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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro cancer cell growth inhibition evaluation of *11*-amino-modified 5-Me-indolo[2,3-*b*]quinolines and their COMPARE analyses

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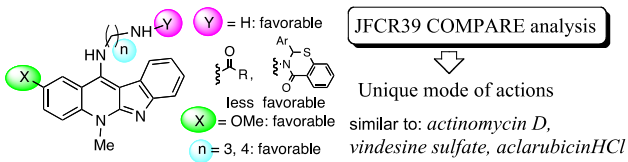
Abstract A plant-derived neocryptolepine core, the 5-Me-indolo[2,3-*b*]quinoline skeleton, was emblazoned with substituents at C11 and C2 and then tested against various cancer cell lines to find potent anticancer agents. In the in vitro antiproliferative activity assay against the breast cancer MDA-MB-453 cell line, the attachment of alkylamino substituents at C11 of the 5-Me-indolo[2,3-*b*]quinoline induced improved activities. Specifically, 11-(3-aminopropylamino) and 11-(4-aminobutylamino) derivatives indicated the highest activity and selectivity against MDA-MB-453 (IC_{50} = 0.3–0.5 μM) and also exhibited a higher cytotoxicity against the colon adenocarcinoma (WiDr) and ovarian cancer (SKOv3) cell lines. A synergistic effect by attachment of substituents at C2 was favorably observed with an electron-donating group, such as CH_3O, and unfavorably observed with an electron-withdrawing one, such as F and CF_3. Further modification of the terminal free amino group of the lariat attachment at C11 into the corresponding acylamides and 2,3-dihydrobenzo[e][1,3]thiazin-4-ones was not effective for the antiproliferative activity. The computer-assisted database analysis, COMPARE, suggested that **14e** and **13b** have a mode of action similar to actinomycin D and **13c** has a mode of actions similar to vindesine sulfate or aclarubicin hydrochloride. However, the new compounds may have other unique mode of actions since the correlation coefficients (*r*) were in relatively low levels.

Graphical Abstract



Keywords 5Me-indolo[2,3-*b*]quinoline · Antiproliferative agent · C11-alkylamino-substituted · MDA-MB-453 · JFCR39 panel · COMPARE study

Introduction

Cancer is a leading cause of death worldwide, accounting for approximately 14 million new cases and 8.2 million deaths in 2012 (data from WHO) (World Cancer Report, 2014; Cancer Fact sheet No 297). A virtually promising strategy for cancer prevention today is chemotherapy (Neidle and Thurston, 2005). DNA has been a major target for anticancer drugs (Gibson, 2002), because the

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interaction ability can interfere with transcription (gene expression and protein synthesis) and DNA replication (Yang and Wang, 1999; Denny, 1989).

Naturally occurring alkaloids are important chemical compounds that serve as a rich reservoir of lead compounds for drug discovery (Kittakoop *et al.*, 2014; Leland, 2006). In a search for such lead compounds, the constituents of the roots of the climbing shrub *Cryptolepis sanguinolenta*, growing in West Africa, such as Ghana, have attracted our attention, since an aqueous macerate or decoction of this root is used for the treatment of endemic disease, such as malaria fever, and various disorders of the body (Alexandra *et al.*, 2000; Cimanga *et al.*, 1996a).

Quinoline–indole motif-containing alkaloids were the main components identified in this plant (Cimanga *et al.*, 1996b). Among the known constituents, cryptolepine (**i**), neocryptolepine (**ii**), and isocryptolepine (**iii**) are promising scaffolds for developing more active derivatives in view of their various biological properties (Fig. 1) (Bracca *et al.*, 2014).

The antiproliferative testing of the neocryptolepine analogues indicated that the derivatives of 5-methylated **ii** are usually more cytotoxic than their respective congeners of 6-methylated **iv** (Wang *et al.*, 2012). A synergistic effect of the substituents at the C2 and/or C9 positions was also observed (Kaczmarek *et al.*, 1999).

Intrigued by the importance of the substituents, such as alkyl and alkylamino groups, on the indolo[2,3-*b*]quinoline scaffolds **ii** and **iv** (Wang *et al.*, 2012; Lu *et al.*, 2013), we attempted to synthesize the 5-Me-indolo[2,3-*b*]quinoline derivatives **11**–**16** with various alkylamino and aminoalkylamino groups at the C11 positions and tested their antiproliferative activities against cancer cell lines, such as human breast cancer (MDA-MB-453), colon adenocarcinoma (WiDr), and ovarian cancer (SKOv3). Although target-based screening is now predominant, cell-based screening is still the main technique for revealing cytotoxic drugs. Thus, we applied the synthesized agents to the different cell lines from that used in our previous testing (Wang *et al.*, 2012).

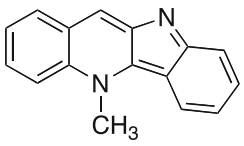
In the meantime, some of the 11-amino-modified 5-Me-indolo[2,3-*b*]quinolines **13** and **14** were examined for their antitumor effects across a panel of 39 human cancer cell lines, termed JFCR39, which is comprised the subpanels of human cancer cell lines derived from the breast, melanoma, lung, colon, central nervous system (CNS), ovary, stomach, kidney, and prostate. An antitumor spectrum of each compound across JFCR39, or *fingerprint*, was calculated, and a COMPARE analysis was carried out to predict the molecular mode of action of a respective antitumor compound by comparing the fingerprints with those of reference compounds possessing a known mechanism of action previously determined (Yamori *et al.*, 1999; Yamori, 2003).

Results and discussion

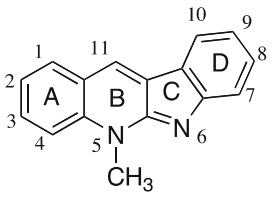
Synthesis

According to the reported procedure, the 11-chloro-neocryptolepine cores with substituents at C2 were synthesized starting from 1H-methyl indole-3-carboxylate (**1**) and *N*-methylanilines **2** (Scheme 1) (Wang *et al.*, 2014). Thus, the intermediate 2-*N*-(*N*-methylanilino)indole-3-carboxylates (**3**) were obtained by the chlorination of **1** with *N*-chlorosuccinimide in the presence of 1,4-dimethylpiperazine followed by the addition of a mixture of **2** and trichloroacetic acid. Cyclization of **3** via intramolecular aromatic acylation was carried out by heating at 250 °C in diphenyl ether to form tetracyclic ketones **4**, which were

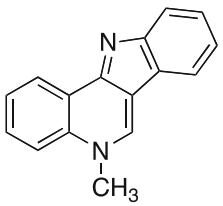
Fig. 1 Representative indoloquinolines from *Cryptolepis sanguinolenta* and congener



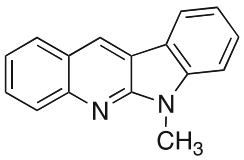
i, Cryptolepine



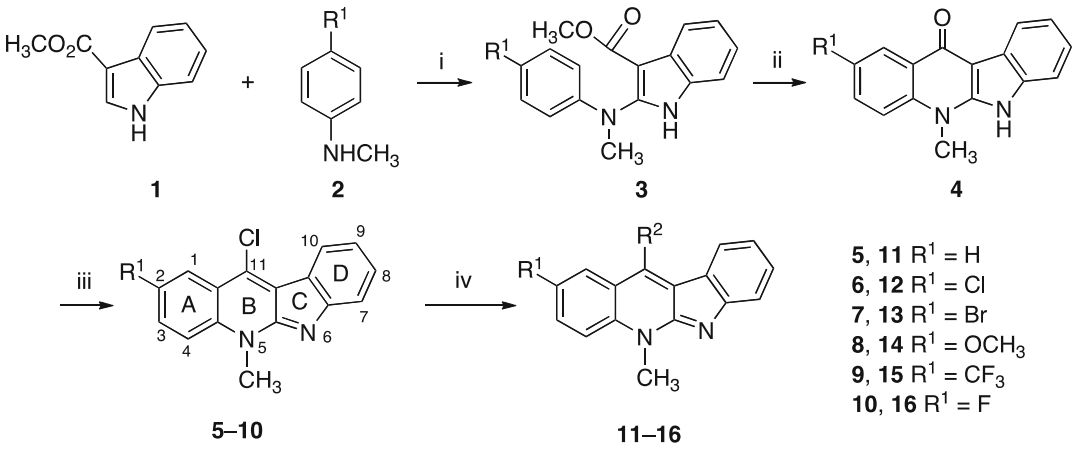
ii, Neocryptolepine



iii, Isocryptolepine



iv, 6-Me-indolo[2,3-*b*]quinoline



Scheme 1 Preparation of 11-chloroneocryptolepines **5–10** and their 11-amino derivatives **11–16**. 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

converted to 11-chloroneocryptolepines **5–10** by chlorination and dehydration with $POCl_3$. Subsequently, amination through the S_NAr reaction of **5–10** was performed by heating with the appropriate amines in DMF to afford the 11-aminoalkylamino-substituted neocryptolepine derivatives **11–16**.

Further modifications at the terminal amino function of the lateral 3-aminopropylamino groups attached at C11 of the indolo[2,3-*b*]quinoline **11e** were carried out as outlined in Scheme 2. First, the terminal amino group at C11 of **11e** was smoothly acylated with acetyl and 2-thienyl chlorides to give the corresponding 11-(3-*N*-acylaminopropylamino) derivatives **17**. In order to connect the sulfur-containing heterocyclic pendant at the C11 attachment, the thiazolidin-4-one skeleton was constructed. Thus, the three-component condensation of the 11-(3-aminopropylamino)-substituted **11e**, the substituted aldehydes, and 2-mercaptobenzoic acid was smoothly achieved with *N*,*N*-dicyclohexylcarbodiimide (DCC) in toluene by heating in good yields.

In vitro antiproliferative activities

The results of the cytotoxic activity in vitro were expressed as the IC_{50} 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.

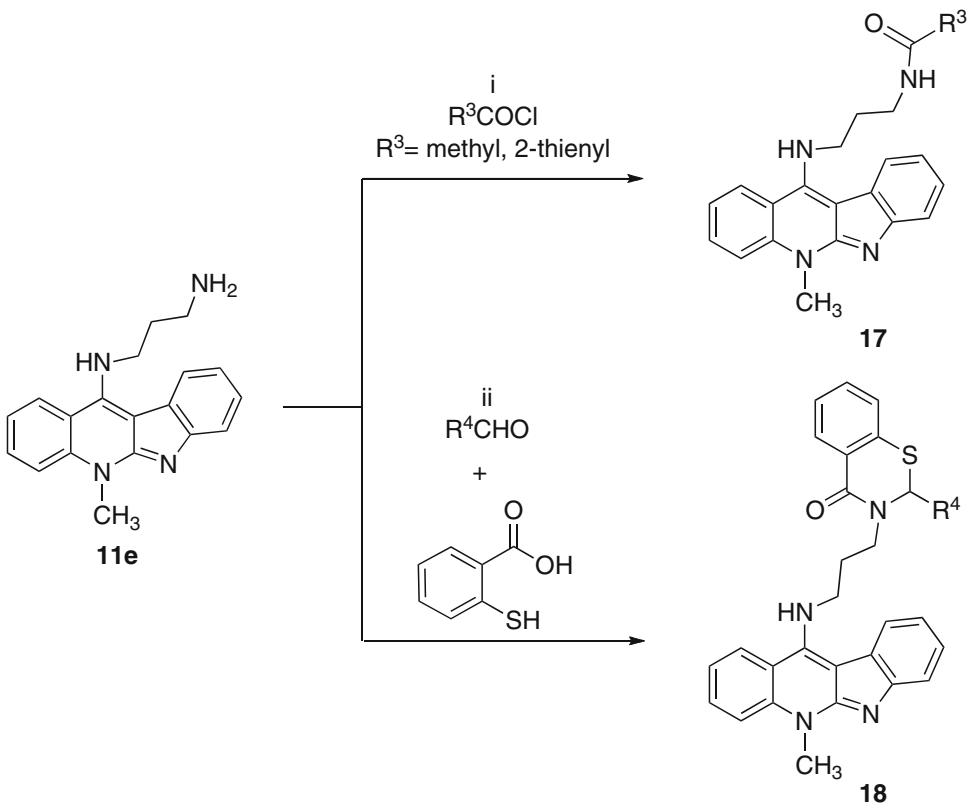
The results on the antiproliferative activity of neocryptolepine and its chemically modified analogues against the human breast cancer MDA-MB-453 cell line are summarized in Table 1, along with the data of the anticancer drugs, cisplatin (Rakic *et al.*, 2012) and doxorubicin HCl (Sandhu *et al.*, 2012).

The natural product neocryptolepine (**ii**) exhibited comparable antiproliferative activities (IC_{50} 7.48 μM) against the MDA-MB-453 cell line with the reference anticancer drug cisplatin (IC_{50} 7.6 ± 0.7 μM), but much weaker than that of doxorubicin HCl (IC_{50} 0.086 ± 0.006 μM). Introduction of the Cl atom at the C11 of neocryptolepine induced a moderate improvement in the activity (IC_{50} 5.16 μM). Strikingly, introduction of an amino group significantly increased the cytotoxic activity compared with the non-substituted neocryptolepine. Accordingly, various kinds of amino groups were then evaluated to assess their antiproliferative activity.

For the SAR study by varying the substituents at C11, we applied three different kinds of amines as the substituents at C11, namely alkylamines, benzenamines, and hydrazines, and the results are given in Table 1 and in the supplementary Table. Among the three different kinds of amines, the alkylamines showed a higher activity than the benzenamines and are more favorable than hydrazines in terms of their availability as structure-tunable materials.

Various alkylamines, which are classified into three categories, i.e., monoamines, 1,n-diamines, and polyamines, were employed as the substituents. In our experiments, the diamines and polyamines show higher activities compared with the monoamines. The 1,ω-diamino-substituted derivatives were applied for screening under varying the size of carbon spacer between two amino groups. Thus, the highest activities were achieved with 4-aminobutylamino- and 3-aminopropylamino-substituents, having LC_{50} values of 0.50 and 0.30 μM, respectively. As shown in the supplementary Table, the hydrazine derivatives show lower activities than that of the 1,3-diaminopropane derivative, i.e., IC_{50} 0.50 versus IC_{50} 1.85–2.34.

We then focused our attention on the effect of the substituent at C2 on the cytotoxicity. Thus, the substituents at C2 were varied from H to Br, OCH_3, CF_3, F in the



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

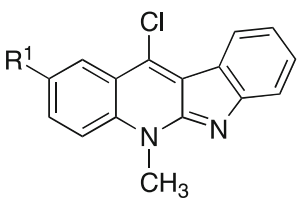
presence of the (5-(diethylamino)pentan-2-yl)amino group at C11 (including the supplementary Table). However, in these cases, the substituent at C2 exerted little influence on the activity. We observed that the strong electron-withdrawing groups, such as CF_3 and F, at C2 are not favorable for the cytotoxicity and, in contrast, the electron-donating group, such as OCH_3, is fairly favorable. Indeed, compound **14e** bearing an OCH_3 at C2 and a (3-aminopropyl)amino group at C11 showed the improved IC_{50} value of 0.20 μM, the highest value of the evaluated data compared with that of compound **11e** bearing no substituent at C2 (0.50 μM).

Subsequently, these compounds, **11a–c**, **11e**, **11g**, **11k**, **11l**, **13a**, and **14e**, which showed a potent antiproliferative activity against the human breast cancer MDA-MB-453 cell line with $IC_{50} < 10$ were then chosen for the next stage, i.e., the antiproliferative activity against WiDr (colon adenocarcinoma cell line), SKOV3 (ovarian cancer cell line), along with the normal mice fibroblast BALB/3T3. These results are summarized in Table 2. All the tested compounds, except for **11l**, were cytotoxic against the WiDr and SKOv3 cancer cells, and their antiproliferative activities against these cancer cells were much higher than that of the control agent, cisplatin (Kuo *et al.*, 2010; Ganta *et al.*, 2014). However, these tested compounds were ca. 5 times less active when compared to anticancer drug doxorubicin (Moon *et al.*, 1999; Molphy *et al.*, 2014) (Table 2). Compounds **11e** and **14e** have much higher antiproliferative activities against the cancer cell lines WiDr and SKOv3, compared with other compounds, and are less cytotoxic against normal mice fibroblast BALB/3T3. Against the normal fibroblast BALB/3T3, the antiproliferative activities of **11e** and **14e** were 1.4–2.6 times lower than that against the cancer cell lines. Compounds **14e** have a selective antiproliferative activity, mostly against the SKOv3 cell line.

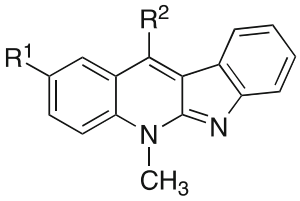
Based on these results, it is quite obvious that the introduction of the proper alkylamino substituents into biologically active derivatives can favorably influence their activities and selectivity in DNA binding (Wang *et al.*, 2012; Kaczmarek *et al.*, 1999).

As the additional SAR study, the C11 terminal amino group modified compounds, such as the acylated **17** and 2,3-dihydrobenzo[e][1,3]thiazin-4-one derivatives **18**, were tested for their antiproliferative activity against the human breast cancer MDA-MB-453 cell line, WiDr (colon adenocarcinoma cell line), and SKOV3 (ovarian cancer cell line). The biological results along with the cytotoxicity of the anticancer drugs, cisplatin and doxorubicin HCl, are summarized in Table 3. However, the chemically modified analogues **17** and **18** showed slightly lower antiproliferative activities compared with the respective precursors **11–16** bearing a terminal free amino group. In the series of modified compounds **18** bearing a 2,3-

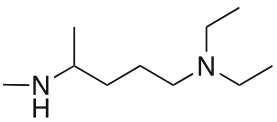
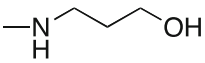
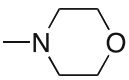
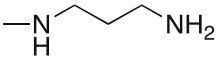
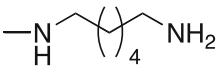
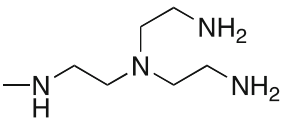
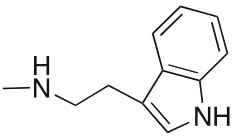
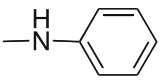
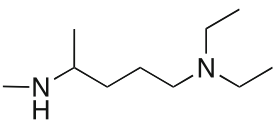
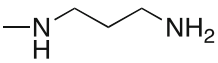
Table 1 Yields of 11-alkylaminated 5-methyl-indolo[2,3-*b*]quinolines and their antiproliferative activities against the human breast cancer MDA-MB-453 cell line



6,10



11, 13, 14

Compound	R^1	R^2	Yield (%)	MDA-MB-453 IC_{50} (μM)
Neocryptolepine (**ii**)	H	H		7.48 ± 4.42
6	H	Cl	75	5.16 ± 2.74
10	F	Cl	95	3.78 ± 0.12
11a	H		99	1.53 ± 1.00
11b	H		94	4.41 ± 3.37
11c	H		77	5.12 ± 0.43
11e	H		96	0.50 ± 0.24
11 g	H		96	1.76 ± 1.08
11 k	H		78	5.41 ± 1.58
11 l	H		92	5.72 ± 1.31
11p	H		95	>10.4
13a	Br		82	1.90 ± 1.15
14e	OCH_3		99	0.20 ± 0.09
Cisplatin		7.6 ± 0.7[a]		
Doxorubicin		0.086 ± 0.006[b]		

[a] Rakic *et al.* (2012)

[b] Sandhu *et al.* (2012)

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 WiDr (colon adenocarcinoma) and SKOv3 (ovarian cancer)

Compound	BALB/3T3 IC_{50} (μM)	WiDr IC_{50} (μM)	SKOv3 IC_{50} (μM)
11a	N.T.	4.18 ± 0.09	10.18 ± 3.07
11b	0.635 ± 0.190	5.12 ± 4.11	>20
11c	1.607 ± 0.624	5.25 ± 0.47	15.55 ± 11.15
11e	0.884 ± 0.115	0.64 ± 0.13	2.7 ± 0.42
11 g	N.T.	2.81 ± 0.55	6.35 ± 4.64
11 k	N.T.	7.20 ± 1.20	>20
11 l	N.T.	>20	>20
13a	N.T.	4.23 ± 0.62	6.22 ± 3.91
14e	0.978 ± 0.021	0.37 ± 0.07	1.3 ± 0.18
Cisplatin	8.700 ± 0.970	8.84 ± 0.52[a]	18.2 ± 0.1[c]
Doxorubicin	1.078 ± 0.033	0.089 μM[b]	0.52[d]

N.T. not tested

[a] Kuo *et al.* (2010)

[b] Moon *et al.* (1999)

[c] Ganta *et al.* (2014)

[d] Molphy *et al.* (2014)

dihydrobenzo[e][1,3]thiazin-4-one pendant, the aryl substituents at the C2 of the 1,3-thiazin-4-one unit revealed that the electron-donating dimethylamino group is favorable, while an electron-withdrawing group, such as F, and the pyridine motif are not favorable.

Cytotoxic and growth inhibitory activities across the JFCR39 cancer cell line panel

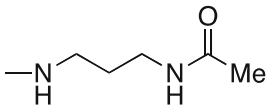
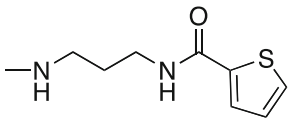
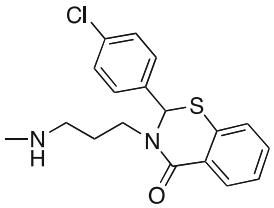
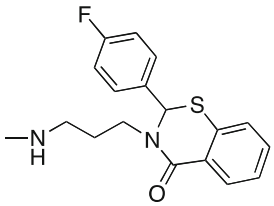
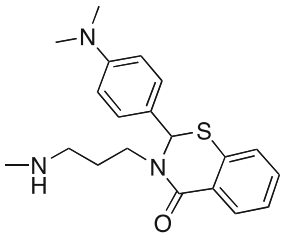
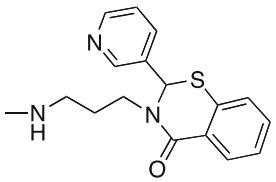
The anticancer screening of compounds **14e**, **13b**, and **13c** was performed in order to determine their cytotoxic and growth inhibitory activities across the JFCR39 cancer cell line panel. The compounds were evaluated at five concentration levels (100, 10, 1.0, 0.1, and 0.01 μM). The results (the means of GI50, TGI, and LC50 values) are summarized in Table 4.

Table 4 shows that all of the compounds are highly potent against the melanoma (LOX-IMVI) and lung (NCI-H522, A549, and DMS273) cancer cell lines in the JFCR39 panels. Compounds **14e** and **13b** are more potent than compound **13c** toward the cancer cell lines in the JFCR39 panel. Compounds **14e** and **13b** show a similar growth inhibitory activity ($GI_{50} > 0.01$ μM) against the 31 and 28 cell lines, respectively, of the JFCR39 panel. Compound **14e** shows a notable cytotoxicity against the breast (BSY-1, $LC_{50} = 0.82$ μM), CNS (SF-539, $LC_{50} = 0.60$ μM, SNB-75, $LC_{50} = 0.91$ μM), colon (HCC2998, $LC_{50} = 0.74$ μM), lung (NCI-H522, $LC_{50} = 0.71$ μM, A549, $LC_{50} = 0.85$, DMS273, $LC_{50} = 0.70$, DMS114, $LC_{50} = 0.72$), and ovarian (VCAR-4, $LC_{50} = 0.48$ μM) cancer cell lines. Compound **13b** shows a mentionable cytotoxicity for the CNS (SF-539, $LC_{50} = 0.63$ μM, SNB-75, $LC_{50} = 0.93$ μM), colon (HCC2998, $LC_{50} = 0.69$ μM), lung (NCI-H522, $IC_{50} = 0.57$ μM, DMS273, $LC_{50} = 0.61$ μM), and melanoma (LOX-IMVI, $LC_{50} = 0.75$ μM) cancer cell lines. Compound **13c** displayed a strong growth inhibitory activity ($GI_{50} < 1$ μM) for 28 panels in the JFCR39 panel of the various cell lines. DMS273 (lung cancer) and NCI-H522 (lung cancer) are the most sensitive cell lines of the 39 cell lines ($GI_{50} = 0.42$ μM for both and $LC_{50} = 4.7$ μM and 5.5 μM, respectively). Compound **C** also showed a strong antitumor effect on LOX-IMVI (melanoma, $GI_{50} = 0.3$ μM, $LC_{50} = 7.6$ μM).

COMPARE study

The COMPARE analysis assesses the correlation coefficient between the fingerprints of the test compounds and those of the various reference compounds (Kong and Yamori, 2012). This system provides an information intensive approach to identifying the molecular targets of new compounds. The JFCR39 and COMPARE analysis-guided assay is a successful means to find new anticancer drug candidates. The COMPARE analysis is carried out by calculation of the Pearson correlation coefficient (r value) between the fingerprints of compounds X and Y. The r value is then used to determine the degree of similarity, that is, the higher the r value, the greater the similarity of X to Y. Generally, an r value of $0.5 < r < 0.75$ between two agents suggests they might have a similar mechanism of action (Fig. 2).

Table 3 Antiproliferative activity of terminal-modified 11-(3-aminopropylamino)-5-methyl-indolo[2,3-*b*]quinolines against MDA-MB-453, WiDr, and SKOv3 cell lines

Compound	R^2	MDA-MB-453 LC_{50} (μM)	WiDr LC_{50} (μM)	SKOv3 LC_{50} (μM)
17a		2.06 ± 1.00	8.43 ± 4.16	13.60 ± 3.96
17b		3.27 ± 1.18	>7.8	>7.8
18a		2.12 ± 1.09	3.39 ± 0.88	4.1 ± 0.07
18b		3.99 ± 0.41	4.05 ± 0.91	10.9 ± 0.26
18c		1.41 ± 1.00	2.19 ± 0.74	>20
18d		4.25 ± 1.18	10.62 ± 1.20	13.1 ± 0.18
Cisplatin		7.6 ± 0.7[a]	8.84 ± 0.52[c]	18.2 ± 0.[e]
Doxorubicin		0.086 ± 0.006[b]	0.089[d]	0.52[f]

[a] Rakic *et al.*, (2012)

[b] Sandhu *et al.*, (2012)

[c] Kuo *et al.*, (2010)

[d] Moon *et al.*, (1999)

[e] Ganta *et al.*, (2014)

[f] Molphy *et al.*, (2014)

The COMPARE analysis revealed that compounds **14e** and **13b** have a very good match to actinomycin D ($r = 0.7$ for both). Similarly, compound **13b** has a slight similarity to paclitaxel ($r = 0.64$). Compound **13c** shows some resemblance to vindesine sulfate ($r = 0.58$) and aclarubicin HCl ($r = 0.57$).

Conclusion

Plant-derived 5-Me-indolo[2,3-b]quinolines with pendant substituents at C11 and/or C2 were assayed for antiproliferative screening against several cancer cell lines. In particular, the 11-(3-aminopropylamino)-substituted **11e** and **14e** and 11-(4-aminobutylamino)-substituted **11f** recorded the highest activity and selectivity against a breast cancer (MDA-MB-453) cell line ($IC_{50} = 0.3$–0.5 μM). A synergistic effect by the additional attachment was observed with an electron-donating group such as CH_3O at C2, i.e., **14e** ($IC_{50} = 0.2$ μM). On the other hand, further modification of the terminal free amino group of the lariat attachment at C11 into the corresponding acylamides and 2,3-dihydrobenzo[e][1,3]thiazin-4-ones **18e** was not effective for the antiproliferative activity. The computer-assisted database analysis, COMPARE, suggested that **14e** and **13b** have a mode of action similar to actinomycin D. It also suggested that **13c** has a mode of actions similar to vindesine sulfate or aclarubicin HCl. However, the new compounds may have other unique mode of actions since the correlation coefficients (r) were in relatively low levels, which are interesting possibility to examine in further studies.

Experimental

Chemistry

General

The commercially obtained reagents were directly used without further purification. The ^{1}H-NMR and ^{13}C-NMR spectra were measured by a Varian INOVA-600 spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent unless otherwise indicated. High-resolution mass spectra were obtained on a Bruker micrOTOF II-SKA spectrometer.

According to the reported procedure, the 11-chloroneocryptolepine cores with substituents at C2 were synthesized starting from 1H-methyl indole-3-carboxylate (**1**) and *N*-methylanilines **2** (Scheme 1) (Wang *et al.*, 2014).

*General procedure for the synthesis of **11–16***

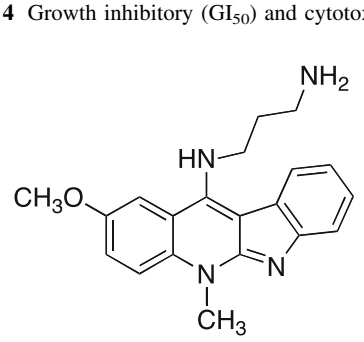
11-Chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolines **5–10** (0.3 mmol) and an excess of the appropriate aminoalkylamine (3.0 mmol) were heated together at 135–155 °C for 1–4 h. Monitoring by TLC was used to ensure the completion of reaction. The resulting brown crude oil was purified by flash chromatography using AcOEt-2N ammonia in MeOH (9:1) as an eluent to yield pure **11–16** as yellowish-orange solids.

N^1,N^1-diethyl-N^4-(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)pentane-1,4-diamine ***11a*** Yield: 99 %; yellowish solids; mp 70–73 °C; IR (KBr) ν_{max} 3242, 3048, 2965, 2801, 2620, 1591, 1566, 1549, 1487, 1441, 1422, 1404, 1373, 1277, 1244, 1192, 1142, 1061, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 0.92$ (6H, t, $J = 7.2$ Hz), 1.36 (3H, d, $J = 6.0$ Hz), 1.50–1.60 (2H, m), 1.64–1.78 (2H, m), 2.34 (2H, m), 2.41 (4H, q, $J = 7.2$ Hz), 4.29 (3H, s), 4.30 (1H, m), 4.91 (1H, d, $J = 10.8$ Hz), 7.21 (1H, td, $J = 7.8$, 0.6 Hz), 7.38 (1H, t, $J = 7.2$ Hz), 7.46 (1H, t, $J = 7.8$ Hz), 7.68–7.73 (2H, m), 7.77 (1H, d, $J = 7.8$ Hz), 7.91 (1H, d, $J = 7.8$ Hz), 8.16 (1H, d, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 11.42$ (2C), 22.35, 23.71, 32.74, 37.20, 46.73 (2C), 52.66, 54.34, 110.75, 114.77, 116.55, 117.50, 118.96, 120.60, 120.67, 124.09, 124.67, 126.32, 130.38, 138.15, 148.49, 153.15, 156.58; HRMS (ESI) calcd for $C_{25}H_{31}N_4$ $[M - H]^-$ exact mass: 387.2554, found 387.2583.

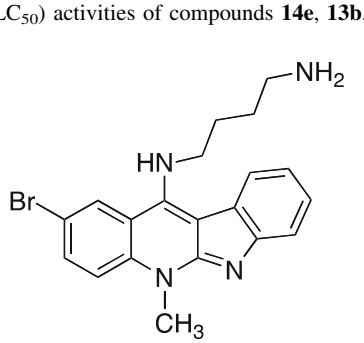
3-((5-Methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propan-1-ol ***11b*** Yield: 94 %, yellowish solids; mp 187–188 °C; IR (KBr) ν_{max} 3391, 3080, 3042, 2922, 2859, 2359, 1912, 1618, 1591, 1570, 1514, 1443, 1416, 1341, 1292, 1248, 1225, 1074, 1063, 864, 748, 714 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 1.95$ (2H, quint, $J = 6.0$ Hz), 3.96–4.02 (4H, m), 4.20 (3H, s), 6.20 (1H, brs), 7.14 (1H, t, $J = 7.2$ Hz), 7.31 (1H, td, $J = 7.8$, 1.2 Hz), 7.37 (1H, t, $J = 7.8$ Hz), 7.60 (1H, d, $J = 8.4$ Hz), 7.67 (1H, t, $J = 7.2$ Hz), 7.73 (1H, d, $J = 8.4$ Hz), 7.91 (1H, d, $J = 7.8$ Hz), 8.11 (1H, d, $J = 7.8$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 32.74$, 32.87, 48.39, 62.20, 107.05, 114.63, 115.87, 117.08, 118.73, 120.50, 121.10, 124.00, 124.29, 125.58, 130.27, 138.06, 148.51, 152.30, 156.56; HRMS (ESI) calcd for $C_{19}H_{18}N_3O$ $[M - H]^-$ exact mass: 304.1455, found 304.1485.

4-(5-Methyl-5H-indolo[2,3-b]quinolin-11-yl)morpholine ***11c*** Yield: 77 %, orange-colored solids; mp 205–207 °C; IR (KBr) ν_{max} 3046, 2951, 2849, 1734, 1614, 1599, 1564, 1520, 1489, 1437, 1416, 1387, 1366, 1292, 1269, 1242, 1194, 1171, 1105, 1063, 1009, 856, 746, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 3.58$ (4H, t, $J = 4.80$ Hz), 4.09 (4H, t, $J = 4.80$ Hz), 4.36 (3H, s), 7.27 (1H, m), 7.46 (1H, m), 7.55 (1H, t, $J = 7.20$ Hz), 7.74–7.78 (3H, m), 8.36 (1H, d, $J = 7.80$ Hz), 8.57 (1H, d, $J = 8.40$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 33.15$, 49.34 (2C), 67.68 (2C), 114.41, 117.60, 119.49, 120.82, 121.38, 122.78,

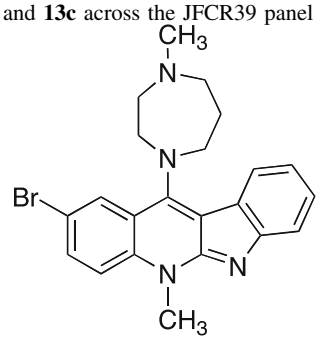
Table 4 Growth inhibitory (GI_{50}) and cytotoxic (LC_{50}) activities of compounds **14e**, **13b**, and **13c** across the JFCR39 panel



14e



13b



13c

Tissue of origin	Cell line	**14e**			**13b**			**13c**		
		GI_{50} (μM)	TGI (μM)	IC_{50} (μM)	GI_{50} (μM)	TGI	IC_{50} (μM)	GI_{50} (μM)	TGI (μM)	IC_{50} (μM)
Breast	HBC-4	0.06	0.80	9.5	0.08	0.61	6.1	1.3	4.3	19
	BSY-1	0.11	0.30	0.82	0.12	0.35	3.5	2.0	5.2	20
	HBC-5	0.06	0.33	3.4	0.09	0.41	4.1	0.86	10	53
	MCF-7	0.04	0.24	17	0.03	0.31	3.1	0.87	7.9	44
	MDA-MB-23	0.08	0.33	6.9	0.07	0.34	3.4	0.50	4.1	44
CNS	U251	0.04	0.47	17	0.05	0.55	7.9	0.7	13	58
	SF-268	0.05	0.59	26	0.06	0.75	4.7	0.94	14	76
	SF-295	0.10	0.81	14	0.11	0.55	2.9	1.3	14	49
	SF-539	0.05	0.20	0.60	0.07	0.23	0.63	0.7	3.9	22
	SNB-75	0.13	0.34	0.91	0.15	0.38	0.93	3.3	15	42
	SNB-78	0.12	0.78	25	0.20	0.98	5.2	2.9	20	62
Colon	HCC2998	0.05	0.22	0.74	0.07	0.23	0.69	1.0	2.9	8.0
	KM-12	0.06	0.64	23	0.09	0.59	4.6	0.72	7.0	64
	HT-29	0.04	0.54	24	0.06	0.64	28	0.56	5.6	46
	HCT-15	0.72	6.9	50	1.0	3.7	100	0.58	5.8	49
	HCT-116	0.04	0.57	22	0.04	0.80	5.1	0.43	2.3	15
Lung	NCI-H23	0.06	0.28	1.8	0.07	0.36	2.5	1.2	4.8	98
	NCI-H226	0.08	0.49	25	0.09	0.63	6.2	0.74	4.8	44
	NCI-H522	0.04	0.20	0.71	0.04	0.18	0.57	0.42	1.8	5.5
	NCI-H460	0.05	0.27	3.4	0.05	0.23	1.7	0.45	2.6	20
	A549	0.06	0.23	0.85	0.05	0.26	1.9	0.68	2.5	8.3
	DMS273	0.04	0.18	0.70	0.05	0.18	0.61	0.42	1.6	4.7
	DMS114	0.08	0.26	0.72	0.10	0.33	1.10	1.1	3.7	18
Melanoma	LOX-IMVI	0.03	0.11	1.1	0.03	0.11	0.75	0.31	1.5	7.6
Ovarian	OVCAR-3	0.06	0.28	8.0	0.05	0.30	42	0.64	4.6	82
	OVCAR-4	0.04	0.15	0.48	0.04	0.16	59	0.52	2.7	19
	OVCAR-5	0.05	0.35	9.5	0.05	0.26	42	0.56	3.1	32
	OVCAR-8	0.05	0.33	23	0.05	0.43	100	0.48	3.4	100
	SK-OV-3	0.13	1.0	20	0.13	1.2	54	1.2	7.4	51
Renal	RXF-631L	0.27	1.4	7.4	0.19	0.67	3.0	1.1	3.0	18
	ACHN	0.27	2.1	20	0.25	2.2	10	0.7	7.1	51
Stomach	St-4	0.06	0.43	3.8	0.12	0.60	3.7	0.72	4.9	44
	MKN1	0.09	0.38	12	0.11	0.47	3.1	1.1	3.6	14
	MKN7	0.04	0.34	23	0.06	0.48	36	0.9	8.0	69
	MKN28	0.05	0.9	37	0.05	1.1	58	0.55	12	100
	MKN45	0.05	1.0	28	0.05	1.1	8.0	0.44	6.2	100
	MKN74	0.05	1.0	52	0.06	0.94	8.0	0.56	6.8	100
Prostate	DU145	0.1	0.34	17	0.09	0.43	3.3	1.2	4.0	22
	PC-3	0.16	1.3	70	0.16	1.3	22	2.0	12	56

CNS central nervous system, *GI_{50}* 50 % growth inhibition concentration (μM), *TGI* total growth inhibition concentration (μM), *LC_{50}* lethal concentration (μM)

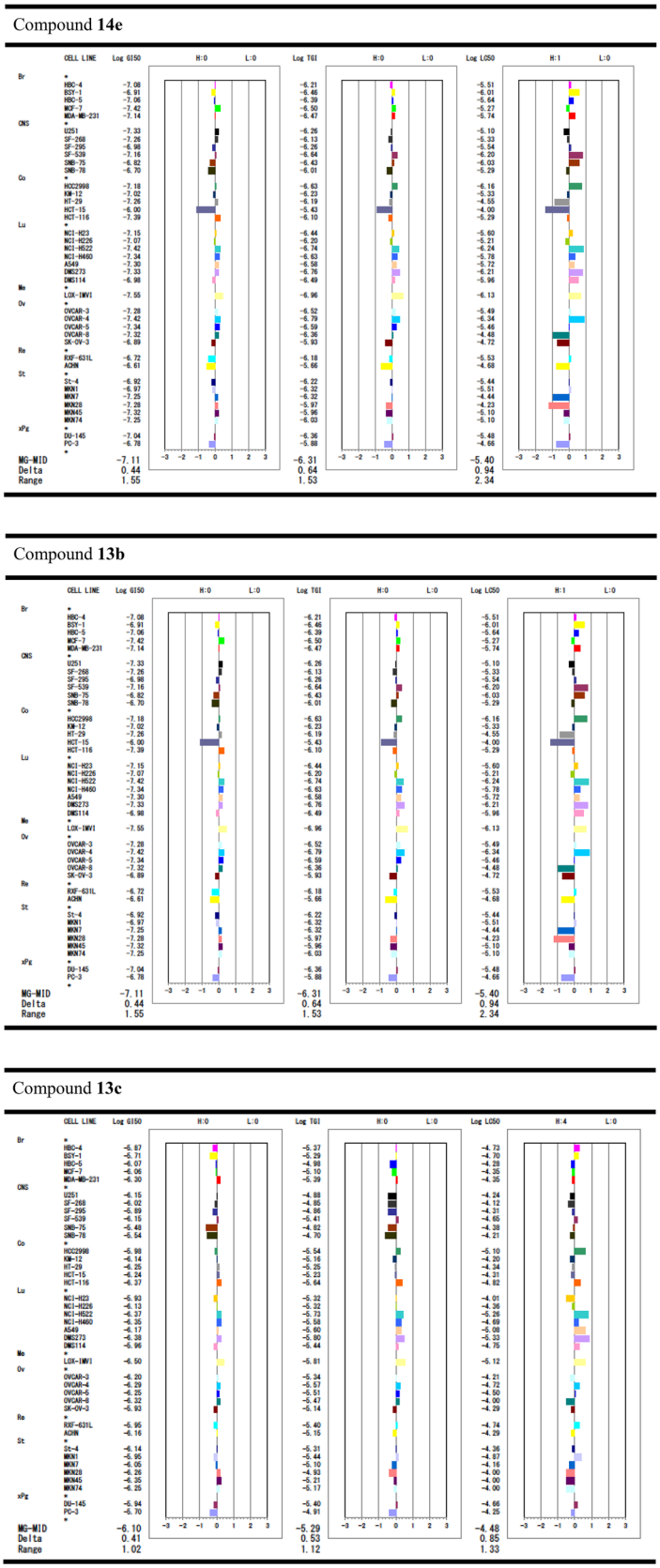


Fig. 2 Growth inhibitory and cytotoxic activities of compounds **14e**, **13b**, and **13c** across a panel of the JFCR39 cell lines. The mean graph was produced by computer processing of the 50 % growth inhibition (GI_{50}) and the 50 % lethal concentration (LC_{50}) values. The logarithm of the GI_{50} and the LC_{50} values for each cell line is indicated. The *X* axis shows the difference on a logarithmic scale between the mean of Log GI_{50}/Log LC_{50} values for all 39-cell lines (MG-MID, expressed as 0 in the fingerprint) and the Log GI_{50}/Log LC_{50} for each cell line in the JFCR39 panel. *Columns to the right* of 0 indicate the sensitivity of the cell lines to a given compound, and *columns to the left* indicate their resistance. The MG-MID mean of the Log GI_{50}/Log LC_{50} values for all 39 cell lines; delta difference between the MG-MID and the Log GI_{50}/Log LC_{50} value for the most sensitive cell line; range difference between the Log GI_{50}/Log LC_{50} values for the most resistant cell line and the most sensitive cell line

123.44, 124.91, 126.20, 128.50, 130.46, 138.03, 149.93, 154.55, 157.81; HRMS (ESI) calcd for $C_{20}H_{20}N_3O$ $[M + H]^+$ exact mass: 318.1601, found 318.1621.

N^1-(5-Methyl-5H-indolo[2,3-b]quinolin-11-yl)propane-1,3-diamine ***11e*** Yield: 96 %, yellowish solids; mp 69–71 °C; IR (KBr) ν_{max} 3435, 2928, 2868, 2359, 2342, 1622, 1559, 1489, 1441, 1418, 1287, 1248, 1057, 750, 669 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): $\delta = 1.80$ (2H, quint, $J = 6.0$ Hz), 3.01 (2 H, t, $J = 6.0$ H), 4.01 (2H, t, $J = 6.0$ Hz), 4.22 (3H, s), 7.17 (1H, t, $J = 7.2$ Hz), 7.18 (1H, brs), 7.31 (1H, t, $J = 7.2$ Hz), 7.41 (1H, t, $J = 7.2$ Hz), 7.61 (1H, d, $J = 9.0$ Hz), 7.67 (1H, td, $J = 7.2$, 1.2 Hz), 7.76 (1H, d, $J = 7.8$ Hz), 7.95 (1H, d, $J = 7.8$ Hz), 8.13 (1H, d, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 32.64$, 32.66, 41.26, 49.25, 105.61, 114.40, 115.86, 116.94, 118.49, 120.35, 121.37, 124.06, 124.12, 125.16, 130.08, 137.79, 148.52, 151.94, 156.55; HRMS (ESI) calcd for $C_{19}H_{21}N_4$ $[M + H]^+$ exact mass: 305.1761, found 305.1765.

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

N^1,N^1-Bis(2-aminoethyl)-N^2-(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)ethane-1,2-diamine ***11k*** Yield: 78 %, yellowish-orange solids; mp 69 °C; IR (KBr) ν_{max} 3354, 3048, 2934, 2855, 2357, 2189, 1616, 1591, 1564, 1495, 1489, 1418, 1279, 1246, 1142, 1103, 1024, 882, 853, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): $\delta = 2.41$ (4H, brs), 2.61 (4H, t, $J = 6.6$ Hz), 2.72 (2H, t, $J = 6.0$ Hz), 2.80 (4H, t, $J = 6.0$ Hz), 3.94 (2H, t, $J = 5.4$ Hz), 4.23 (3H, s), 7.16 (1H, t, $J = 7.2$ Hz), 7.33 (1H, t, $J = 7.2$ Hz), 7.41 (1H, t, $J = 7.2$ Hz), 7.63–7.69 (2H, m), 7.74 (1H, d, $J = 7.8$ Hz), 7.95 (1H, d, $J = 7.2$ Hz), 8.31 (1H, d, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 32.73$, 39.71 (2C), 45.66, 55.29, 56.56 (2C), 106.73, 114.68, 115.94, 117.18, 118.60, 120.45, 121.31, 123.99, 124.15, 125.57, 130.35, 137.97, 148.38, 152.37, 156.67; HRMS (ESI) calcd for $C_{22}H_{27}N_6$ $[M - H]^-$ exact mass: 375.2303, found 375.2324.

N-(2-(1H-Indol-3-yl)ethyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine ***11l*** Yield: 92 %, yellow solids; mp 198–200 °C; IR (KBr) ν_{max} 3410, 3053, 2918, 2862, 1717, 1622, 1593, 1568, 1499, 1441, 1418, 1343, 1290, 1248, 1109, 1071, 887, 745 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): $\delta = 3.18$ (2H, t, $J = 6.6$ Hz), 4.20–4.23 (2H, m), 4.22 (3H, s), 5.44 (1H, brs), 7.04 (2H, m), 7.11 (1H, t, $J = 7.8$ Hz), 7.23 (1H, t, $J = 8.4$ Hz), 7.29 (1H, t, $J = 7.8$ Hz), 7.36 (1H, t, $J = 7.8$ Hz), 7.42 (1H, dd, $J = 8.4$, 0.6 Hz), 7.48 (1H, d, $J = 8.4$ Hz), 7.58–7.62 (2H, m), 7.68 (1H, t, $J = 7.8$ Hz), 7.75 (1H, d, $J = 8.4$ Hz), 8.02 (1H, d, $J = 8.4$ Hz), 8.54 (1H, brs); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 26.62$, 32.52, 48.61, 103.99, 110.81, 111.35, 115.18, 115.70, 115.96, 118.14, 118.25, 118.38. 120.87, 120.94, 122.02, 122.97 (2C), 123.62, 124.14, 124.73, 126.99, 130.84, 136.12, 137.26, 148.60, 155.31; HRMS (ESI) calcd for $C_{26}H_{23}N_4$ $[M + H]^+$ exact mass: 391.1917, found 391.1943.

5-Methyl-N-phenyl-5H-indolo[2,3-b]quinolin-11-amine ***11p*** Yield: 95 %, yellowish solids; mp 187–188 °C; IR (KBr) ν_{max} 3296, 3053, 2943, 1620, 1589, 1568, 1524, 1497, 1485, 1439, 1416, 1402, 1317, 1261, 1242, 1171, 1142, 1099, 897, 858, 746, 721, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): $\delta = 4.27$ (3H, s), 6.89 (3H, m), 7.00 (2H, t, $J = 7.20$ Hz), 7.19–7.25 (3H, m), 7.33 (1H, t, $J = 7.20$ Hz), 7.43 (1H, t, $J = 7.8$ Hz), 7.68 (1H, m), 7.73 (2H, m), 8.05 (1H, d, $J = 7.80$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 32.99$, 114.60, 114.88, 117.07, 117.16, 118.21 (2C), 119.32, 121.14, 122.25, 123.21, 123.45, 124.72, 127.44, 129.42 (2C), 130.54, 137.75, 140.05, 142.50, 153.30, 156.75; HRMS (ESI) calcd for $C_{22}H_{18}N_3$ $[M + H]^+$ exact mass: 324.1495, found 324.1509.

N^4-(2-Bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-yl)-N^1,N^1-diethylpentane-1,4-diamine ***13a*** Yield: 82 %, reddish solids; mp 39 °C; IR (KBr) ν_{max} 3296, 3050, 2967, 2934, 2799, 1622, 1559, 1489, 1443, 1418, 1379, 1273, 1242, 1188, 1107, 1059, 909, 802, 760, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): $\delta = 0.93$ (6H, t, $J = 7.2$ Hz), 1.35 (3H, d, $J = 6.6$ Hz), 1.58 (2H, m), 1.65–1.78 (2H, m), 2.37 (2H, m), 2.44 (4H, q, $J = 7.2$ Hz), 4.21 (1H, m), 4.25 (3H, s), 4.87 (1H, d, $J = 10.8$ Hz), 7.22 (1H, td, $J = 7.8$, 1.2 Hz), 7.47 (1H, t, $J = 7.8$ Hz), 7.56 (1H, d, $J = 9.0$ Hz), 7.73–7.80 (2H, m), 7.90 (1H, d, $J = 7.2$ Hz), 8.25 (1H, d, $J = 2.4$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 11.38$ (2C), 22.37, 23.65, 32.82, 37.14, 46.74 (2C), 52.57, 54.47, 111.65, 113.45, 116.40, 117.71, 118.13, 119.29, 120.83, 123.95, 126.79, 127.17, 132.98, 136.90, 147.18, 153.35, 156.30; HRMS (ESI) calcd for $C_{25}H_{32}BrN_4$ $[M + H]^+$ exact mass: 467.1805, found 467.1807.

N^1-(2-Methoxy-5-methyl-5H-indolo[2,3-b]quinolin-11-yl)propane-1,3-diamine ***14e*** Yield: 99 %, yellowish solids; mp

105–106 °C; IR (KBr) ν_{max} 3381, 3268, 2934, 1614, 1593, 1568, 1489, 1445, 1424, 1348, 1288, 1246, 1184, 1140, 1038, 937, 810, 760 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz): $\delta = 1.72$ (2H, quint, $J = 6.6$ Hz), 2.64 (2H, t, $J = 6.6$ Hz), 3.91 (3H, s), 3.94 (2H, t, $J = 6.6$ Hz), 4.14 (3H, s), 7.04 (1H, t, $J = 7.2$ Hz), 7.26 (1H, t, $J = 7.2$ Hz), 7.43–7.47 (2H, m), 7.79 (1H, d, $J = 9.6$ Hz), 7.92 (2H, m); ^{13}C NMR (DMSO-d_6, 150.8 MHz): $\delta = 32.23$, 33.44, 39.82, 47.18, 55.80, 104.47, 105.88, 116.18, 116.28, 116.43, 117.47, 119.52, 122.15, 123.98, 124.43, 132.18, 147.73, 152.50, 153.55, 156.26; HRMS (ESI) calcd for $C_{20}H_{23}N_4O$ $[M + H]^+$ exact mass: 335.1866, found 335.1864.

General procedure for the preparation of compound ***17***

N^1-(5-Methyl-5*H*-indolo[2,3-*b*]quinolin-11-yl)propane-1,3-diamine (**11e**, 50 mg) was completely dissolved in dry DMF (2 ml), then a mixture solution of R^3COCl (1.2 equiv.) and dry DMF (1 ml) was added drop by drop under stirring, finally 2.0 equiv of triethylamine was added, and the reaction was carried out at room temperature for 2–4 h. TLC monitoring was used to ensure the completion of reaction. The crude product was purified by flash chromatography using AcOEt-2 N ammonia in MeOH (9:1 v/v) as an eluent to yield pure products **17** as yellowish-orange solids.

N-(3-((5-Methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl) thiophene-2-carboxamide ***17b*** Yield: 95 %, yellowish solids; mp 204–205 °C; IR (KBr) ν_{max} 3424, 3333, 3059, 2930, 1732, 1622, 1593, 1566, 1545, 1499, 1439, 1418, 1358, 1294, 1248, 1204, 1144, 1071, 862, 745, 719 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz): $\delta = 1.92$ (2H, quint, $J = 7.2$ Hz,), 3.26 (2H, q, $J = 6.6$ Hz,), 3.88 (2H, q, $J = 7.2$ Hz), 4.16 (3H, s), 7.02–7.07 (2H, m), 7.10 (1H, dd, $J = 4.8$, 2.4 Hz), 7.27 (1H, t, $J = 7.2$ Hz), 7.41 (1H, t, $J = 7.2$ Hz), 7.49 (1H, d, $J = 7.8$ Hz), 7.64 (1H, dd, $J = 3.6$, 1.2 Hz), 7.72 (1H, dd, $J = 5.4$, 1.2 Hz), 7.79 (1H, td, $J = 7.2$ 1.2 Hz), 7.85 (1H, d, $J = 7.8$ Hz), 7.94 (1H, d, $J = 7.2$ Hz), 8.52 (2H, d, $J = 6.6$ Hz); ^{13}C NMR (DMSO-d_6, 150.8 MHz): $\delta = 30.92$, 32.22, 36.69, 45.39, 105.08, 115.06, 115.65, 116.55, 118.00, 120.58, 122.08, 123.97, 124.02, 124.68, 127.81, 127.93, 130.64 (2C), 137.39, 139.84, 148.14, 152.38, 156.40, 161.33; HRMS (ESI) calcd for $C_{24}H_{23}N_4OS$ $[M + H]^+$ exact mass: 415.1587, found 415.1597.

General procedure for the synthesis of compound ***18a–d***

N^1-(5-Methyl-5*H*-indolo[2,3-*b*]quinolin-11-yl)propane-1,3-diamine (**11e**, 100 mg) and R^4CHO (2.0 equiv.) was stirred in dry toluene at 70–80 °C for 5 min, followed by addition of thiosalicylic acid (3.0 equiv.) and DCC (1.2 equiv.). Then the reaction mixture was heated to reflux for 10–15 h. After cooling to room temperature, the mixture was concentrated to dryness under reduced pressure, and the residue was taken up in chloroform and washed with aq. 5 % $NaHCO_3$ and then finally with brine. The organic layer was dried over $MgSO_4$ and evaporated to get a crude product, which was followed purification by flash chromatography using ethyl acetate (AcOEt)–2 N ammonia in MeOH (9:1 v/v) as the eluent to yield pure products as yellowish-orange solids (**18a–d**).

2-(4-Chlorophenyl)-3-(3-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl)-2H-benzo[e][1,3]thiazin-4(3H)-one ***18a*** Yield: 49.7 %, yellowish solids; mp 124–126 °C; IR (KBr) ν_{max} 3349, 3055, 2932, 1622, 1591, 1564, 1489, 1456, 1441, 1422, 1310, 1277, 1246, 1204, 1146, 1092, 1013, 841, 748 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz): $\delta = 2.06$ (1H, m), 2.13 (1H, m), 3.23 (1H, dt, $J = 15.0$, 5.4 Hz), 3.82 (1H, m), 4.22 (1H, m), 4.34 (3H, s), 4.51 (1H, m), 5.73 (1H, s), 7.11 (1H, d, $J = 7.8$ Hz), 7.15–7.22 (5H, m), 7.29 (1H, t, $J = 7.8$ Hz), 7.32–7.36 (2H, m), 7.58 (1H, t, $J = 7.2$ Hz), 7.68 (1H, d, $J = 9.0$ Hz), 7.70 (1H, brs), 7.81 (1 H, t, $J = 7.8$ Hz), 7.86 (1H, d, $J = 8.4$ Hz), 7.91 (1H, d, $J = 7.8$ Hz), 8.16 (1H, dd, $J = 7.8$, 1.2 Hz), 8.63 (1H, d, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 29.56$, 35.64, 44.08, 45.23, 61.03, 102.48, 114.67, 115.41, 116.73, 120.94, 121.41, 121.77, 123.46, 124.67, 126.25, 126.71, 127.45 (2C), 127.79, 128.49, 128.82 (2C), 129.01, 129.95, 131.99, 132.53, 132.74, 134.51, 136.77, 136.97, 150.09, 151.09, 165.22; HRMS (ESI) calcd for $C_{33}H_{26}ClN_4OS$ $[M - H]^-$ exact mass: 561.1516, found 561.1517.

2-(4-Fluorophenyl)-3-(3-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl)-2H-benzo[e][1,3]thiazin-4(3H)-one ***18b*** Yield: 69 %, yellowish solids; mp 104–108 °C; IR (KBr) ν_{max} 3349, 3055, 2934, 1622, 1593, 1564, 1506, 1456, 1441, 1422, 1279, 1244, 1229, 1159, 1098, 845, 746 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): $\delta = 2.13$ (1H, m), 2.19 (1H, m), 3.25 (1H, dt, $J = 14.4$, 4.8 Hz), 3.85 (1H, m), 4.22 (1H, m), 4.35 (3H, s), 4.50 (1H, m), 5.82 (1H, m), 6.91 (2H, t, $J = 8.4$ Hz), 7.10 (1H, d, $J = 7.8$ Hz), 7.16–7.33 (5H, m), 7.35 (1H, t, $J = 7.2$ Hz), 7.61 (1H, t, $J = 7.8$ Hz), 7.67 (1H, d, $J = 8.4$ Hz), 7.82 (2H, t, $J = 7.8$ Hz), 7.93 (1H, d, $J = 7.8$ Hz), 8.10 (1H, m), 8.14 (1H, d, $J = 8.4$ Hz), 8.68 (1H, d, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 29.52$, 36.51, 44.33, 45.07, 60.98, 100.67, 113.75, 115.63, 115.64 (d, $J = 21.9$ Hz, 2C), 116.77, 120.01, 121.72, 122.21, 124.23, 125.02, 126.31, 126.61, 127.78, 127.92 (d, $J = 8.3$ Hz, 2C), 128.43, 129.88, 132.50, 132.72, 132.74, 133.92, 136.68, 138.05, 147.76, 151.99, 161.75, 165.25; ^{19}F NMR ($CDCl_3$, 564 MHz): $\delta = -112.96$; HRMS (ESI) calcd for $C_{33}H_{26}FN_4OS$ $[M - H]^-$ exact mass: 545.1817, found 545.1842.

2-(4-(Dimethylamino)phenyl)-3-(3-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl)-2H-benzo[e][1,3]thiazin-4(3H)-one ***18c*** Yield: 81 %, yellowish solids; mp 119–121 °C; IR (KBr) ν_{max} 3364, 3053, 2934, 1614, 1593, 1564, 1520, 1495, 1456, 1441, 1420, 1360, 1281, 1246, 1188, 1165, 1065, 947, 748 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): $\delta = 1.82–1.94$ (2H, m), 2.88 (6H, s), 3.32 (1H, m), 3.74 (1H, m), 4.11 (1H, m), 4.28 (3H, s), 4.36 (1H, m), 5.61 (1H, s), 6.54 (2H, d, $J = 8.4$ Hz), 7.01 (1H, brs), 7.09 (2H, d, $J = 9.0$ Hz), 7.14 (1H, d, $J = 7.8$ Hz), 7.20 (1H, t, $J = 7.2$ Hz), 7.28 (1H, t, $J = 7.2$ Hz), 7.34 (1H, t, $J = 7.2$ Hz), 7.41 (1H, t, $J = 7.2$ Hz), 7.46 (1H, t, $J = 7.2$ Hz), 7.65 (1H, m), 7.73 (1H, m), 7.81 (1H, d, $J = 7.8$ Hz), 7.95 (1H, d, $J = 7.8$ Hz), 8.25 (1H, dd, $J = 7.8, 1.2$ Hz), 8.53 (1H, d, $J = 8.4$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 29.70$, 33.42, 40.14 (2C), 43.71, 44.59, 62.10, 106.04, 111.85 (2C), 114.66, 116.48, 116.58, 119.29, 121.49, 121.95, 123.27, 124.04, 124.55, 125.68, 126.14, 127.25 (2C), 127.59, 128.69, 130.00, 130.64, 132.24, 133.82, 137.52, 149.05, 149.89, 150.40, 155.38, 165.34; HRMS (ESI) calcd for $C_{35}H_{32}N_5OS$ $[M - H]^-$ exact mass: 570.2333, found 570.2346.

3-(3-((5-Methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl)-2-(pyridin-3-yl)-2H-benzo[e][1,3]thiazin-4(3H)-one ***18d*** Yield: 90 %, yellowish solids; mp 96–97 °C; IR (KBr) ν_{max} 3418, 3053, 2934, 1622, 1591, 1564, 1495, 1456, 1441, 1418, 1283, 1246, 1202, 1153, 1024, 795, 748, 710 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): $\delta = 1.92–2.02$ (2H, m), 3.16 (1H, dt, $J = 14.4, 5.4$ Hz), 3.75 (1H, m), 4.17 (1H, m), 4.25 (3H, s), 4.52 (1H, m), 5.63 (1H, s), 6.80 (1H, brs), 7.09–7.19 (3H, m), 7.29 (1H, t, $J = 7.8$ Hz), 7.33 (1H, t, $J = 7.8$ Hz), 7.38 (1H, t, $J = 7.8$ Hz), 7.46 (1H, t, $J = 7.2$ Hz), 7.51 (1H, d, $J = 7.8$ Hz), 7.65 (1H, d, $J = 8.4$ Hz), 7.73 (1H, t, $J = 7.2$ Hz), 7.77 (1H, d, $J = 7.8$ Hz), 7.92 (1H, d, $J = 7.8$ Hz), 8.20 (1H, d, $J = 8.4$ Hz), 8.44 (1H, d, $J = 4.2$ Hz), 8.48 (1H, d, $J = 7.8$ Hz), 8.51 (1H, d, $J = 2.4$ Hz); ^{13}C NMR ($CDCl_3$, 150.8 MHz): $\delta = 29.66$, 33.49, 43.81, 45.51, 59.65, 106.35, 114.78, 116.43, 116.49, 119.49, 121.65, 121.86, 123.07, 123.22, 124.06, 125.91, 126.87, 127.83, 128.66, 130.13, 130.80, 132.06, 132.69, 133.47, 134.25, 137.52, 147.49 (2C), 148.96, 149.67, 155.09, 164.85; HRMS (ESI) calcd for $C_{32}H_{26}N_5OS$ $[M - H]^-$ exact mass: 528.1864, found 528.1870.

Pharmacology

In vitro antiproliferative assay

Cell culture

All the cell lines were purchased from American Tissue Culture Center (ATCC, Manassas, VA, USA). Human breast cancer-derived MDA-MB-453 cells and human colon cancer-derived WiDr cells were cultured in DMEM (Wako Pure Chemicals, Osaka, Japan) supplemented with 10 % heat-inactivated fetal bovine serum (FBS) and 100 μg/ml kanamycin at 37 °C under atmosphere of 5 % CO_2/95 % air. Human ovary cancer-derived SKOv3 cells and human leukemia-derived K562 cells were cultured in medium for RPMI-1640 (Wako Pure Chemicals, Osaka, Japan) supplemented with 10 % FBS and 100 μg/ml kanamycin at 37 °C under atmosphere of 5 % CO_2/95 % air.

Cytotoxic assay

Neocryptolepine derivatives were evaluated for anticancer activity in vitro by MTT assay. Cells in logarithmic growth phase were suspended at 100,000 cells/ml in the culture medium. The cell suspension solutions in 100 μl were dispensed into each well of 96-well plate. The plate was incubated at 37 °C and 5 % CO_2 overnight. Then sequentially diluted solution of compounds was added to each well. Then after 72 h, 20 μl of 5 mg/ml MTT dissolved in PBS was added to each well. The plate was further incubated at 37 °C for 4 h, and formazan crystal was dissolved by 10 % SDS supplemented with 20 μM of hydrochloride. Absorbance at 570 nm was observed followed by incubation overnight.

The results of the cytotoxic activity in vitro were expressed as the IC_{50} 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.

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