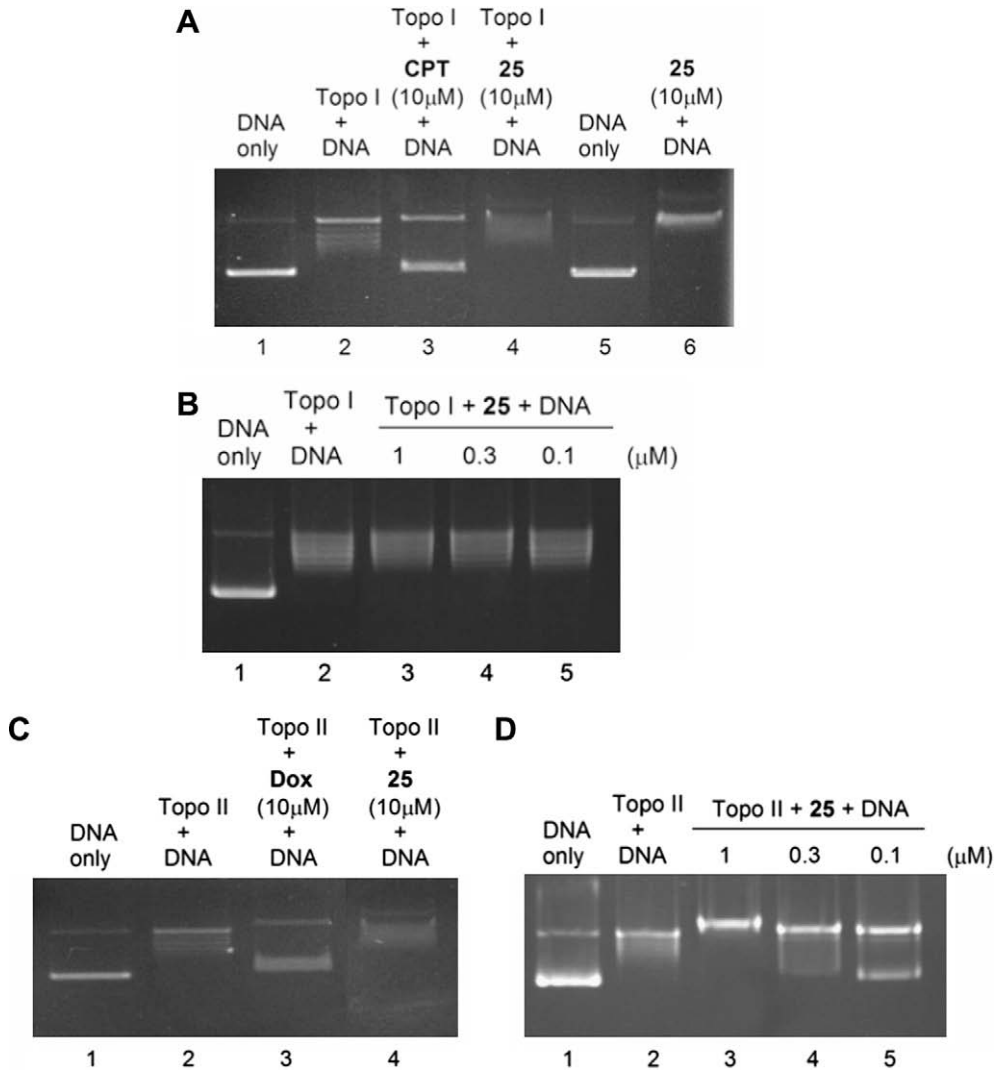


Figure 1. Effect of indolo[3,2-c]quinolines on DNA topoisomerases I and II. (A) Topo I inhibitory activities and the unwinding assay of supercoiled plasmid DNA by camptothecin (CPT in 10 μM) and **25** (10 μM). (B) Similar reactions were carried out with **25** (1.0, 0.3, and 0.1 μM in lines 3–5). (C) Topo II inhibitory activity of compound **25**. Lines 1 and 2 are both control experiments with supercoiled DNA, untreated or treated with topo II, respectively; lines 3–4 correspond to supercoiled pBR322 reacted with topo II and 10 μM of doxorubicin and **25**. (D) Similar reactions were carried out with **25** (1.0, 0.3, and 0.1 μM in lines 3–5).

resonance (^{1}H and ^{13}C) spectra were recorded on a Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co., The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Taiwan University using Heraeus CHN-O Rapid EA, and all values are within ± 0.4% of the theoretical compositions.

5.1.1. 6-Chloro-2-fluoro-11*H*-indolo[3,2-*c*]quinoline (5b)

A mixture of 5-fluoroisatin (0.83 g, 5 mmol), 2-aminobenzylamine (1.22 g, 10 mmol), and acetic acid (30 mL) was refluxed for 8 h (TLC monitoring), the reaction mixture was poured into water. The solid thus formed was collected and crystallized from MeOH to give 0.85 g (67%) of 2-fluoro-11*H*-indolo[3,2-*c*]quinolin-6-one (**4b**). Mp: >350 °C. IR (KBr): 3240, 1623, 1556, 1452, 1202. ^{1}H NMR (DMSO-d_6): 7.28–7.32 (m, 1H, Ar-H), 7.39–7.45 (m, 2H, Ar-H), 7.51 (dd, 1H, J = 8.8, 4.8 Hz, 4-H), 7.66 (d, 1H, J = 8.4 Hz, Ar-H), 8.02 (dd, 1H, J = 9.6, 2.8 Hz, 1-H), 8.23 (d, 1H, J = 7.6 Hz, Ar-H), 11.54 (br s, 1H, NH), 12.60 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 107.08, 107.45 (J = 24.2 Hz), 111.84, 112.60 (J = 9.1 Hz), 117.04 (J = 24.3 Hz), 117.94 (J = 8.4 Hz), 120.97, 121.27, 124.22, 124.44, 134.64, 137.75, 139.95 (J = 3.1 Hz), 156.98 (J = 235.7 Hz), 159.65. Anal. Calcd for $C_{15}H_9FN_2O$: C, 71.42; H, 3.60; N, 11.11. Found: C, 71.11; H, 3.80; N 10.92.

A mixture of **4b** (1.51 g, 6.0 mmol) and $POCl_3$ (30 mL) was refluxed for 8 h (TLC monitoring). After cooling, the reaction mixture was poured into ice-H_2O (150 mL) and the concentrated NaOH solution was added until a pH of 10 was reached. The precipitate thus formed was collected and crystallized from MeOH/DMF to give **5b** (1.48 g, 91% yield) as a yellow solid. Mp: 250–251 °C. IR (KBr): 3265, 1630, 1591, 1513, 1458, 1357, 1241, 1191. ^{1}H NMR (DMSO-d_6): 7.41–7.45 (m, 1H, Ar-H), 7.57–7.62 (m, 1H, Ar-H), 7.68 (ddd, 1H, J = 8.8, 8.8, 2.4 Hz, 3-H), 7.79 (d, 1H, J = 8.0 Hz, Ar-H), 8.11 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.30 (dd, 1H, J = 9.2, 2.4 Hz, 1-H), 8.43 (d, 1H, J = 8.0 Hz, Ar-H), 13.11 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 106.46 (J = 24.2 Hz), 111.59, 112.30, 117.30 (J = 10.6 Hz), 118.50 (J = 25.3 Hz), 120.58, 121.42 (J = 2.3 Hz), 126.43, 131.18 (J = 10.1 Hz), 138.80, 141.31, 141.45, 141.50, 143.92, 159.53 (J = 243.3 Hz). Anal. Calcd for $C_{15}H_8FClN_2 \cdot 0.3H_2O$: C, 65.23; H, 3.14; N, 10.14. Found: C, 65.21; H, 3.52; N, 10.20.

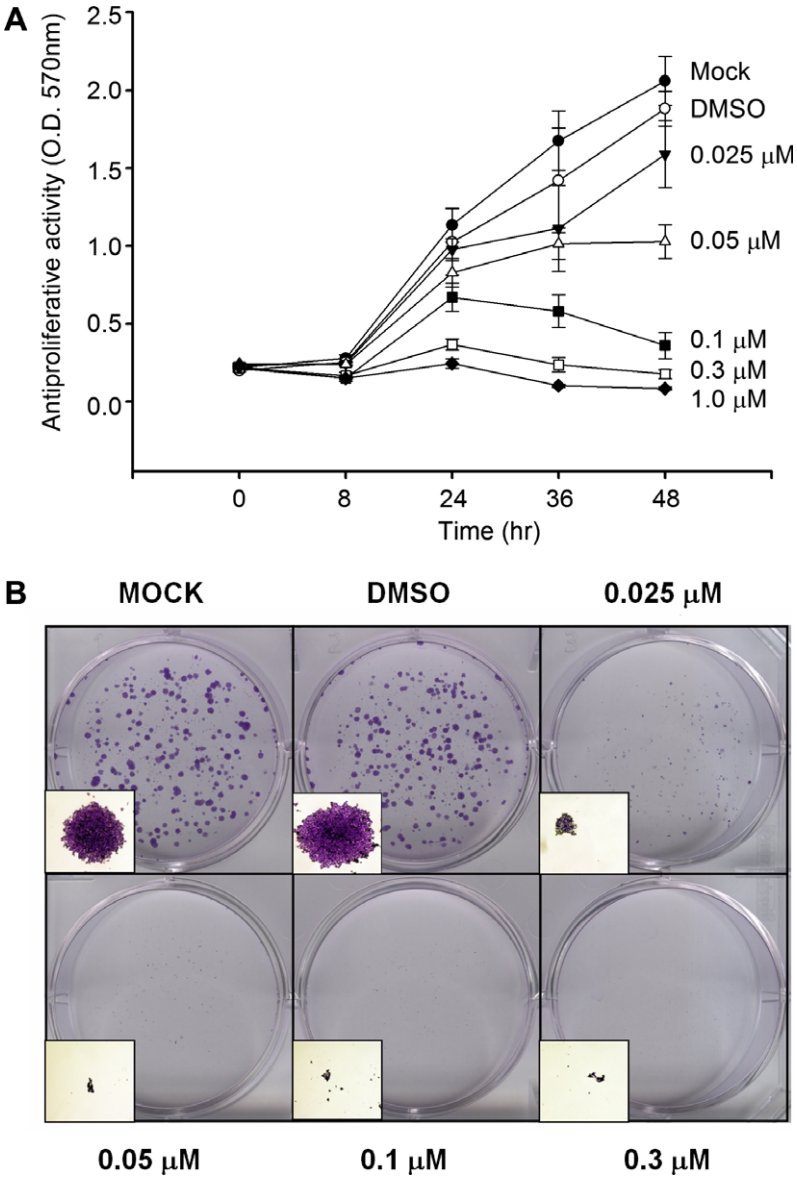


Figure 2. The effects of **25** on cell proliferative inhibition and colony formation in HeLa cells. (A) Cell proliferative inhibition effect of **25** in HeLa. Cell growth inhibition activity of **25** was assessed by MTT. Adherent cells proliferated in 96-well plates (3000 cells/well) were incubated with different concentrations of (0.025, 0.05, 0.1, 0.3 and 1.0 μM) at various time intervals. (B) Representative dishes by colony forming assay.

5.1.2. (11*H*-Indolo[3,2-*c*]quinolin-6-yl)phenylamine (6a)

A mixture of 6-chloro-2-methoxy-11*H*-indolo[3,2-*c*]quinoline (**5a**, 0.51 g, 2 mmol),[12] aniline (0.28 g, 3 mmol), and 2-butanol (20 mL) was refluxed for 18 h (TLC monitoring). The solvent was removed in vacuo and the residue was suspended in H_2O (50 mL). The resulting precipitate that separated was collected, washed with H_2O, and dried to give a crude solid, which was recrystallized from MeOH to give **6a** (0.40 g, 65%). Mp: 339–340 °C. IR (KBr): 3059, 1643, 1587, 1539, 1496, 1434, 1388, 1355, 1216, 1155. ^{1}H NMR (DMSO-d_6): 7.36–7.41 (m, 2H, Ar-H), 7.54–7.67 (m, 6H, Ar-H), 7.76–7.85 (m, 2H, Ar-H), 8.03 (d, 1H, J = 8.0 Hz, Ar-H), 8.32 (d, 1H, J = 8.4 Hz, Ar-H), 8.69 (d, 1H, J = 7.2 Hz, Ar-H), 10.20 (br s, 1H, NH), 12.53 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 102.16, 112.60, 113.52, 120.00, 121.19, 121.87 (2C), 123.01, 124.18, 125.14, 125.94, 126.32, 129.20, 129.82 (2C), 130.80, 136.03, 137.45, 138.94, 143.06, 148.34. Anal. Calcd for $C_{21}H_{15}N_3 \cdot 0.8H_2O$: C, 71.06; H, 4.83; N, 11.84. Found: C, 71.41; H, 4.75; N, 11.88.

Compounds **7a–10a** were prepared from **5a** in analogy to that described for compound **6a**, using the appropriate substituted-aniline.

5.1.3. 4-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)phenol hydrochloride (7a)

Yield (60%). Mp: 310–311 °C (MeOH). IR (KBr): 3083, 1637, 1608, 1510, 1455, 1398, 1266, 1228, 1163. ^{1}H NMR (DMSO-d_6): 7.98–7.01 (m, 2H, Ar-H), 7.39–7.45 (m, 3H, Ar-H), 7.54–7.75 (m, 3H, Ar-H), 7.82 (d, 1H, J = 8.0 Hz, Ar-H), 8.04 (d, 1H, J = 8.4 Hz, Ar-H), 8.54 (d, 1H, J = 8.4 Hz, Ar-H), 8.63 (d, 1H, J = 8.0 Hz, Ar-H), 9.95 (br s, 2H, NH, OH), 12.21 (br s, 1H, NH), 14.02 (br s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 101.00, 112.57, 113.16, 116.45 (2C), 119.55, 121.25, 121.51, 121.91, 122.88, 124.84, 125.75, 126.98, 127.70, 130.63, 135.62, 138.76(2C), 142.45, 149.15, 156.99. Anal. Calcd for $C_{21}H_{15}N_3O \cdot 1.0H_2O \cdot 1.0HCl$: C, 66.38; H, 4.43; N, 11.06. Found: C, 66.01; H, 4.77; N, 11.00.

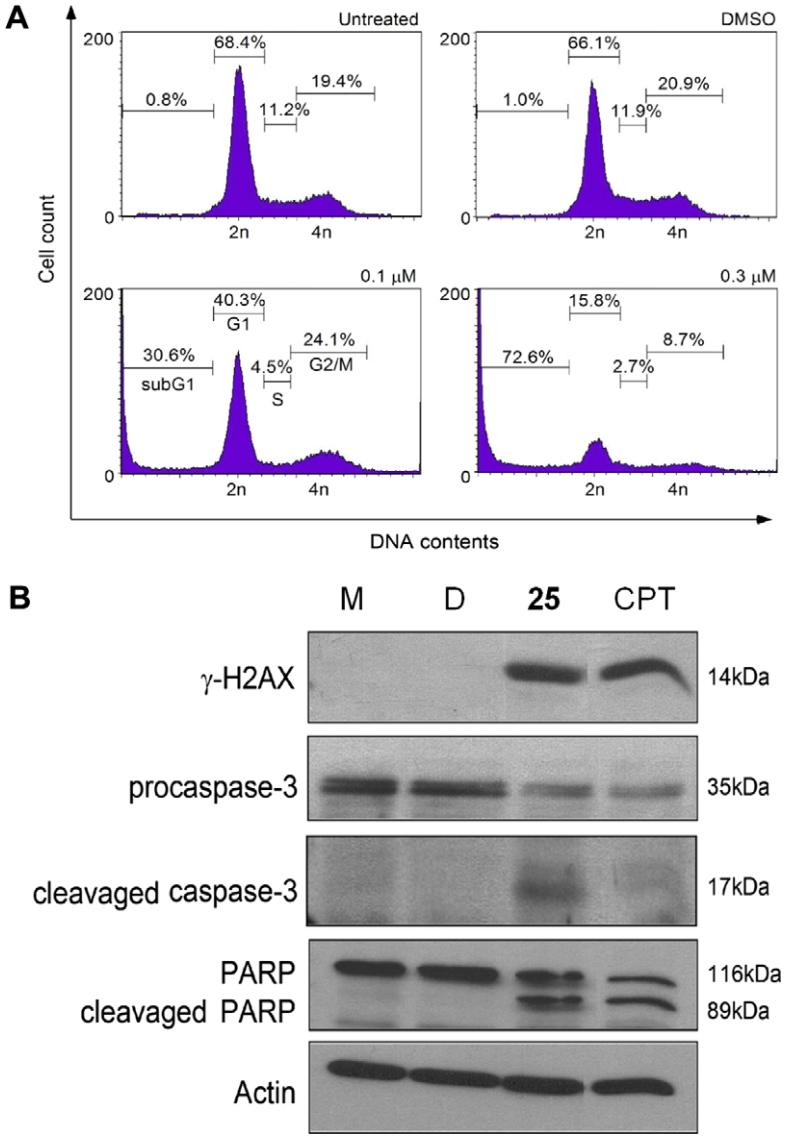


Figure 3. Effect of **25**-mediated apoptosis and cell death in PC-3 cells associated with the apoptotic pathways. (A) Flow cytometric analysis of PC-3 cells. After treatment with DMSO, 0.1, or 0.3 μM of **25** for 48 h, cells were harvested, fixed, and stained with propidium iodide as described in the Experimental Section prior to analysis by flow cytometry. (B) Western blot analysis of the effect of **25** on DNA damage (phophorylation of γ-H2AX), induce caspase-3 activation and PARP cleavage in PC-3 cells. Cells were treated with 0.1 μM of **25** and 10 μM of CPT (camptothecin) for 48 h, respectively. The protein contents were normalized by probing the same membrane with β-actin antibody. M: mock, D: DMSO.

5.1.4. 3-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)phenol hydrochloride (8a)

Yield (62%). Mp: 319–320 °C (MeOH). IR (KBr): 3426, 3178, 1639, 1594, 1457, 1408, 1250, 1215, 1186. ^{1}H NMR (DMSO-d_6): 6.84–6.87 (m, 1H, Ar-H), 7.01–7.04 (m, 2H, Ar-H), 7.33–7.85 (m, 6H, Ar-H), 8.07 (d, 1H, J = 8.0 Hz, Ar-H), 8.32 (d, 1H, J = 8.0 Hz, Ar-H), 8.69 (dd, 1H, J = 8.0, 0.8 Hz, Ar-H), 9.98 (br s, 1H, OH), 10.27 (br s, 1H, NH), 12.95 (br s, 1H, NH), 14.18 (br s. 1H, HCl). ^{13}C NMR (DMSO-d_6): 101.99, 111.53, 112.69, 113.34, 114.07, 114.89, 119.29, 121.26, 121.95, 122.07, 123.11, 125.41, 126.10. 130.72, 131.05, 135.13, 137.81, 138.97, 143.16, 148.12, 158.77. Anal. Calcd for $C_{21}H_{15}N_3O{\cdot}1.5H_2O{\cdot}1.0HCl$: C, 64.54; H, 4.96; N, 10.75. Found: C, 64.27; H, 5.00; N, 10.65.

5.1.5. 2-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)phenol (9a)

Yield (61%). Mp: 339–340 °C (MeOH). IR (KBr): 3176, 1641, 1604, 1508, 1456, 1402, 1296, 1243, 1213, 1107. ^{1}H NMR (DMSO-d_6): 7.00–7.77 (m, 8H, Ar-H), 7.84 (d, 1H, J = 8.0 Hz, Ar-H), 8.09 (d, 1H, J = 8.0 Hz, Ar-H), 8.57 (d, 1H, J = 8.0 Hz, Ar-H), 8.66 (d, 1H, J = 7.2 Hz, Ar-H), 9.85 (br s, 1H, OH), 10.28 (br s, 1H, NH), 12.20 (br s, 1H, NH), 14.07 (br s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 100.98, 112.68, 113.06, 117.09, 119.17, 119.77, 121.28, 121.48, 122.04, 122.56, 123.03, 125.05, 125.89, 128.42, 129.15, 130.82, 135.23, 138.82, 142.34, 148.74, 152.87. Anal. Calcd for $C_{21}H_{15}N_3O{\cdot}1.9H_2O{\cdot}1.0HCl$: C, 63.66; H, 5.04; N, 10.61. Found: C, 63.50; H, 5.07; N, 10.58.

5.1.6. (11*H*-Indolo[3,2-*c*]quinolin-6-yl)-(4-methoxyphenyl)amine (10a)

Yield (60%). Mp: 317–318 °C (MeOH). IR (KBr): 3064, 1639, 1605, 1509, 1455, 1397, 1247, 1175. ^{1}H NMR (DMSO-d_6): 3.85 (s, 1H, OCH_3), 7.11–7.15 (m, 2H, Ar-H), 7.38–7.75 (m, 5H, Ar-H), 7.82 (d, 1H, J = 8.0 Hz, Ar-H), 8.00 (d, 1H, J = 8.0 Hz, Ar-H), 8.52 (d, 1H, J = 8.4 Hz, Ar-H), 8.62 (d, 1H, J = 7.2 Hz, Ar-H), 9.83 (br s, 1H, NH), 12.38 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 55.44, 101.44, 112.48, 113.39, 114.93 (2C), 120.28, 121.21, 121.54,

121.73, 122.77, 124.62, 125.66, 126.93, 129.54, 130.43, 135.21, 138.74 (2C), 142.43, 149.13, 157.95. Anal. Calcd for $C_{22}H_{17}N_3O{\cdot}1.1H_2O{\cdot}1.0HCl$: C, 66.76; H, 5.11; N, 10.62. Found: C, 66.91; H, 5.15; N, 10.56.

5.1.7. 2-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)ethanol hydrochloride (11a)

A mixture of **5a** (0.51 g, 2 mmol), ethanolamine (0.24 g, 4 mmol), and 2-ethoxyethanol (20 mL) was heated at 140–150 °C for 48 h (TLC monitoring). The solvent was removed in vacuo and the residue was suspended in MeOH (50 mL). The resulting precipitate that separated was collected, washed with H_2O, and dried to give a crude solid, which was recrystallized from MeOH to give **11a** (0.38 g, 69%). Mp: 339–340 °C. IR (KBr): 3383, 3080, 1647, 1616, 1457, 1419, 1348, 1224, 1180. ^{1}H NMR (DMSO-d_6 + TFA-*d*): 3.86–3.89 (m, 2H, CH_2O), 4.00–4.03 (m, 2H, CH_2N), 7.44–7.81 (m, 5H, Ar-H), 8.18 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.38 (br s, 1H, NH), 8.47–8.50 (m, 1H, Ar-H), 8.58 (d, 1H, *J* = 8.0 Hz, Ar-H), 12.40 (br s, 1H, NH), 13.59 (s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 43.91, 60.63, 101.83, 111.88, 113.71, 120.62 (2C), 120.69, 121.29, 122.06, 123.66, 124.47, 128.91, 138.27, 140.95, 142.44, 152.16. Anal. Calcd for $C_{17}H_{15}N_3O{\cdot}1.0HCl$: C, 65.07; H, 5.14; N, 13.39. Found: C, 65.02; H, 5.22; N, 13.36.

Compounds **12a–14a**, **14b**, **15a–20a**, and **16b**–**18b** were prepared from **5a** or **5b** in analogy to that described for compound **11a**, using the appropriate substituted-alkylamines.

5.1.8. *N*-(2-(Dimethylamino)ethyl)-(11*H*-indolo[3,2-*c*]quinolin-6-yl)amine (12a)

The title compound was crystallized from EtOH in 69% yield. Mp: 85–87 °C. ^{1}H NMR (DMSO-d_6): 2.32 (s, 6H, $N(CH_3)_2$), 2.68 (t, 2H, *J* = 6.4 Hz, CH_2N), 3.80 (q, 2H, *J* = 6.4 Hz, $NHCH_2$), 6.55 (br s, 1H, NH), 7.27–7.53 (m, 4H, Ar-H), 7.66–7.68 (m, 2H, Ar-H), 8.24–8.27 (m, 2H, Ar-H), 12.53 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 38.29, 45.21 (2C), 58.31, 102.68, 111.58, 114.15, 120.26 (2C), 120.92, 121.39, 121.60, 123.98, 126.23, 128.11, 138.15, 140.75, 145.98, 152.90. Anal. Calcd for $C_{19}H_{20}N_4{\cdot}0.7H_2O$: C, 71.79; H, 6.80; N, 17.67. Found: C, 71.94; H, 6.77; N, 17.80.

5.1.9. *N*-(3-(Dimethylamino)propyl)-(11*H*-indolo[3,2-*c*]quinolin-6-yl)amine (13a)

The title compound was crystallized from EtOH in 66% yield. Mp: 73–75 °C. ^{1}H NMR (DMSO-d_6): 1.90 (quin, 2H, *J* = 6.4 Hz, CH_2), 2.27 (s, 6H, $N(CH_3)_2$), 2.34–2.48 (m, 2H, CH_2N), 3.73–3.78 (m, 2H, $NHCH_2$), 7.14 (br s, 1H, NH), 7.25–7.52 (m, 4H, Ar-H), 7.65–7.68 (m, 2H, Ar-H), 8.24–8.28 (m, 2H, Ar-H), 12.48 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 26.18, 40.46, 45.33 (2C), 58.31, 102.71, 111.48, 114.10, 120.10, 120.14, 120.69, 121.48, 121.54, 123.86, 126.18, 128.00, 138.09, 140.67, 146.16, 152.87. Anal. Calcd for $C_{20}H_{22}N_4{\cdot}0.2H_2O$: C, 74.60; H, 7.01; N, 17.40. Found: C, 74.57; H, 7.15; N, 17.45.

5.1.10. 2-(2-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)ethylamino)ethanol hydrochloride (14a)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/10) and crystallized from EtOH in 61% yield. Mp: 273–274 °C (dec). IR (KBr): 3337, 3077, 1643, 1612, 1456, 1419, 1343, 1219, 1086. ^{1}H NMR (DMSO-d_6): 3.14 (br s, 2H, CH_2), 3.41–3.42 (m, 2H, CH_2), 3.73 (t, 2H, *J* = 5.6 Hz, $NHCH_2$), 4.43–4.45 (m, 2H, CH_2O), 5.34 (br s, 1H, OH), 7.42–7.86 (m, 5H, Ar-H), 8.57–8.74 (m, 4H, Ar-H) 9.18 (br s, 2H, NH), 13.01 (br s, 1H, NH), 13.90 (br s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 30.69, 45.88, 49.28, 56.44, 100.55, 112.63, 112.84, 118.93, 121.25 (2C), 121.93, 122.78, 125.12, 125.76, 130.60, 135.30, 138.53, 141.48, 149.33. Anal. Calcd for $C_{19}H_{20}N_4{\cdot}1.5HCl{\cdot}1.5H_2O$: C, 56.75; H, 6.15; N, 13.93. Found: C, 56.59; H, 5.76; N, 14.21.

5.1.11. 2-((2-Fluoro-11*H*-indolo[3,2-*c*]quinolin-6-ylamino)methylamino)ethanol hydrochloride (14b)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/10) and crystallized from EtOH in 76% yield. Mp: 260 °C (dec). IR (KBr): 3386, 3179, 1585, 1530, 1501, 1458, 1416, 1371, 1220, 1196. ^{1}H NMR (DMSO-d_6): 3.13–3.17 (m, 2H, CH_2), 3.365–3.38 (m, 2H, CH_2), 3.72 (br s, 2H, $NHCH_2$), 3.97–4.00 (m, 2H, CH_2O), 5.34 (br s, 1H, OH), 7.05 (br s, 1H, NH), 7.31–7.48 (m, 3H, Ar-H), 7.70 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.81 (dd, 1H, *J* = 8.8, 5.2 Hz, 4-H), 8.18 (dd, 1H, *J* = 10.0, 2.8 Hz, 1-H), 8.50 (d, 1H, *J* = 8.4 Hz, Ar-H), 9.28 (br s, 1H, NH), 12.87 (br s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 37.76, 47.69, 48.99, 56.35, 103.08, 106.15 (*J* = 22.8 Hz), 111.56, 114.30 (*J* = 9.9 Hz), 116.69 (*J* = 23.5 Hz), 120.22, 120.95, 121.09, 124.43, 127.96 (*J* = 8.3 Hz), 138.11, 140.39 (*J* = 3.8 Hz), 141.87, 152.44, 156.93 (*J* = 236.5 Hz). Anal. Calcd for $C_{19}H_{19}FN_4O{\cdot}1.0HCl$: C, 60.88; H, 5.38; N, 14.95. Found: C, 61.00; H, 5.59; N, 14.57.

5.1.12. *N*-(2-(2-Aminoethylamino)ethyl)-(11*H*-indolo[3,2-*c*]quinolin-6-yl)amine hydrochloride (15a)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/3) and crystallized from EtOH in 57% yield. Mp: 202–203 °C. IR (KBr): 3351, 1647, 1615, 1526, 1453, 1343, 1216. ^{1}H NMR (DMSO-d_6): 2.93–2.97 (m, 4H, 2 × CH_2), 3.06 (t, 2H, *J* = 6.0 Hz, CH_2NH_2), 3.85 (t, 2H, *J* = 6.0 Hz, CH_2NH), 7.27–7.53 (m, 4H, Ar-H), 7.66–7.70 (m, 2H, Ar-H), 8.31–8.42 (m, 2H, Ar-H), 12.71 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 37.97, 39.66, 45.94, 48.06, 102.69, 111.49, 114.21, 120.10, 120.80, 121.03, 121.33, 121.76, 123.96, 126.02, 128.10, 138.13, 140.81, 145.64, 152.94. Anal. Calcd for $C_{19}H_{20}N_4{\cdot}2.0HCl{\cdot}0.4H_2O$: C, 57.11; H, 6.00; N, 17.53. Found: C, 57.47; H, 6.30; N, 17.21.

5.1.13. *N*-(11*H*-Indolo[3,2-*c*]quinolin-6-yl)-2-(piperazin-1-yl)ethanamine (16a)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/10 to 1/3) and crystallized from MeOH in 29% yield. Mp: 210–211 °C. IR (KBr): 3402, 1570, 1526, 1451, 1344, 1267, 1120. ^{1}H NMR (DMSO-d_6): 2.47 (br s, 4H, piperazinyl-H), 2.68 (t, 2H, *J* = 6.4 Hz, CH_2N), 2.77 (br s, 4H, piperazinyl-H), 3.75–3.80 (m, 2H, $NHCH_2$), 6.56 (t, 1H, *J* = 4.8 Hz, NH), 7.26–7.52 (m, 4H, Ar-H), 7.65–7.68 (m, 2H, Ar-H), 8.23–8.26 (m, 2H, Ar-H), 12.50 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 37.37, 45.85 (2C), 54.01 (2C), 57.27, 102.70, 111.68, 114.17, 120.02, 120.26, 120.88, 121.38, 121.58, 123.98, 126.28, 128.08, 138.19, 140.69, 146.14, 152.91. Anal. Calcd for $C_{21}H_{23}N_5{\cdot}1.0H_2O$: C, 69.40; H, 6.93; N, 19.27. Found: C, 69.68; H, 6.92; N, 19.00.

5.1.14. 2-Fluoro-*N*-(piperazin-1-ylethyl)-11*H*-indolo[3,2-*c*]quinolin-6-amine hydrochloride (16b)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/10 to 1/3) and crystallized from MeOH in 31% yield. Mp: 278–279 °C. IR (KBr): 3383, 1645, 1614, 1458, 1418, 1115. ^{1}H NMR (DMSO-d_6): 2.77–2.87 (m, 8H, piperazinyl-H), 3.05 (br s, 2H, CH_2N), 3.52 (m, 2H, $NHCH_2$), 7.36–7.54 (m, 3H, Ar-H), 7.74 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.91 (dd, 1H, *J* = 9.2, 5.2 Hz, 4-H), 7.98 (d, 1H, *J* = 8.0 Hz, Ar-H), 8.28 (dd, 1H, *J* = 9.6, 2.8 Hz, 1-H), 12.45 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 35.58, 48.59, 48.93, 52.50 (2C), 54.30, 106.25 (*J* = 24.2 Hz), 106.37, 111.94, 115.95 (*J* = 9.8 Hz), 117.17 (*J* = 25.1 Hz), 120.76, 120.94, 121.67, 124.93, 130.09 (*J* = 8.4 Hz), 138.57, 141.46, 141.66 (*J* =

4.5 Hz), 156.83, 158.15 (J = 238.7 Hz). Anal. Calcd for $C_{21}H_{22}FN_5 \cdot 0.5HCl \cdot 2.0H_2O$: C, 60.39; H, 6.39; N, 16.77. Found: C, 60.52; H, 6.40; N, 16.81.

5.1.15. *N*-(Morpholinoethyl)-11*H*-indolo[3,2-*c*]quinolin-6-amine (17a)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/5) and crystallized from EtOH in 26% yield. Mp: 132–133 °C. IR (KBr): 3432, 3154, 1592, 1518, 1449, 1402, 1366, 1224, 1197, 1126. ^1H NMR (DMSO-d_6): 2.49–2.54 (m, 4H, morpholinyl-H), 2.72 (t, 2H, J = 6.4 Hz, CH_2N), 3.62–3.66 (m, 4H, morpholinyl-H), 3.76–3.86 (m, 2H, $NHCH_2$), 6.56 (t, 1H, J = 5.2 Hz, NH), 7.25–7.70 (m, 6H, Ar-H), 8.23–8.30 (m, 2H, Ar-H) 12.48 (br s, 1 h, NH). ^{13}C NMR (DMSO-d_6): 37.22, 53.22 (2C), 57.10, 66.36 (2C), 102.59, 111.51, 114.04, 120.05, 120.14, 120.76, 121.28, 121.44, 123.86, 126.17, 127.95, 138.04, 140.59, 145.98, 152.76. Anal. Calcd for $C_{21}H_{22}N_5O \cdot 1.0H_2O$: C, 69.21; H, 6.64; N, 15.37. Found: C, 69.07; H, 6.65; N, 15.38.

5.1.16. 2-Fluoro-*N*-(morpholinoethyl)-11*H*-indolo[3,2-*c*]quinolin-6-amine (17b)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/5) and crystallized from EtOH in 38% yield. Mp: 116–117 °C. IR (KBr): 3355, 3282, 1595, 1527, 1454, 1413, 1338, 1195, 1112. ^1H NMR (DMSO-d_6): 2.60 (br s, 4H, morpholinyl-H), 2.77 (m, 2H, CH_2N), 3.64–3.67 (m, 4H, morpholinyl-H), 3.80–3.84 (m, 2H, $NHCH_2$), 6.62 (br s, 1H, NH), 7.33–7.48 (m, 3H, Ar-H), 7.68–7.73 (m, 2H, Ar-H), 8.06 (dd, 1H, J = 9.6, 3.2 Hz, 1-H), 8.31 (d, 1H, J = 8.0 Hz, Ar-H), 12.54 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 37.26, 53.21 (2C), 57.12 (2C), 66.24, 103.15, 105.96 (J = 22.7 Hz), 111.73, 114.11 (J = 9.1 Hz), 116.65 (J = 23.5 Hz), 102.43 (2C), 121.13, 124.40, 128.15 (J = 7.6 Hz), 138.17, 140.22 (J = 3.8 Hz), 142.68, 152.45, 156.77 (J = 235.7 Hz). Anal. Calcd for $C_{21}H_{21}FN_4O \cdot 2.0H_2O$: C, 62.99; H, 6.29; N, 13.99. Found: C, 62.63; H, 6.50; N, 13.79.

5.1.17. 6-[2-(4-Hydroxyphenyl)ethylamino]-11*H*-indolo[3,2-*c*]quinoline hydrochloride (18a)

The title compound was crystallized from MeOH in 30% yield. Mp: 320–321 °C. IR (KBr): 3415, 3088, 1645, 1611, 1513, 1458, 1426, 1345, 1218, 1170. ^1H NMR (DMSO-d_6): 3.03 (t, 2H, J = 7.4 Hz, CH_2), 4.10–4.16 (m, 2H, NCH_2), 6.67–6.71 (m, 2H, Ar-H), 7.18–7.22 (m, 2H, Ar-H), 7.42–7.47 (m, 1H, Ar-H), 7.54–7.63 (m, 2H, Ar-H), 7.76–7.82 (m, 2H, Ar-H), 8.32 (d, 1H, J = 8.4 Hz, Ar-H), 8.38 (br s, 1H, NH), 8.50–8.54 (m, 2H, Ar-H), 9.29 (br s, 1H, OH), 12.47 (br s, 1H, NH), 13.80 (br s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 33.69, 43.96, 100.16, 112.74, 113.08, 115.14 (2C), 118.74, 120.73, 121.20, 122.04, 122.79, 124.94, 125.74, 128.39, 129.96 (2C), 130.72, 135.21, 138.56, 141.42, 149.16, 155.93. Anal. Calcd for $C_{23}H_{19}N_3O \cdot 1.0HCl \cdot 1.2H_2O$: C, 67.14; H, 5.49; N, 10.21. Found: C, 67.14; H, 5.47; N, 10.20.

5.1.18. 4-[2-(2-Fluoro-11*H*-indolo[3,2-*c*]quinolin-6-ylamino)ethyl]phenol (18b)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/10) and crystallized from EtOH in 13% yield. Mp: 252–253 °C. IR (KBr): 3458, 3328, 1585, 1535, 1513, 1455, 1415, 1239, 1192. ^1H NMR (DMSO-d_6): 2.97 (t, 2H, J = 7.2 Hz, CH_2), 4.81–4.87 (m, 2H, NCH_2), 6.63 (br s, 1H, NH), 6.71–6.75 (m, 2H, Ar-H), 7.15–7.18 (m, 2H, Ar-H), 7.29–7.47 (m, 3H, Ar-H), 7.67 (d, 1H, J = 8.0 Hz, Ar-H), 7.74 (dd, 1H, J = 9.2, 5.6 Hz, 4-H), 8.05 (dd, 1H, J = 9.6, 2.8 Hz, 1r-H), 8.32 (d, 1H, J = 8.0 Hz, Ar-H), 9.20 (s, 1H, OH), 12.48 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): 34.51, 42.88, 103.26, 105.88 (J = 22.7 Hz), 111.56, 114.06 (J = 9.1 Hz), 115.18 (2C), 116.61 (J = 24.3 Hz), 102.26, 120.87, 121.17, 124.32, 128.33 (J = 7.6 Hz), 129.63 (2C), 130.33, 138.12, 140.26 (J = 3.0 Hz), 142.90, 152.43, 155.57, 156.74 (J = 235.0 Hz). Anal. Calcd for $C_{23}H_{18}FN_3O \cdot 1.5H_2O$: C, 69.33; H, 5.31; N, 10.55. Found: C, 69.15; H, 5.31; N, 10.22.

5.1.19. 6-[2-(3,4-Dihydroxyphenyl)ethylamino]-11*H*-indolo[3,2-*c*]quinoline hydrochloride (19a)

The title compound was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/10) and crystallized from MeOH in 15% yield. Mp: 202–203 °C. IR (KBr): 3418, 3180, 1648, 1613, 1526, 1459, 1346, 1251, 1116. ^1H NMR (DMSO-d_6): 2.96 (t, 2H, J = 7.4 Hz, CH_2), 4.05–4.10 (m, 2H, NCH_2), 6.62–6.68 (m, 2H, Ar-H), 6.80–6.81 (d, 1H, J = 1.6 Hz, Ar-H), 7.42–7.81 (m, 5H, Ar-H), 8.27–8.36 (m, 2H, Ar-H and NH), 8.50 (d, 1H, J = 8.0 Hz, Ar-H), 8.54 (d, 1H, J = 8.0 Hz, Ar-H), 8.80 (br s, 1H, OH), 8.84 (br s, 1H, OH), 12.44 (br s, 1H, NH), 13.80 (br s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 33.88, 43.93, 100.22, 112.66, 112.80, 115.51, 116.45, 118.86, 119.62, 120.74, 121.19, 121.95, 122.78, 124.81, 125.64, 129.14, 130.64, 138.53, 141.37, 143.81, 145.18, 149.24, 155.96. Anal. Calcd for $C_{23}H_{19}N_3O_2 \cdot 1.0HCl \cdot 2.0H_2O$: C, 62.52; H, 5.47; N, 9.51. Found: C, 62.58; H, 5.45; N, 9.63.

5.1.20. 6-[2-(3,4-Dimethoxyphenyl)ethylamino]-11*H*-indolo[3,2-*c*]quinoline hydrochloride (20a)

The title compound was crystallized from MeOH in 66% yield. Mp: 231–232 °C. IR (KBr): 3411, 3055, 1648, 1612, 1514, 1458, 1421, 1347, 1261, 1157. ^1H NMR (DMSO-d_6): 3.07 (t, 2H, J = 7.4 Hz), 3.01 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 4.21–4.26 (m, 2H, NCH_2), 6.78 (d, 1H, J = 8.0 Hz, Ar-H), 6.86 (dd, 1H, J = 1.6, 8.0 Hz, Ar-H), 7.05 (d, 1H, J = 1.6 Hz, Ar-H), 7.42–7.81 (m, 5H, Ar-H), 8.34 (br s, 1H, NH), 8.38 (d, 1H, J = 8.0 Hz, Ar-H), 8.51–8.55 (m, 2H, Ar-H), 12.60 (br s, 1H, NH), 13.80 (br s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 34.32, 43.89, 55.28, 55.49, 100.14, 111.88, 112.66, 112.70, 113.06, 118.74, 120.68, 120.98, 121.18, 121.97, 122.72, 124.84, 125.71, 130.61, 130.86, 135.24, 138.53, 141.38, 147.37, 148.58, 149.21. Anal. Calcd for $C_{25}H_{23}N_3O_2 \cdot 1.0HCl \cdot 1.2H_2O$: C, 65.92; H, 5.84; N, 9.22. Found: C, 65.89; H, 5.93; N, 9.29.

5.1.21. *N*-[6-(11*H*-indolo[3,2-*c*]quinolin-6-ylamino)hexyl]-11*H*-indolo[3,2-*c*]quinolin-6-amine (21) and *N*-(6-aminohexyl)-11*H*-indolo[3,2-*c*]quinolin-6-amine (23)

A mixture of **5a** (0.51 g, 2 mmol), 1,6-diaminohexane (0.47 g, 4 mmol), and 2-ethoxyethanol (20 mL) was heated in a sealed steel bomb at 130–140 °C for 4 days (TLC monitoring). The reaction mixture was partitioned between H_2O (50 mL) and CH_2Cl_2 (50 mL). The organic layer was separated, dried over $MgSO_4$, and evaporated. The resulting residue was purified by column chromatography ($MeOH/CH_2Cl_2$ 1/5 to 1/3) to give two fractions. The first fraction was crystallized from EtOH to give bis-substituted product **21** (0.14 g, 13%) as a yellow solid. Mp: 249–250 °C. IR (KBr): 3338,3173, 1643, 1614, 1528, 1455, 1417, 1217. ^1H NMR (DMSO-d_6): 1.57–1.84 (m, 8H, 4 × CH_2), 3.91 (q, 4H, J = 6.0 Hz, 2 × $NHCH_2$), 7.34–7.38 (m, 2H, Ar-H), 7.47–7.51 (m, 4H, Ar-H), 7.63–7.67 (m, 2H, Ar-H), 8.23 (br s, 2H, 2 × NH), 8.47 (d, 2H, J = 8.0, 1.6 Hz, Ar-H), 8.55 (d, 2H, J = 8.0 Hz, Ar-H), 12.60 (br s, 1H, HCl), 13.45 (br s, 2H, 2 × NH). ^{13}C NMR (DMSO-d_6): 26.05, 28.71, 41.84, 100.86, 112.30, 113.18, 120.78, 121.28, 121.39, 122.50, 123.60, 125.10, 129.81, 138.40, 141.17, 150.29. Anal. Calcd for $C_{36}H_{32}N_6 \cdot 2.0HCl \cdot 4.0H_2O$: C, 62.33; H, 6.10; N, 12.12. Found: C, 62.51; H, 6.11; N, 12.01.

The second fraction was crystallized from EtOH to give mono-substituted product **23** (0.08 g, 12%) as a yellow solid. Mp: 84–85 °C. IR (KBr): 3430, 3165, 1618, 1575, 1529, 1453, 1215. ^1H NMR (DMSO-d_6): 1.41–1.78 (m, 8H, 4 × CH_2), 2.76 (t, 2H, J = 7.2 Hz, CH_2NH_2), 3.70 (m, 2H, $NHCH_2$), 5.59 (br s, 1H, NH), 7.24–7.30 (m, 2H, Ar-H), 7.41 (m, 1H, Ar-H), 7.48 (m, 1H, Ar-H), 7.63–7.67 (m, 2H, Ar-H), 8.28 (m, 1H, Ar-H), 8.40 (m, 1H, Ar-H).

^{13}C NMR (DMSO-d_6): 25.84, 26.32, 27.29, 29.24, 38.87, 40.36, 102.69, 111.45, 114.13, 120.05, 120.69, 120.77, 121.43, 121.69, 123.87, 126.22, 128.02, 138.12, 140.75, 146.10, 152.98. Anal. Calcd for $C_{21}H_{24}N_4 \cdot 1.0HCl \cdot 2.0H_2O$: C, 62.29; H, 7.229; N, 13.84. Found: C, 62.52; H, 7.26; N, 13.62.

5.1.22. *N*-[7-(11*H*-Indolo[3,2-*c*]quinolin-6-ylamino)heptyl]-11*H*-indolo[3,2-*c*]quinolin-6-amine (22) and *N*-(7-aminoheptyl)-11*H*-indolo[3,2-*c*]quinolin-6-amine (24)

From **5a** and 1,7-diaminoheptane as described for **21** and **23**. Compound **22** was obtained in 8% yield (0.09 g) as a pale-yellow solid. Mp: 330 °C (dec). IR (KBr): 3368, 3066, 1643, 1613, 1458, 1417, 1218. Mp: 330 °C (dec). IR (KBr): 1614, 1643, 3338. UV (MeOH): 258 (4.89), 295 (4.31), 323 (4.31, 338 (4.39). ^{1}H NMR (DMSO-d_6): 1.48 (br s, 6H, 3 × CH_2), 1.81 (br s, 4H, 2 × CH_2), 3.96 (q, 4H, J = 6.4 Hz, 2 × NHCH_2), 7.36–7.58 (m, 6H, Ar-H), 7.71–7.79 (m, 4H, Ar-H), 8.33 (br s, 2H, 2 × NH), 8.43 (d, 2H, J = 8.0, 1.6 Hz, Ar-H), 8.53–8.62 (m, 4H, Ar-H), 12.62 (br s, 2H, HCl), 13.82 (br s, 2H, 2 X NH). ^{13}C NMR (DMSO-d_6): 26.07, 28.57, 42.27, 100.27, 112.59, 112.87, 119.28, 120.78, 121.24, 121.82, 122.79, 124.57, 125.50, 130.41, 138.51, 141.31, 149.38. Anal. Calcd for $C_{37}H_{34}N_6 \cdot 2.0HCl \cdot 2.0H_2O$: C, 66.16; H, 6.00; N, 12.51. Found: C, 65.95; H, 6.16; N, 12.41.

Compound **24** was obtained in 32% yield (0.22 g) as a pale-yellow solid. Mp: 141–142 °C. IR (KBr): 3412, 3146, 1625, 1573, 1528, 1453, 1214. ^{1}H NMR (DMSO-d_6): 1.32–1.43 (m, 8H, 4 × CH_2), 1.76 (quin, 2H, J = 7.2 Hz, CH_2), 2.52 (m, 2H, CH_2), 3.69 (q, 2H, J = 6.8 Hz, NHCH_2), 6.56 (br s, 1H, NH), 7.24–7.31 (m, 2H, Ar-H), 7.38–7.50 (m, 2H, Ar-H), 7.63–7.66 (m, 2H, Ar-H), 8.23 (dd, 1H, J = 8.0, 1.6 Hz, Ar-H), 8.38 (d, 1H, J = 8.0 Hz, Ar-H), 12.20–12.80 (br s, 2H, NH_2). ^{13}C NMR (DMSO-d_6): 26.47, 26.77, 28.95, 29.35, 32.92, 40.48, 41.50, 102.74, 111.41, 114.09, 120.07, 120.68, 120.76, 121.46, 121.54, 123.88, 126.24, 128.02, 138.09, 140.74, 146.13, 153.00. Anal. Calcd for $C_{22}H_{26}N_4 \cdot 0.3HCl \cdot 0.4H_2O$: C, 72.47; H, 7.49; N, 15.37. Found: C, 72.47; H, 7.49; N, 15.36.

5.1.23. *N,N*-Bis-[3-(11*H*-indolo[3,2-*c*]quinolin-6-yl)aminopropyl]amine hydrochloride (25)

A mixture of **5a** (1.26 g, 5 mmol), dipropylenetriamine (1.31 g, 10 mmol), pyridine (0.8 mL), and ethoxyethanol (20 mL) was heated in a sealed steel bomb at 100–120 °C for 4 days (TLC monitoring). The reaction mixture was then cooled and evaporated in vacuo to give a residue which was treated with 1 N HCl (30 mL) and stirred at room temperature overnight. The resulting residue was filtered off and the filtrate was treated with $NaHCO_3$ to neutralize HCl. The precipitate thus obtained was collected, purified by FC (MeOH/CH_2Cl_2 = 1/5), and crystallized from EtOH to give **25** (0.45 g, 16%) as a white powder. Mp: 107–108 °C. IR (KBr): 3385, 3179, 1645, 1612, 1458, 1416, 1217. ^{1}H NMR (DMSO-d_6): 2.21 (m, 4H), 3.16 (m, 4H), 4.16 (m, 4H), 7.38 (m, 2H), 7.51 (m, 2H), 7.57 (m, 2H), 7.70–7.78 (m, 4H), 8.52 (m, 4H), 8.75 (m, 2H), 9.41 (br s, 2H), 12.71 (br s, 2H), 13.84 (br s, 2H, HCl). ^{13}C NMR (DMSO-d_6): 25.37, 42.46, 44.18, 100.39, 112.60, 112.88, 118.94, 121.02, 121.24, 121.96, 122.72, 124.87, 125.63, 130.52, 135.48, 138.52, 141.44, 149.22. Anal. Calcd for $C_{36}H_{33}N_7 \cdot 2.4HCl \cdot 3.0H_2O$: C, 61.31; H, 5.92; N, 13.90. Found: C, 61.33; H, 5.72; N, 13.83.

5.1.24. *N,N*-Bis-[3-(11*H*-indolo[3,2-*c*]quinolin-6-yl)aminopropyl]-*N*-methylamine hydrochloride (26)

This compound was obtained from **5a** and *N,N*-bis(3-aminopropyl)methylamine as described for **25**, which was crystallized from EtOH to give **25** as a white powder. Yield: 21%; Mp: 76–77 °C. IR (KBr): 3398, 1650, 1614, 1576, 1528, 1452, 1215. ^{1}H NMR (DMSO-d_6): 2.31 (m, 4H), 2.89 (s, 3H), 3.35–3.49 (m, 4H), 4.11 (m, 4H), 7.39 (m, 2H, Ar-H), 7.51 (m, 2H, Ar-H), 7.61 (m, 2H, Ar-H), 7.71–7.78 (m, 4H, Ar-H),), 8.45–8.50 (m, 4H, Ar-H), 8.55 (br s, 2H, NH), 8.66 (m, 2H, Ar-H), 10.60 (br s, 1H, NH), 12.71 (br s, 1H, NH), 13.64 (br s, 1H, HCl). ^{13}C NMR (DMSO-d_6): 23.42, 40.21, 42.06, 52.19, 100.24, 112.49, 112.82, 118.88, 120.86, 121.15, 121.81, 122.65, 124.72, 125.48, 130.32, 135.40, 138.44, 141.36, 149.13. Anal. Calcd for $C_{37}H_{35}N_7 \cdot 1.2HCl \cdot 0.9H_2O$: C, 70.30; H, 6.11; N, 15.51. Found: C, 70.34; H, 6.04; N, 15.42.

Acknowledgments

Financial support of this work by the National Science Council of the Republic of China is gratefully acknowledged. We also thank National Cancer Institute (NCI) of the United States for the anticancer screenings and the National Center for High-Performance Computing for providing computer resources and chemical database services.

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