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SYNTHESIS AND EVALUATION OF TOPOISOMERASE I INHIBITORS POSSESSING THE 5,13-DIHYDRO-6*H*-BENZO[6,7]INDOLO[3,2-*c*]-QUINOLIN-6-ONE SCAFFOLD

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Abstract – Novel topoisomerase I inhibitors possessing the 5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (BIQ) scaffold were designed and synthesized. This scaffold was constructed using sequential and regioselective functionalization of the pyrrole core through palladium-catalyzed cross-coupling, conventional electrophilic substitution, directed lithiation, and subsequent diphenylphosphoryl azide (DPPA)-mediated lactam ring construction. The obtained BIQs were evaluated for their topoisomerase I inhibitory activities and their antiproliferative activities in the panel of 39 human cancer cell lines established by the Japanese Foundation for Cancer Research (JFCR39).

INTRODUCTION

Lamellarin D (**1**) is a marine alkaloid that was isolated from the marine prosobranch mollusc *Lamellaria* sp. by Faulkner and co-workers in 1985 (Figure 1).[1] Since then, it has attracted considerable attention due to possessing a unique 14-phenyl-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinoline scaffold, in addition to its potent antitumor activity.[2] For example, in 1996, Quesada *et al.* reported that the triacetate of lamellarin D exhibits potent cytotoxicity against a range of cancer cell lines, including multidrug-resistant (MDR) phenotypes.[3] In addition, in 1997, we achieved the first total synthesis of **1** using an *N*-ylide-mediated cyclization as the key reaction step.[4] This synthetic method allowed the

This paper is dedicated to Professor Dr. Tohru Fukuyama on the occasion of his 70th birthday.

preparation of ten non-natural analogues of **1**, the cytotoxicities of which were evaluated against the HeLa cell line.[5] Examination of the structure-activity relationship (SAR) revealed that the hydroxy groups at the C8 and C20 (lamellarin numbering[1]) positions of **1** are essential for such potent cytotoxicity, whereas the hydroxy group at the C14 position is less important. Later, Ploypradith and co-workers performed a more precise SAR study using twenty-two naturally occurring and three unnatural lamellarins, where they employed eleven cancer cell lines to confirm our results.[6]

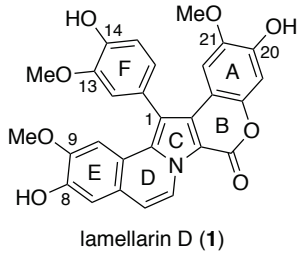


Figure 1. The structure of lamellarin D (**1**)

In 2003, Bailly and coworkers suggested that DNA topoisomerase I is a major molecular target of **1** in cancer cells owing to the strong correlation observed between the cytotoxicity and topoisomerase I inhibition.[7] They also proposed a theoretical model of a **1**–DNA–topoisomerase I ternary complex,[7,8] where **1** intercalates at the site of DNA cleavage and is stabilized with both the +1(C·G) and the –1(A·T) base pairs to form stacking interactions. Hydrogen bonds between **1** and the specific amino acid residues of topoisomerase I further stabilize the ternary complex. More specifically, from their predicted distances, the hydroxy groups at the C8 and C20 positions and the carbonyl oxygen atom appear to hydrogen bond to the Asn722, Glu356, and Arg364 residues, respectively. In contrast, the aryl group at the C1 position (i.e., the F-ring) is directed toward the major groove cavity, and does not exhibit any direct interaction with the protein, thereby suggesting that the F-ring of **1** may not be essential for topoisomerase I inhibition and could be replaced by other groups.

To confirm this speculation, we synthesized a series of F-ring-defected lamellarin D analogues **2** (R = H, Me, CH_2NMe_2, CHO, F, Cl, Br) (Figure 2).[9] Indeed, the antiproliferative activities of **2** were found to be as potent as that of **1**[10] in the panel of 39 human cancer cell lines established by the Japanese Foundation for Cancer Research (JFCR39).[11] Based on these results, we also designed a benzo[*g*][1]benzo-pyrano[4,3-*b*]indol-6(13*H*)-one (BBPI) scaffold through scaffold-hopping of **2**.[12] This scaffold can be regarded as a regioisomer of the pentacyclic core (ABCDE-ring) of **2** with respect to the position of the ring-nitrogen atom (Figure 2). Since the positions of the hydroxy groups in the BBPI scaffold (i.e., at the C10 and C3 positions) are similar to those of the hydroxy groups at the C8 and C20 positions of **2**, we expected that the BBPI derivatives could maintain the potent cytotoxicity of **1** and **2**. Indeed,

N13-substituted BBPI derivatives **3** [R = Me, Et, allyl, propargyl, $(CH_2)_2NMe_2$] exhibited potent antiproliferative activities at low nanomolar concentrations in addition to a potent topoisomerase I inhibitory activity in a DNA relaxation assay.[13,14] In contrast, Ruchirawat and co-workers designed and synthesized azalamellarins, which are artificial analogues of lamellarins, through simple replacement of the lactone moiety with a lactam moiety (Figure 2).[15,16] The screening of these azalamellarins against four cancer cell lines revealed that azalamellarin D (**4**) exhibits a potent cytotoxicity comparable to that of **1**.[15,16] Thus, from a series of studies into BBPIs and azalamellarins, we speculated that a 5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (BIQ) scaffold (a lactam congener of BBPIs) would also exhibit an antiproliferative activity based on topoisomerase I inhibition (Figure 2). Thus, we herein describe the synthesis and evaluation of BIQ derivatives **5**.

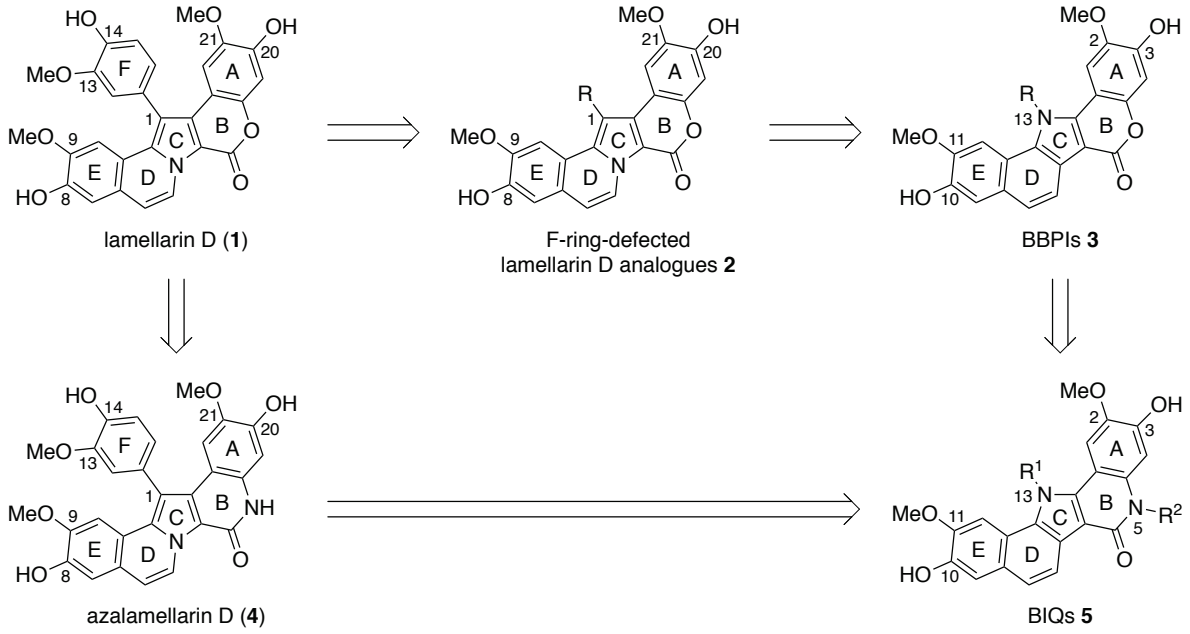
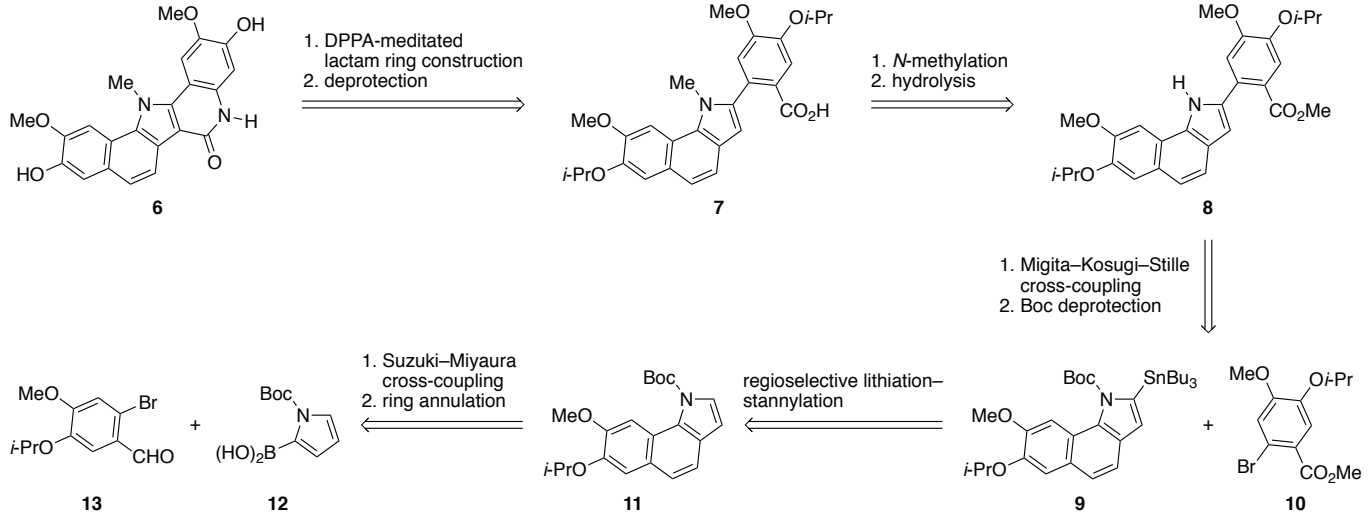


Figure 2. Structures of the various compounds of interest

RESULTS AND DISCUSSION

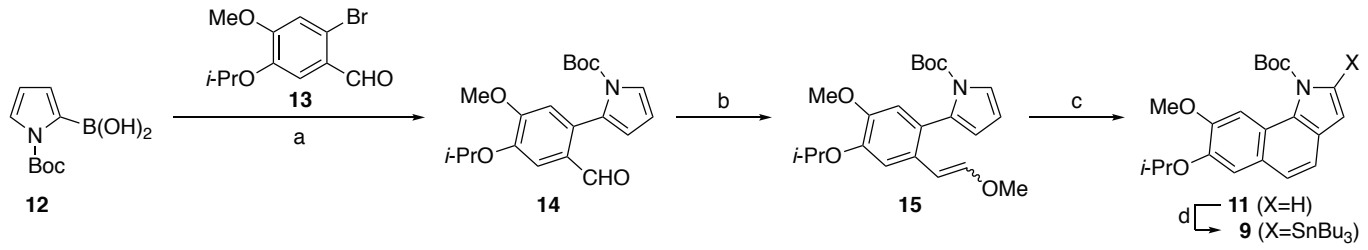
Initially, we selected BIQ **6** as the synthetic target, and the corresponding retrosynthetic analysis is outlined in Scheme 1. Thus, the synthesis of BIQ **6** can be completed by diphenylphosphoryl azide (DPPA)[17]-mediated lactam ring construction involving acyl azide formation from carboxylic acid **7**. This can be followed by a subsequent cascade Curtius rearrangement/6π-electrocyclization under thermal conditions[18] and deprotection of the *O*-isopropyl groups. The carboxylic acid **7** can be produced by the *N*-methylation of **8** and subsequent hydrolysis, where compound **8** can be prepared from stannane **9** and bromide **10** via a Migita–Kosugi–Stille cross-coupling reaction followed by *tert*-butoxycarbonyl (Boc) deprotection. In addition, stannane **9** can be prepared from tricyclic compound **11** through a regioselective lithiation–stannylation sequence. Finally, the tricyclic compound **11** can be obtained from the known

N-Boc-2-pyrroleboronic acid **12**[19] and bromide **13**[20] by a Suzuki–Miyaura cross-coupling reaction and subsequent ring annulation.[21]



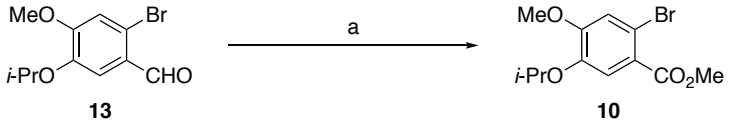
Scheme 1. Retrosynthetic analysis of BIQ **6**

Based on the above retrosynthetic analysis, we initially prepared stannane **9**, as outlined in Scheme 2. A Suzuki–Miyaura cross-coupling of **12** with bromide **13** under standard conditions [i.e., $Pd(PPh_3)_4$ (10 mol%), Na_2CO_3, THF, water, reflux] afforded **14** in 94% yield. Tricyclic compound **11** was then obtained from **14** in two steps by applying the method employed for the construction of polyaromatic hydrocarbons.[21] Thus, a Wittig reaction of **14** with (methoxymethyl)triphenylphosphonium chloride in the presence of *t*-BuOK as a base afforded methyl enol ether **15** in 93% yield as a 64:36 mixture of the *E*- and *Z*-isomers. Subsequent treatment of methyl enol ether **15** with a catalytic amount of methanesulfonic acid produced the tricyclic compound **11** in 92% yield, which was converted to stannane **9** in 99% yield by regioselective lithiation followed by treatment with tributyltin chloride.



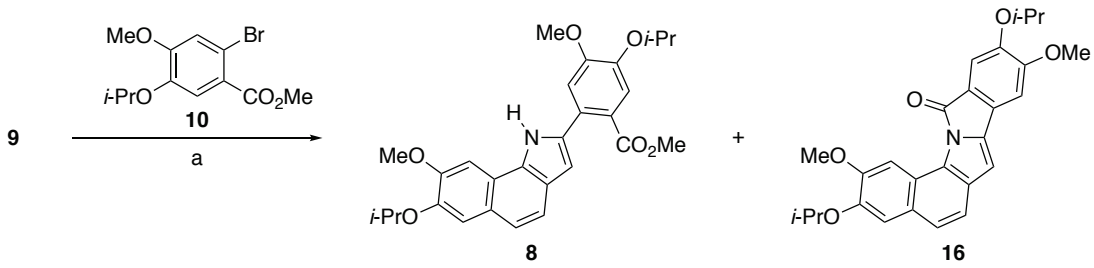
Scheme 2. *Reagents and conditions:* (a) **13**, $Pd(PPh_3)_4$, Na_2CO_3, THF, water, reflux, 18 h (94%); (b) $MeOCH_2P^+Ph_3 \cdot Cl^-$ (1.25 equiv), *t*-BuOK (1.5 equiv), THF, 0 °C, 3 h (93%, *E*:*Z* = 64:36); (c) $MeSO_3H$ (9.5 mol%), CH_2Cl_2, 0 °C, 23 h (92%); (d) (1) *t*-BuLi (1.2 equiv), THF, −78 °C, 1 h, (2) Bu_3SnCl (1.5 equiv), −78 °C, 1 h, then rt, 20 h (99%).

The preparation of coupling partner **10** was then examined. As Yamada and co-workers previously reported that aldehydes can be converted to the corresponding methyl esters through a one-step oxidation process (i.e., iodine, KOH, MeOH),[22] we applied their conditions to the synthesis of **10**. Thus, benzaldehyde **13** was treated with iodine and KOH in MeOH to afford the corresponding methyl benzoate **10** in 89% yield (Scheme 3).



Scheme 3. *Reagents and conditions:* (a) I_2 (1.4 equiv), KOH (2.3 equiv), MeOH, 0 °C, 1 h then rt, 21.5 h (89%).

With both coupling partners **9** and **10** in hand, a Migita–Kosugi–Stille cross-coupling was attempted (Scheme 4). More specifically, the treatment of stannane **9** with 1.5 equiv of bromide **10** in the presence of 10 mol% $Pd(PPh_3)_4$ in *N*,*N*-dimethylacetamide (DMA) at 100 °C for 13 h gave the Boc-deprotected coupling product **8** in 77% yield, along with the lactamized by-product **16** in 20% yield.

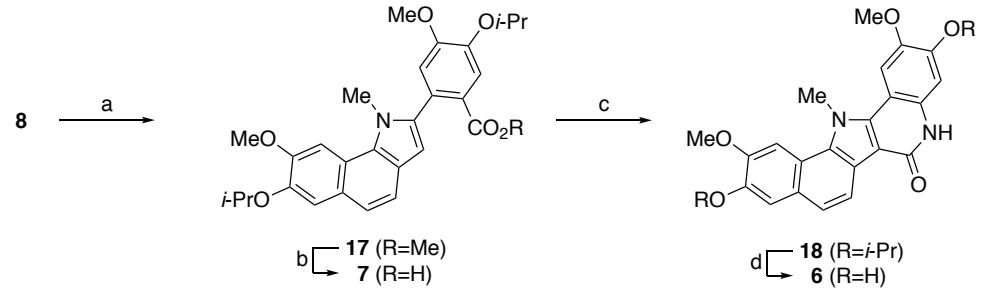


Scheme 4. *Reagents and conditions:* (a) **10** (1.5 equiv), $Pd(PPh_3)_4$ (10 mol%), DMA, 100 °C, 13 h (**8**: 77%, **16**: 20%).

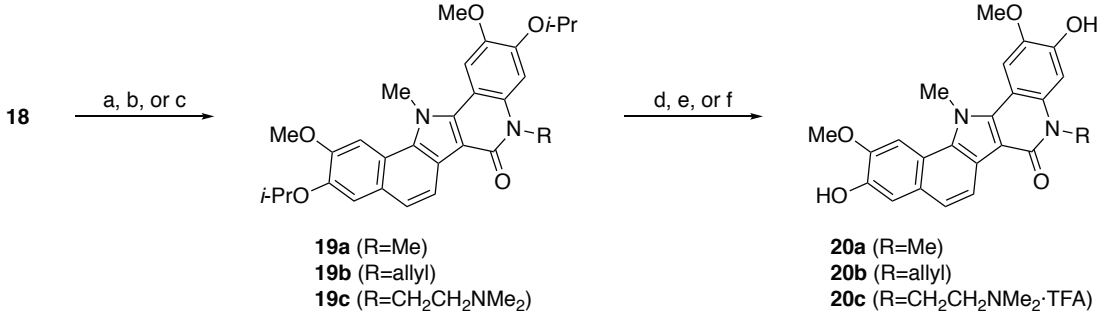
Further conversion of **8** to give BIQ **6** was then examined (Scheme 5) through the initial treatment of **8** with iodomethane in the presence of *t*-BuOK as a base in DMF to give *N*-methylated **17** in 89% yield. Subsequent alkaline hydrolysis of **17** afforded carboxylic acid **7** in 95% yield, and treatment of **7** with 1.0 equiv of DPPA followed by heating gave **18**, possessing the BIQ scaffold, in good yield (86%). Deprotection of the *O*-isopropyl groups of **18** using excess $AlCl_3$ produced the desired BIQ **6** in 95% yield.[23]

Having established a method for the construction of the BIQ scaffold, we then attempted the synthesis of N5-substituted BIQ analogues **20a**–**20c** (Scheme 6). Thus, treatment of **18** with iodomethane or allyl bromide in the presence of *t*-BuOK in DMF gave methylated **19a** and allylated **19b** in yields of 89 and 66%, respectively. In the case of **19c**, this compound was obtained by the reaction of **18** with

2-(dimethylamino)ethyl chloride hydrochloride and sodium hydride in DMF. Subsequent deprotection of the *O*-isopropyl groups of **19a**–**19c** using $AlCl_3$ gave the corresponding BIQ analogues **20a**–**20c** in moderate to good yields.

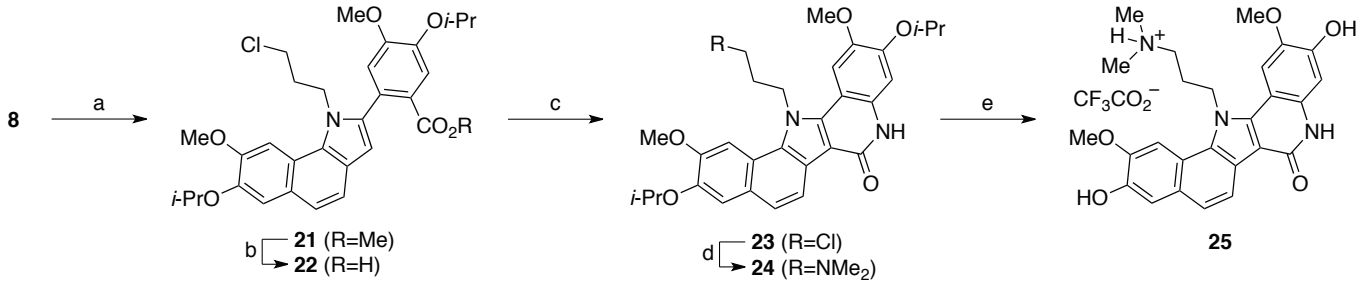


Scheme 5. *Reagents and conditions:* (a) *t*-BuOK (2.0 equiv), MeI (5.0 equiv), DMF, 0 °C, 1 h then rt, 3 h (89%); (b) 40% aqueous KOH, EtOH, reflux, 0.5 h, (95%); (c) DPPA (1.0 equiv), Et_3N (0.86 equiv), Ph_2O, 35 °C, 3 h then 100 °C, 2 h then 220 °C, 1 h (86%); (d) $AlCl_3$ (5.4 equiv), $CHCl_3$, rt, 48 h (95%).



Scheme 6. *Reagents and conditions:* (a) *t*-BuOK (2.0 equiv), MeI (5.1 equiv), DMF, 0 °C, 1 h then rt, 18 h (**19a**: 89%); (b) *t*-BuOK (2.0 equiv), allyl bromide (5.1 equiv), DMF, 0 °C, 1 h then rt, 24 h (**19b**: 66%); (c) NaH (6.0 equiv), $Me_2N(CH_2)_2Cl{\cdot}HCl$ (1.7 equiv), DMF, 0 °C, 0.5 h then rt, 4 h then 65 °C, 14 h (**19c**: 68%); (d) $AlCl_3$ (5.4 equiv), $CHCl_3$, rt, 48 h (**20a**: 65%); (e) $AlCl_3$ (5.4 equiv), CH_2Cl_2, rt, 24 h (**20b**: 77%); (f) (1) $AlCl_3$ (5.4 equiv), CH_2Cl_2, rt, 70 h, (2) TFA, rt, 0.5 h (**20c**: 93%).

Subsequently, the synthesis of BIQ **25** possessing a 3-(dimethylamino)propyl group at the N13 position was attempted (Scheme 7). Initially, the 3-chloropropylation of **8** was examined through the one-pot reaction of **8** with 2.0 equiv of *t*-BuOK and 5.0 equiv of 1-chloro-3-iodopropane, which gave the 3-chloropropylated **21** in 43% yield. The yield of **21** improved to 81% by the two-step addition of *t*-BuOK (1.0 equiv) and 1-chloro-3-iodopropane (2.4 equiv). Subsequent alkaline hydrolysis of **21** afforded the carboxylic acid **22** in 91% yield, and the DPPA-mediated lactam ring construction of **22** gave the BIQ scaffold **23** in 76% yield. Treatment of the chloride **23** with 10 equiv of dimethylamine in the presence of potassium iodide in dimethyl sulfoxide (DMSO) at 80 °C for 20 h afforded the amine **24**, and a final deprotection of the *O*-isopropyl groups of **24** using excess $AlCl_3$ produced the desired BIQ **25** in 94% yield.



Scheme 7. *Reagents and conditions:* (a) (1) *t*-BuOK (1.0 equiv), $Cl(CH_2)_3I$ (2.4 equiv), DMF, 0 °C, 1 h then rt, 5 h, (2) *t*-BuOK (1.0 equiv), $Cl(CH_2)_3I$ (2.4 equiv), 0 °C then rt, 18 h, (81%); (b) 40% aqueous KOH, EtOH, 50 °C, 2 h, (91%); (c) DPPA (1.0 equiv), Et_3N (0.86 equiv), Ph_2O, 35 °C, 3 h then 100 °C, 2 h then 220 °C, 1 h (76%); (d) Me_2NH (10 equiv), KI (5.0 equiv), DMSO, 80 °C, 20 h (51%); (e) (1) $AlCl_3$ (6.4 equiv), CH_2Cl_2, rt, 72 h, (2) TFA (94%).

Following the preparation of a range of BIQs (**6**, **20a**, **20b**, **20c**, and **25**), we then evaluated the topoisomerase I inhibitory activities of these compounds using a DNA relaxation assay[24,25] with supercoiled pBR322 plasmid DNA and topoisomerase I isolated from calf thymus (Figure 3). The experiments were performed at a concentration of 2 μM for all BIQs. Camptothecin (CPT), a typical topoisomerase I inhibitor, was used as the reference compound at the same concentration. In the absence of an inhibitor, the supercoiled DNA changed to relaxed DNA, which appeared as multiple bands corresponding to the topoisomers of the different linking numbers. Since similar multiple bands were observed in the presence of CPT and BIQ **20b**, it appeared that their topoisomerase I inhibitory activities were weak at the concentration examined herein. In contrast, treatment with BIQs **6**, **20a**, **20c**, and **25** resulted in the accumulation of nicked DNA, thereby confirming their inhibitory activity at 2 μM. As the topoisomerase I inhibitory activity of **1** was reported to be as potent as that of CPT,[25] it was concluded that the topoisomerase I inhibitory activities of BIQs **6**, **20a**, **20c**, and **25** are more potent than those of CPT and **1**.

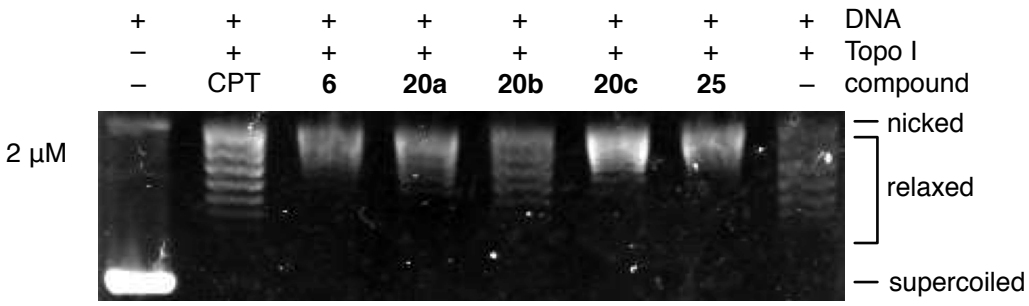


Figure 3. DNA relaxation assay of BIQs **6**, **20a**, **20b**, **20c**, and **25**

We then moved on to evaluate the antiproliferative activities of BIQs **6**, **20a**, and **20c** against the JFCR39 panel.[11] Thus, the 50% growth-inhibitory concentration (GI_{50}) values of the selected nine cell lines and

the mean-graph midpoints (MG-MID) of the average GI_{50} values across the entire JFCR39 panel are shown in Table 1 (a full record of the cytotoxicity data can be found in the Supporting Information). For comparison, lamellarin D (**1**) was also included. Among the three BIQs examined, BIQ **20a**, which possesses methyl groups at the N5 and N13 positions, showed strong antiproliferative activity at a nanomolar concentration (MG-MID = 74.1 nM), which was comparable to that of lamellarin D (**1**). Compared to BIQ **20a**, the activities of the N5-unsubstituted **6** and N5-2-(dimethylamino)ethylated **20c** were significantly lower (MG-MID = 501 and 1318 nM). This result can likely be attributed to factors in the living cells, such as the cell membrane permeability, since BIQs **6** and **20c** showed potent topoisomerase I inhibition at 2 μM, at which concentrations lamellarin D (**1**) and CPT were essentially inactive. The COMPARE analysis[11] of the cytotoxicity profiles of the different BIQs suggested that the major cellular target of these compounds was topoisomerase I, since the profiles of these compounds showed a good correlation to those of known topoisomerase I inhibitors such as SN-38[26] and TAS-103[27] (r = 0.718–0.85).

Table 1. *In vitro* antiproliferative activities of BIQs **6**, **20a**, and **20c** against selected human cancer cell lines

Human tumor cell lines		Antiproliferative activity (GI_{50} in nM)[a]			
		6[e]	**20a**[e]	**20c**[f]	lamellarin D (**1**)[e]
Breast	MCF-7	<10	12	650	<10
CNS	U251	<10	<10	710	<10
Colon	HCT-116	42	17	1100	<10
Lung	NCI-H522	15	<10	410	<10
Melanoma	LOX-IMVI	20	<10	370	<10
Ovarian	OVCAR-8	150	34	640	12
Renal	ACHN	53	10	1000	<10
Stomach	MKN45	190	69	370	110
Prostate	DU-145	110	27	1500	<10
MG-MID[b]		501	74.1	1318	41.7
Delta[c]		1.7	0.87	0.55	0.62
Range[d]		4.00	3.58	1.44	2.30

[a] Concentration for 50% inhibition of cell growth relative to the control. Cell growth was determined according to the sulforhodamine B assay.
[b] Mean GI_{50} value in all cell lines tested.
[c] Difference in log GI_{50} values between the most sensitive cells and the MG-MID value.
[d] Difference in log GI_{50} values between the most and least sensitive cells.
[e] The GI_{50} value was obtained from the dose-response curve in the test range between 10^{-4} and -10^{-8} M.
[f] The GI_{50} value was obtained from the dose-response curve in the test range between 10^{-5} and -10^{-9} M.

In conclusion, we designed and synthesized a series of 5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (BIQ) scaffolds as novel topoisomerase I inhibitors. The synthesis of the BIQs was achieved by sequential and regioselective functionalization of the pyrrole core, which involved palladium-catalyzed cross-coupling, conventional electrophilic substitution, directed lithiation, and subsequent

diphenylphosphoryl azide (DPPA)-mediated lactam ring construction as the key reactions. The obtained BIQs exhibited similar or more potent topoisomerase I inhibitory activities compared to camptothecin and the parent compound lamellarin D (**1**). In addition, BIQ **20a** exhibited a good activity in the low nanomolar GI_{50} range, which was comparable to that of lamellarin D (**1**). Further modifications to improve the antiproliferative activities of the BIQs against cancer cell lines are currently underway in our laboratory.

EXPERIMENTAL

Melting points were determined with a Yanagimoto micro melting point apparatus and are reported uncorrected. IR spectra were obtained with a Thermo Nicolet Nexus 670 NT FT-IR instrument and are reported in terms of absorption frequency (cm^{-1}). NMR spectra were recorded on a JEOL JNM-AL400 instrument (400 MHz for ^1H and 100 MHz for ^{13}C) or a Varian NMR System 500PS SN instrument (500 MHz for ^1H and 126 MHz for ^{13}C). Chemical shifts for ^1H NMR are expressed in parts per million (ppm) relative to the following internal standards: $CDCl_3$ (tetramethylsilane, δ 0.0 ppm); DMSO-d_6 (DMSO, δ 2.50 ppm). Data for ^1H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, dd = double doublet, t = triplet, sep = septet, m = multiplet, br s = broad singlet), coupling constant (Hz), and integration. Chemical shifts for ^{13}C NMR are expressed in ppm relative to the following internal standards: $CDCl_3$ (tetramethylsilane, δ 0.0 ppm); DMSO-d_6 (DMSO-d_6, δ 39.52 ppm). High-resolution mass spectra were recorded on a JEOL JMS-T100TD (direct analysis by real-time mass spectrometry, DARTMS). Column chromatography was conducted using silica gel 60N, 63–210 μm (Kanto Chemical Co., Inc.), Chromatorex NH-DM1020 (Fuji Silysia Chemical Ltd.), or aluminium oxide 90 (Merck KGaA). Flash chromatography was conducted using silica gel 60N, 40–50 μm (Kanto Chemical Co., Inc.).

[1-(*tert*-Butoxycarbonyl)-1*H*-pyrrol-2-yl]boronic acid (12). Under an argon atmosphere, to a solution of diisopropylamine (18.2 mL, 130 mmol) in THF (450 mL), was added dropwise a hexane solution of BuLi (1.61 M, 74.8 mL, 120 mmol) at −78 °C. The mixture was stirred at −78 °C for 5 min, gradually warmed up to 0 °C, and kept at the same temperature for 10 min. The whole was again cooled to −78 °C and a solution of *N*-Boc-pyrrole (16.7 g, 100 mmol) in THF (30 mL) was added dropwise. After 1 h at −78 °C, trimethyl borate (16.7 mL, 150 mmol) was added dropwise. After 1 h, the mixture was gradually warmed up to rt and stirred for 15 h. The reaction was then quenched by adding saturated aqueous NH_4Cl solution and the THF was removed *in vacuo*. The pH of the residual liquid was made 3 with AcOH and the whole was extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residual solid was triturated with hexane and filtered to give **12** as pale brown granules

(15.6 g, 75%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.62 (s, 9H), 6.26 (t, J = 3.2 Hz, 1H), 7.10 (dd, J = 1.6 and 3.2 Hz, 1H), 7.18 (br s, 2H), 7.45 (dd, J = 1.6 and 3.2 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 28.0, 85.6, 112.0, 127.1, 128.7, 152.2. These physical and spectroscopic data are in good agreement with those previously reported.[19]

2-Bromo-5-isopropoxy-4-methoxybenzaldehyde (13). Under an argon atmosphere, a solution of NBS (35.6 g, 199 mmol) in DMF (100 mL) was added dropwise to a solution of 3-isopropoxy-4-methoxybenzaldehyde (19.4 g, 99.9 mmol) in DMF (50 mL) at rt. After stirring for 19.5 h, the mixture was quenched with 10% aqueous Na_2SO_3 and then diluted with water. The products were extracted with Et_2O and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by recrystallization from Et_2O-hexane to give **13** as pale brown needles (17.0 g, 62%). The mother liquor was evaporated and the residue was purified by column chromatography over silica gel 60N (hexane–EtOAc = 10:1) to give an additional **13** as pale brown solid (3.36 g, 12%). Mp 102.5–103 °C. IR (KBr): 1681, 1588, 1508, 1269, 1217, 1158, 1021 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.38 (d, J = 6.1 Hz, 6H), 3.94 (s, 3H), 4.63 (sep, J = 6.1 Hz, 1H), 7.05 (s, 1H), 7.43 (s, 1H), 10.18 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 21.8, 56.5, 71.5, 113.6, 115.9, 120.1, 126.5, 147.2, 155.7, 190.9. Anal. Calcd for $C_{11}H_{13}BrO_3$: C, 48.37; H, 4.80. Found: C, 48.23; H, 4.72. These physical and spectroscopic data are in good agreement with those previously reported.[20]

***tert*-Butyl 2-(2-formyl-4-isopropoxy-5-methoxyphenyl)-1*H*-pyrrole-1-carboxylate (14).** Under an argon atmosphere, a mixture of **13** (5.49 g, 20.1 mmol), **12** (5.06 g, 24.0 mmol), $Pd(PPh_3)_4$ (2.42 g, 2.10 mmol), and Na_2CO_3 (12.9 g, 126 mmol), THF (420 mL), and degassed water (38 mL) was refluxed for 18 h. After cooling to rt, the solvent was removed *in vacuo* and the residue was diluted with water and extracted CH_2Cl_2. The extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography over silica gel 60N (hexane–EtOAc = 10:1) to give **14** as a reddish viscous oil (6.78 g, 94%). IR (KBr): 1743, 1683, 1511, 1332, 1155, 1124 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (s, 9H), 1.41 (d, J = 6.1 Hz, 6H), 3.92 (s, 3H), 4.70 (sep, J = 6.1 Hz, 1H), 6.24 (dd, J = 1.8 and 3.3 Hz, 1H), 6.29 (t, J = 3.3 Hz, 1H), 6.82 (s, 1H), 7.44 (dd, J = 1.8 and 3.3 Hz, 1H), 7.47 (s, 1H), 9.74 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.9, 27.6, 56.2, 71.2, 84.0, 110.6, 111.2, 113.9, 116.9, 122.6, 128.7, 129.3, 132.8, 147.4, 148.9, 154.0, 190.7. HRFABMS (*m/z*) Calcd for $C_{20}H_{25}NO_5$ (M^+): 359.1733. Found: 359.1738.

***tert*-Butyl 2-[2-(2-methoxyethenyl)-4-isopropoxy-5-methoxyphenyl]-1*H*-pyrrole-1-carboxylate (15).** Under an argon atmosphere, to a mixture of (methoxymethyl)triphenylphosphonium chloride (2.68 g, 7.82 mmol) in THF (39 mL) cooled to 0 °C was added dropwise a suspension of *t*-BuOK (1.05 g, 9.36 mmol) in THF (9.4 ml). After stirring for 10 min at 0 °C, a solution of **14** (2.31 g, 6.26 mmol) in THF (27 mL) was added dropwise. After stirring for 3 h at 0 °C, the reaction was quenched by adding

water (100 mL). The THF was removed *in vacuo* and the residue was extracted with Et_2O. The extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography over silica gel 60N (hexane–EtOAc = 10:1) to give **15** as an *E*/*Z* mixture (*E*:*Z* = 64:36, based on NMR) as a reddish viscous oil (2.26 g, 93%). IR (KBr): 1736, 1509, 1336, 1158, 1125 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.22 (s, 3.28H), 1.26 (s, 5.72H), 1.38 (d, *J* = 6.1 Hz, 3.82H), 1.40 (d, *J* = 6.2 Hz, 2.18H), 3.50 (s, 1.91H), 3.71 (s, 1.09H), 3.82 (s, 1.91H), 3.82 (s, 1.09H), 4.50–4.61 (m, 1H), 4.84 (d, *J* = 7.2 Hz, 0.364H), 5.47 (d, *J* = 12.9 Hz, 0.636H), 5.95 (d, *J* = 7.2 Hz, 0.364H), 6.11 (dd, *J* = 1.8 and 3.4 Hz, 0.364H), 6.12 (dd, *J* = 1.9 and 3.3 Hz, 0.636H), 6.24 (t, *J* = 3.4 Hz, 0.364H), 6.24 (t, *J* = 3.3 Hz, 0.636H), 6.72 (d, *J* = 12.9 Hz, 0.636H), 6.74 (s, 0.364H), 6.76 (s, 0.636H), 6.87 (s, 0.636H), 7.37 (dd, *J* = 1.9 and 3.3 Hz, 0.636H), 7.38 (dd, *J* = 1.8 and 3.4 Hz, 0.364H), 7.70 (s, 0.364H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.2, 27.3, 27.5, 56.0, 56.1, 56.3, 60.4, 71.4, 71.7, 83.1, 83.1, 103.3, 103.4, 110.3, 110.4, 112.1, 114.0, 114.2, 114.3, 114.6, 116.1, 121.3, 121.4, 125.8, 128.8, 129.0, 132.9, 133.1, 146.4, 146.5, 147.0, 147.7, 148.1, 148.3, 149.4, 149.6. HRFABMS (*m/z*) Calcd for $C_{22}H_{29}NO_5$ (M^+): 387.2046. Found: 387.2053.

***tert*-Butyl 7-isopropoxy-8-methoxy-1*H*-benzo[*g*]indole-1-carboxylate (11).** Under an argon atmosphere, a solution of **15** (1.49 g, 3.85 mmol) in CH_2Cl_2 (25 mL) was cooled to 0 °C and methanesulfonic acid (0.025 mL, 0.365 mmol) was added. After stirring for 23 h at 0 °C, Na_2CO_3 (103.7 mg, 0.948 mmol) and $MgSO_4$ (101.8 mg, 0.846 mmol) were added as solids and the mixture was stirred for a while and filtered. The filtrate was concentrated and the residue was purified by column chromatography over silica gel 60N (hexane–EtOAc = 10:1) to give **11** as a white solid (1.26 g, 92%). Recrystallization from CH_2Cl_2–hexane gave colorless needles. Mp 102.5–103 °C. IR (KBr): 1740, 1491, 1389, 1308, 1250, 1165, 1112 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.45 (d, *J* = 6.1 Hz, 6H), 1.68 (s, 9H), 4.02 (s, 3H), 4.72 (sep, *J* = 6.1 Hz, 1H), 6.61 (d, *J* = 3.7 Hz, 1H), 7.25 (s, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 3.7 Hz, 1H), 8.43 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.0, 28.1, 55.8, 70.9, 83.6, 105.8, 108.0, 111.6, 118.0, 118.8, 123.8, 126.9, 127.8, 128.2, 130.5, 146.0, 149.2, 150.5. Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.06; H, 7.26; N, 3.76.

***tert*-Butyl 7-isopropoxy-8-methoxy-2-(tributylstannyl)-1*H*-benzo[*g*]indole-1-carboxylate (9).** Under an argon atmosphere, a pentane solution of *t*-BuLi (1.60 M, 4.40 mL, 7.04 mmol) was added dropwise to a solution of **11** (2.08 g, 5.85 mmol) in THF (30 mL) at −78 °C. After stirring for 1 h at −78 °C, tributyltin chloride (2.38 mL, 8.77 mmol) was added. After stirring for 1 h at −78 °C, the mixture was allowed to warm to rt and stirred for 20 h. The reaction was quenched by adding saturated aqueous NH_4Cl and the THF was removed *in vacuo*. The residue was extracted with CH_2Cl_2 and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography over aluminium oxide 90 (hexane–EtOAc = 10:1) to give **9** as a colorless powder (3.76 g,

99%). Recrystallization from Et_2O–hexane gave a colorless powder. Mp 98.0–99.5 °C. IR (KBr): 1712, 1495, 1318, 1146, 1109 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, J = 7.3 Hz, 9H), 1.09–1.15 (m, 6H), 1.28–1.40 (m, 6H), 1.46 (d, J = 6.1 Hz, 6H), 1.51–1.60 (m, 6H), 1.63 (s, 9H), 4.04 (s, 3H), 4.72 (sep, J = 6.1 Hz, 1H), 6.76 (t, J = 7.6 Hz, 1H), 7.25 (s, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.66 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.5, 13.7, 22.0, 27.4, 28.2, 29.2, 56.8, 70.9, 84.3, 106.8, 111.6, 117.7, 119.2, 119.6, 123.3, 127.9, 129.3, 133.3, 144.9, 146.1, 149.0, 153.0. HRFABMS (*m/z*) Calcd for $C_{33}H_{52}NO_4Sn$ [$(M+H)^+$]: 646.2918. Found: 646.2918.

Methyl 2-bromo-5-isopropoxy-4-methoxybenzoate (10). Under an argon atmosphere, to a solution of **13** (2.74 g, 10.0 mmol) in MeOH (10 mL) was added KOH (1.26 g, 22.5 mmol) and iodine (3.43 g, 13.5 mmol) at 0 °C. After stirring for 1 h at 0 °C, the mixture was allowed to warm to rt. After stirring for 21.5 h at rt, the reaction was quenched by adding aqueous $Na_2S_2O_3$ and the MeOH was removed *in vacuo*. The products were extracted with $CHCl_3$ and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography over Chromatorex NH-DM1020 (hexane–EtOAc = 10:1) to give **10** as a colorless solid (2.71 g, 89%). Recrystallization from Et_2O–hexane gave colorless granules. Mp 77.5–78.5 °C. IR (KBr): 1724, 1510, 1259, 1205, 1182, 1110 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.37 (d, J = 6.1 Hz, 6H), 3.89 (s, 3H), 3.90 (s, 3H), 4.56 (sep, J = 6.1 Hz, 1H), 7.10 (s, 1H), 7.44 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.9, 52.2, 56.3, 71.9, 114.2, 117.5, 118.2, 122.8, 146.0, 153.5, 165.9. Anal. Calcd for $C_{12}H_{15}BrO_4$: C, 47.54; H, 4.99. Found: C, 47.25; H, 4.69.

Migita–Kosugi–Stille cross-coupling of 9 and 10. Under an argon atmosphere, a solution of **9** (649 mg, 1.01 mmol), **10** (460 mg, 1.52 mmol), and $Pd(PPh_3)_4$ (118 mg, 0.102 mmol) in DMA (15 mL) was heated at 100 °C for 13 h. After cooling to rt, the reaction was quenched by adding water. The products were extracted with CH_2Cl_2 and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography over silica gel 60N (hexane–EtOAc = 5:1 to 1:1) to give **8** as a pale yellow solid (369 mg, 77%) and **16** as a yellwo solid (88.7 mg, 20%).

Methyl 5-isopropoxy-2-{7-isopropoxy-8-methoxy-1*H*-benzo[*g*]indol-2-yl}-4-methoxybenzoate (8). Recrystallization from CH_2Cl_2–hexane gave pale yellow granules. Mp 169.5–170.5 °C. IR (KBr): 3355, 1673, 1501, 1247, 1216, 1116 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.37 (d, J = 6.1 Hz, 6H), 1.46 (d, J = 6.1 Hz, 6H), 3.85 (s, 3H), 3.94 (s, 3H), 4.05 (s, 3H), 4.53 (sep, J = 6.1 Hz, 1H), 4.70 (sep, J = 6.1 Hz, 1H), 6.77 (d, J = 2.1 Hz, 1H), 7.22 (s, 1H), 7.30 (s, 1H), 7.33 (d, J = 8.5 Hz, 1H), 7.40 (s, 1H), 7.46 (s, 1H), 7.55 (d, J = 8.5 Hz, 1H), 10.92 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.0, 22.1, 52.7, 56.0, 56.1, 71.3, 71.7, 100.6, 104.0, 112.8, 113.9, 117.0, 117.5, 118.4, 119.6, 120.8, 123.5, 125.7, 128.3, 131.1, 134.9, 145.9, 145.9, 150.3, 153.0, 169.7. HRDARTMS (*m/z*) Calcd for $C_{28}H_{32}NO_6$ [$(M+H)^+$]: 478.2230. Found: 478.2251.

3,10-Diisopropoxy-2,9-dimethoxy-12*H*-benzo[*g*]isoindolo[2,1-*a*]indol-12-one (16). Recrystallization from CH_2Cl_2–hexane gave orange granules. Mp 151.5–153.0 °C. IR (KBr): 1730, 1489, 1308, 1228, 1210 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.41 (d, J = 6.1 Hz, 6H), 1.47 (d, J = 6.1 Hz, 6H), 3.94 (s, 3H), 4.13 (s, 3H), 4.57 (sep, J = 6.1 Hz, 1H), 4.72 (sep, J = 6.1 Hz, 1H), 6.51 (s, 1H), 6.87 (s, 1H), 7.15 (s, 1H), 7.22 (s, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 9.06 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.0, 22.0, 56.1, 56.3, 70.9, 71.7, 103.4, 103.7, 105.9, 110.6, 111.0, 118.0, 118.4, 123.1, 125.0, 128.6, 129.5, 130.1, 131.5, 138.5, 147.0, 147.8, 150.1, 155.5, 163.4. HRDARTMS (*m/z*) Calcd for $C_{27}H_{28}NO_5$ [$(M+H)^+$]: 446.1968. Found: 446.1994.

Methyl 5-isopropoxy-2-{7-isopropoxy-8-methoxy-1-methyl-1*H*-benzo[*g*]indol-2-yl}-4-methoxybenzoate (17). Under an argon atmosphere, a THF solution of *t*-BuOK (1.0 M, 1.68 mL, 1.68 mmol) was added to a solution of **8** (0.404 g, 0.845 mmol) in DMF (10 mL) at 0 °C. After stirring for 0.5 h at 0 °C, iodomethane (265 μL, 4.25 mmol) was added. After stirring for 1 h at 0 °C, the mixture was allowed to warm to rt and stirred for 3 h at rt. The reaction was quenched by addition of saturated aqueous NH_4Cl and 28% aqueous ammonia. The products were extracted with EtOAc and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography over silica gel 60N (hexane–EtOAc = 3:1) to give **17** as colorless solid (0.368 g, 89%). Recrystallization from CH_2Cl_2–hexane gave pale yellow granules. Mp 184–185.5 °C. IR (KBr): 1691, 1496, 1267, 1213, 1115 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.46 (d, J = 6.1 Hz, 6H), 1.46 (d, J = 6.1 Hz, 6H), 3.62 (s, 3H), 3.90 (s, 3H), 3.99 (s, 3H), 4.02 (s, 3H), 4.71 (sep, J = 6.1 Hz, 1H), 4.72 (sep, J = 6.1 Hz, 1H), 6.51 (s, 1H), 6.93 (s, 1H), 7.35 (s, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.61 (s, 1H), 7.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.1, 22.1, 35.0, 52.1, 56.1, 56.2, 71.1, 71.6, 102.3, 102.4, 113.1, 116.0, 116.4, 118.4, 118.9, 119.8, 123.6, 123.9, 127.0, 127.8, 130.5, 139.1, 145.1, 147.0, 149.5, 152.6, 167.0. HRDARTMS (*m/z*) Calcd for $C_{29}H_{34}NO_6$ [$(M+H)^+$]: 492.2386. Found: 492.2393.

5-Isopropoxy-2-{7-isopropoxy-8-methoxy-1-methyl-1*H*-benzo[*g*]indol-2-yl}-4-methoxybenzoic acid (7). Under an argon atmosphere, a suspension of **17** (0.253 g, 0.515 mmol) in a degassed mixture of 40% aqueous KOH (24 mL) and EtOH (24 mL) was refluxed for 0.5 h. The solution was cooled to rt and concentrated. The pH of the solution was adjusted to pH 1 with concd HCl, and the product was extracted with CH_2Cl_2. The extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography over silica gel 60N (CH_2Cl_2–MeOH = 10:1) to give **7** as pale yellow solid (0.234 g, 95%). Recrystallization from CH_2Cl_2–hexane gave a colorless powder. Mp 222–223 °C. IR (KBr): 3449, 1681, 1492, 1356, 1267, 1217, 1115 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (d, J = 6.1 Hz, 6H), 1.46 (d, J = 6.1 Hz, 6H), 3.87 (s, 3H), 3.93 (s, 3H), 3.98 (s, 3H), 4.64 (sep, J = 6.1 Hz, 1H), 4.72 (sep, J = 6.1 Hz, 1H), 6.47 (s, 1H), 6.85 (s, 1H), 7.34 (s, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.61 (s, 1H), 7.91 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.9, 22.1, 35.0, 56.1,

56.2, 71.1, 71.4, 102.4, 102.5, 113.0, 116.1, 116.7, 118.3, 118.9, 119.8, 123.8, 125.3, 126.9, 128.2, 129.0, 130.6, 138.9, 145.2, 147.1, 149.6, 153.1. HRDARTMS (*m/z*) Calcd for $C_{28}H_{32}NO_6$ [$(M+H)^+$]: 478.2230. Found: 478.2220.

3,10-Diisopropoxy-2,11-dimethoxy-13-methyl-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (18). Under an argon atmosphere, a mixture of **7** (103 mg, 0.216 mmol), diphenylphosphoryl azide (48.0 μL, 0.223 mmol), triethylamine (26.0 μL, 0.187 mmol), and diphenyl ether (5.0 mL) was stirred in a sealed tube at 35 °C. After stirring for 3 h at 35 °C, the mixture was heated to 100 °C. After stirring for 2 h at 100 °C, the mixture was heated to 220 °C. After stirring for 1 h at 220 °C, the mixture was cooled to rt. The volatiles were removed by bulb-to-bulb distillation. The residue was purified by column chromatography over silica gel 60N (CH_2Cl_2–EtOAc = 1:1) to give **18** as pale yellow solid (88.4 mg, 86%). Mp >300 °C. IR (KBr): 1649, 1498, 1263, 1213, 1112 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.36 (d, J = 6.1 Hz, 12H), 3.93 (s, 3H), 4.01 (s, 3H), 4.59 (sep, J = 6.1 Hz, 1H), 4.63 (s, 3H), 4.77 (sep, J = 6.1 Hz, 1H), 7.15 (s, 1H), 7.53 (s, 1H), 7.61 (d, J = 8.5 Hz, 1H), 7.73 (s, 1H), 7.90 (s, 1H), 8.24 (d, J = 8.5 Hz, 1H), 11.26 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.8, 21.9, 38.2, 55.3, 55.8, 70.0, 70.5, 101.9, 102.3, 105.1, 105.2, 107.1, 111.8, 116.6, 117.7, 120.2, 121.6, 127.4, 133.4, 134.5, 141.0, 144.9, 145.6, 148.1, 149.3, 159.3. HRFABMS (*m/z*) Calcd for $C_{28}H_{31}N_2O_5$ [$(M+H)^+$]: 475.2233. Found: 475.2234.

3,10-Dihydroxy-2,11-dimethoxy-13-methyl-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (6). Under an argon atmosphere, a nitrobenzene solution of $AlCl_3$ (1.0 M, 380 μL, 0.380 mmol) was added dropwise to a solution of **18** (33.3 mg, 70.2 μmol) in $CHCl_3$ (6.0 mL) at rt. After stirring for 48 h at rt, a solution of $NaHCO_3$ (97.4 mg, 1.16 mmol) and Rochelle salt (325 mg, 1.15 mmol) in water (2.3 mL) was added. The mixture was stirred for an additional 1 h and then evaporated. The nitrobenzene was removed azeotropically with water under reduced pressure. To the residue was added water and the precipitate was collected by filtration, washed with water, and dried under reduced pressure to give **6** as a pale brown powder (25.9 mg, 95%). Mp >300 °C (sealed capillary). IR (KBr): 3425, 1649, 1611, 1432, 1270, 1229 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.95 (s, 3H), 4.04 (s, 3H), 4.64 (s, 3H), 7.00 (s, 1H), 7.35 (s, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.73 (s, 1H), 7.91 (s, 1H), 8.19 (d, J = 8.5 Hz, 1H), 9.49 (s, 1H), 9.84 (s, 1H), 11.28 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.3, 55.5, 56.0, 102.2, 102.7, 104.4, 105.3, 106.7, 112.1, 116.1, 117.7, 119.7, 121.0, 127.8, 133.7, 134.6, 141.2, 143.6, 145.5, 148.1, 148.3, 159.5. HRFABMS (*m/z*) Calcd for $C_{22}H_{19}N_2O_5$ [$(M+H)^+$]: 391.1294. Found: 391.1292.

3,10-Diisopropoxy-2,11-dimethoxy-5,13-dimethyl-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (19a). Under an argon atmosphere, a THF solution of *t*-BuOK (1.0 M, 240 μL, 0.240 mmol) was added to a solution of **18** (57.1 mg, 0.120 mmol) in DMF (4.0 mL) at 0 °C. After stirring for 0.5 h at 0 °C, iodomethane (38.0 μL, 0.610 mmol) was added. After stirring for 1 h at 0 °C, the mixture was allowed to

warm to rt and stirred for 18 h at rt. The reaction was quenched by addition of saturated aqueous NH_4Cl and 28% aqueous ammonia. The products were extracted with CH_2Cl_2 and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography over silica gel 60N (CH_2Cl_2–EtOAc = 5:1) to give **19a** as pale yellow solid (52.3 mg, 89%). Recrystallization from CH_2Cl_2–hexane gave a colorless powder. IR (KBr): 1639, 1269, 1231, 1093 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.45 (d, J = 6.1 Hz, 12H), 3.46 (s, 3H), 3.85 (s, 3H), 3.91 (s, 6H), 4.60 (sep, J = 6.1 Hz, 1H), 4.61 (sep, J = 6.1 Hz, 1H), 6.59 (s, 1H), 6.98 (s, 1H), 7.10 (s, 1H), 7.12 (s, 1H), 7.24 (d, J = 8.5 Hz, 1H), 8.28 (d, J = 8.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.1, 22.2, 28.7, 37.6, 55.5, 56.2, 70.8, 72.0, 101.6, 102.9, 105.3, 106.9, 106.9, 111.9, 116.3, 118.1, 120.4, 121.5, 127.7, 133.8, 134.1, 138.8, 144.9, 145.5, 147.6, 149.0, 159.3. HRDARTMS (*m/z*) Calcd for $C_{29}H_{33}N_2O_5$ [$(M+H)^+$]: 489.2390. Found: 489.2402.

5-Allyl-3,10-diisopropoxy-2,11-dimethoxy-13-methyl-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]-quinolin-6-one (19b). According to the procedure described for the preparation of **19a**, **18** (53.1 mg, 0.112 mmol) and allyl bromide (50.0 μL, 0.578 mmol) were reacted. After purification by column chromatography over silica gel 60N (CH_2Cl_2–EtOAc = 20:1), **19b** was obtained as a pale yellow solid (38.0 mg, 66%). Recrystallization from CH_2Cl_2–hexane gave a colorless powder. IR (KBr): 1637, 1261, 1234, 1167, 1114 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.46 (d, J = 6.1 Hz, 6H), 1.48 (d, J = 6.1 Hz, 6H), 4.01 (s, 3H), 4.03 (s, 3H), 4.47 (s, 3H), 4.63 (sep, J = 6.1 Hz, 1H), 4.72 (sep, J = 6.1 Hz, 1H), 5.06 (br s, 2H), 5.13 (d, J = 17.3 Hz, 1H), 5.23 (d, J = 10.4 Hz, 1H), 5.95–6.08 (m, 1H), 6.95 (s, 1H), 7.29 (s, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.59 (s, 1H), 7.66 (s, 1H), 8.49 (d, J = 8.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.0, 38.5, 44.4, 56.0, 56.6, 71.0, 71.7, 102.2, 103.6, 106.1, 107.4, 107.7, 112.2, 116.6, 116.7, 118.8, 121.2, 122.1, 128.3, 133.4, 133.9, 135.3, 140.4, 145.2, 146.1, 148.2, 149.5, 159.5. HRDARTMS (*m/z*) Calcd for $C_{31}H_{34}N_2O_5$ (M^+): 514.2468. Found: 514.2465.

5-[2-(Dimethylamino)ethyl]-3,10-diisopropoxy-2,11-dimethoxy-13-methyl-5,13-dihydro-6*H*-benzo-[6,7]indolo[3,2-*c*]quinolin-6-one (19c). Under an argon atmosphere, to a suspension of sodium hydride (60% dispersion in mineral oil, 26.1 mg, ca 0.653 mmol, prewashed with hexane) in DMF (2.0 mL) was added dropwise a solution of **18** (51.3 mg, 0.108 mmol) in DMF (3.0 mL) at 0 °C. After stirring for 0.5 h at 0 °C, a solution of 2-(dimethylamino)ethyl chloride hydrochloride (26.0 mg, 0.181 mmol) in DMF (3.0 mL) was added and the mixture was allowed to warm to rt. After stirring for 4 h at rt, the mixture was warmed to 65 °C. After stirring for 14 h at 65 °C, the mixture was cooled to rt. The reaction was quenched by addition of saturated aqueous NH_4Cl and 28% aqueous ammonia. The products were extracted with CH_2Cl_2 and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography over silica gel 60N (CH_2Cl_2–EtOAc = 5:1) to give **19c** as pale yellow solid (40.0 mg, 68%). Recrystallization from CH_2Cl_2–hexane

gave a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48 (d, J = 6.1 Hz, 6H), 1.49 (d, J = 6.1 Hz, 6H), 2.42 (s, 6H), 2.69 (t, J = 7.7 Hz, 2H), 4.02 (s, 3H), 4.04 (s, 3H), 4.50 (s, 3H), 4.53 (t, J = 7.3 Hz, 2H), 4.73 (sep, J = 6.1 Hz, 1H), 4.73 (sep, J = 6.1 Hz, 1H), 7.13 (s, 1H), 7.32 (s, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.64 (s, 1H), 7.71 (s, 1H), 8.49 (d, J = 8.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.0, 22.1, 38.6, 40.6, 45.9, 56.0, 56.6, 56.7, 71.0, 71.7, 102.2, 102.9, 106.3, 107.6, 107.9, 112.3, 116.7, 118.9, 121.2, 122.1, 128.3, 133.8, 135.5, 140.5, 145.3, 146.1, 148.5, 149.6, 159.7. HRDARTMS (*m/z*) Calcd for $C_{32}H_{40}N_3O_5$ [$(M+H)^+$]: 546.2968. Found: 546.2967.

3,10-Dihydroxy-2,11-dimethoxy-5,13-dimethyl-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (20a). According to the procedure described for the preparation of **6**, **19a** (52.3 mg, 0.107 mmol) and $AlCl_3$ (1.0 M, 580 μL, 0.580 mmol) were reacted to give **20a** as a pale brown powder (28.2 mg, 65%). Mp >300 °C (sealed capillary). IR (KBr): 3288, 1622, 1579, 1447, 1267, 1227 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.66 (s, 3H), 3.99 (s, 3H), 4.03 (s, 3H), 4.60 (s, 3H), 7.09 (s, 1H), 7.35 (s, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.79 (s, 1H), 7.87 (s, 1H), 8.24 (d, J = 8.5 Hz, 1H), 9.49 (br s, 1H), 9.91 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 28.8, 38.5, 55.4, 56.0, 102.2, 102.9, 105.4, 105.9, 106.3, 112.1, 116.1, 117.7, 119.9, 121.1, 127.9, 134.4, 134.9, 140.0, 143.2, 145.6, 148.1, 148.2, 158.7. HRDARTMS (*m/z*) Calcd for $C_{23}H_{21}N_2O_5$ [$(M+H)^+$]: 405.1451. Found: 405.1428.

5-Allyl-3,10-dihydroxy-2,11-dimethoxy-13-methyl-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]-quinolin-6-one (20b). Under an argon atmosphere, a nitrobenzene solution of $AlCl_3$ (1.0 M, 380 μL, 0.380 mmol) was added dropwise to a solution of **19b** (35.9 mg, 69.8 μmol) in CH_2Cl_2 (5.0 mL) at rt. After stirring for 24 h at rt, a solution of $NaHCO_3$ (95.8 mg, 1.14 mmol) and Rochelle salt (322 mg, 1.14 mmol) in water (2.3 mL) was added. The mixture was stirred for an additional 1 h and then evaporated. The nitrobenzene was removed azeotropically with water under reduced pressure. To the residue was added water and the precipitate was collected by filtration, washed with water, and dried under reduced pressure to give **20b** as a pale brown powder (23.1 mg, 77%). Mp >300 °C (sealed capillary). IR (KBr): 3423, 1628, 1575, 1428, 1272, 1230 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.99 (s, 3H), 4.04 (s, 3H), 4.63 (s, 3H), 4.98 (br s, 2H), 5.00 (d, J = 17.6 Hz, 1H), 5.17 (d, J = 10.5 Hz, 1H), 5.93–6.06 (m, 1H), 7.03 (s, 1H), 7.36 (s, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.81 (s, 1H), 7.90 (s, 1H), 8.23 (d, J = 8.5 Hz, 1H), 9.50 (s, 1H), 9.87 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.6, 43.4, 55.4, 55.9, 102.2, 103.5, 105.6, 106.0, 106.1, 112.1, 116.1, 116.1, 117.7, 119.9, 121.2, 127.9, 133.4, 133.6, 135.0, 140.3, 143.3, 145.6, 148.1, 148.2, 158.5. HRDARTMS (*m/z*) Calcd for $C_{25}H_{23}N_2O_5$ [$(M+H)^+$]: 431.1607. Found: 431.1595.

Trifluoroacetic acid salt of 5-[2-(dimethylamino)ethyl]-3,10-dihydroxy-2,11-dimethoxy-13-methyl-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (20c). Under an argon atmosphere, a nitrobenzene solution of $AlCl_3$ (1.0 M, 306 μL, 0.306 mmol) was added dropwise to a solution of **19c**

(30.9 mg, 56.6 μmol) in CH_2Cl_2 (9.1 mL) at rt. After stirring for 70 h at rt, a solution of $NaHCO_3$ (77.1 mg, 0.917 mmol) and Rochelle salt (259 mg, 0.917 mmol) in water (1.7 mL) was added. The mixture was stirred for an additional 1 h and then evaporated. The nitrobenzene was removed azeotropically with water under reduced pressure. To the residue was added water and the precipitate was collected by filtration, washed with water, and dried under reduced pressure to give 5-[2-(dimethylamino)ethyl]-3,10-dihydroxy-2,11-dimethoxy-13-methyl-5,13-dihydro-6*H*-benzo[6,7]-indolo-[3,2-*c*]quinolin-6-one (**20c′**) as a pale brown powder (24.4 mg, 93%). Mp >300 °C (sealed capillary). IR (KBr): 3384, 1628, 1469, 1428, 1275, 1216 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 2.29 (s, 6H), 2.53 (t, J = 7.4 Hz, 2H), 3.98 (s, 3H), 4.04 (s, 3H), 4.40 (t, J = 6.9 Hz, 2H), 4.61 (s, 3H), 7.11 (s, 1H), 7.35 (s, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.79 (s, 1H), 7.89 (s, 1H), 8.23 (d, J = 8.5 Hz, 1H), 9.50 (br s, 1H), 9.97 (br s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 38.6, 39.4, 45.6, 55.4, 55.9, 56.2, 102.2, 102.6, 105.6, 106.1, 106.2, 112.1, 116.0, 117.7, 119.9, 121.1, 127.9, 133.5, 135.1, 140.2, 143.2, 145.6, 148.2, 148.3, 158.5. HRDARTMS (*m/z*) Calcd for $C_{26}H_{28}N_3O_5$ [$(M+H)^+$]: 462.2029. Found: 462.2013.

To a suspension of **20c′** (12.0 mg, 21.0 μmol) in CH_2Cl_2 (2.0 mL) was added trifluoroacetic acid (2.0 mL) at rt. After stirring for 0.5 h at rt, the mixture was evaporated. The crude product was purified by column chromatography over Sephadex LH-20 (MeOH containing 0.1% TFA) to give **20c** as a brown solid (15.2 mg, quant). ^1H NMR (500 MHz, DMSO-d_6): δ 2.98 (s, 6H), 3.46 (br s, 2H), 4.02 (s, 3H), 4.05 (s, 3H), 4.63 (s, 3H), 4.69 (t, J = 5.9 Hz, 2H), 7.22 (s, 1H), 7.36 (s, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.84 (s, 1H), 7.90 (s, 1H), 8.23 (d, J = 8.5 Hz, 1H), 9.61 (br s, 1H), 10.05 (br s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 36.7, 38.7, 42.9, 54.5, 55.5, 56.0, 102.2, 102.9, 106.0, 106.0, 106.2, 112.1, 116.0, 117.6, 119.8, 121.4, 128.0, 133.0, 135.2, 140.6, 143.6, 145.8, 148.3, 148.6, 159.2. HRFABMS (*m/z*) Calcd for $C_{26}H_{28}N_3O_5$ [$(M-CF_3CO_2)^+$]: 462.2029. Found: 462.2030.

Methyl 5-isopropoxy-2-{1-(3-chloropropyl)-7-isopropoxy-8-methoxy-1*H*-benzo[*g*]indol-2-yl}-4-methoxybenzoate (21). Under an argon atmosphere, a THF solution of *t*-BuOK (1.0 M, 209 μL, 0.209 mmol) was added to a solution of **8** (0.100 g, 0.209 mmol) in DMF (10 mL) at 0 °C. After stirring for 0.5 h at 0 °C, 1-chloro-3-iodopropane (55.0 μL, 0.512 mmol) was added. After stirring for 1 h at 0 °C, the mixture was allowed to warm to rt. After stirring for 5 h at rt, the mixture was cooled to 0 °C. To the mixture was successively added a THF solution of *t*-BuOK (1.0 M, 209 μL, 0.209 mmol) and 1-chloro-3-iodopropane (55.0 μL, 0.512 mmol). The mixture was allowed to warm to rt and stirred for 18 h. The reaction was quenched by addition of saturated aqueous NH_4Cl and 28% aqueous ammonia. The products were extracted with EtOAc and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography over silica gel 60N (hexane–EtOAc = 2:1) to give **21** as yellow solid (94.0 mg, 81%). Recrystallization from Et_2O–hexane gave a colorless powder. Mp 134.5–135.5 °C. IR (KBr): 1696, 1523, 1496, 1261, 1218 cm^{-1}. ^1H NMR

(400 MHz, $CDCl_3$): δ 1.46 (d, J = 6.0 Hz, 6H), 1.47 (d, J = 6.0 Hz, 6H), 2.20–2.40 (m, 2H), 3.28–3.48 (m, 2H), 3.62 (s, 3H), 3.90 (s, 3H), 4.03 (s, 3H), 4.37–4.50 (m, 1H), 4.57–4.70 (m, 1H), 4.72 (sep, J = 6.0 Hz, 1H), 4.73 (sep, J = 6.0 Hz, 1H), 6.49 (s, 1H), 6.92 (s, 1H), 7.35 (s, 1H), 7.38 (d, J = 8.5 Hz, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.60 (s, 1H), 7.69 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.1, 22.1, 32.9, 42.1, 44.4, 52.1, 56.2, 56.3, 71.1, 71.6, 101.8, 103.4, 113.2, 115.9, 116.5, 117.7, 118.9, 120.3, 123.9, 124.7, 127.0, 127.6, 129.4, 138.9, 145.0, 147.1, 149.9, 152.5, 167.0. HRDARTMS (*m/z*) Calcd for $C_{31}H_{37}ClNO_6$ [$(M+H)^+$]: 554.2309. Found: 554.2319.

5-Isopropoxy-2-{1-(3-chloropropyl)-7-isopropoxy-8-methoxy-1*H*-benzo[*g*]indol-2-yl}-4-methoxy-benzoic acid (22). Under an argon atmosphere, a suspension of **21** (0.300 g, 0.541 mmol) in a degassed mixture of 40% aqueous KOH (10 mL) and EtOH (10 mL) was heated at 50 °C for 2 h. The solution was cooled to rt and concentrated. The pH of the solution was adjusted to pH 1 with concd HCl, and the products were extracted with CH_2Cl_2. The extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography over silica gel 60N (CH_2Cl_2–MeOH = 10:1) to give **22** as pale brown solid (0.267 g, 91%). Recrystallization from CH_2Cl_2–toluene gave a colorless powder. Mp 99–100.5 °C. IR (KBr): 3421, 1707, 1494, 1257, 1209, 1165 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.32 (d, J = 6.0 Hz, 6H), 1.34 (d, J = 6.0 Hz, 6H), 2.07–2.35 (m, 2H), 3.50–3.66 (m, 2H), 3.83 (s, 3H), 3.94 (s, 3H), 4.32–4.64 (m, 2H), 4.68 (sep, J = 6.0 Hz, 1H), 4.72 (sep, J = 6.0 Hz, 1H), 6.42 (s, 1H), 7.03 (s, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.45 (s, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.51 (s, 1H), 7.62 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 21.9, 22.0, 32.9, 42.6, 43.7, 55.6, 55.9, 70.0, 70.8, 101.5, 102.8, 112.5, 116.3, 116.4, 117.1, 118.6, 119.9, 124.2, 124.8, 126.2, 126.8, 128.7, 139.1, 144.4, 146.3, 149.5, 152.0, 167.5. HRDARTMS (*m/z*) Calcd for $C_{30}H_{35}ClNO_6$ [$(M+H)^+$]: 540.2153. Found: 540.2134.

13-(3-Chloropropyl)-3,10-diisopropoxy-2,11-dimethoxy-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]-quinolin-6-one (23). According to the procedure described for the preparation of **18**, **22** (137 mg, 0.254 mmol) was reacted. After chromatographic purification over silica gel 60N (hexane–EtOAc = 2:1 to EtOAc to CH_2Cl_2–MeOH = 10:1), **23** was obtained as a brown powder (104 mg, 76%). Recrystallization from CH_2Cl_2–hexane gave a colorless powder. Mp 295.5–297 °C. IR (KBr): 1660, 1501, 1437, 1257, 1228 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.36 (d, J = 6.0 Hz, 12H), 2.62–2.73 (m, 2H), 3.88–4.00 (m, 2H), 3.94 (s, 3H), 4.01 (s, 3H), 4.60 (sep, J = 6.0 Hz, 1H), 4.78 (sep, J = 6.0 Hz, 1H), 5.19 (t, J = 8.0 Hz, 2H), 7.17 (s, 1H), 7.55 (s, 1H), 7.57 (s, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.71 (s, 1H), 8.35 (d, J = 8.5 Hz, 1H), 11.31 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.7, 21.9, 32.7, 42.4, 45.9, 55.6, 56.2, 70.0, 70.6, 101.7, 102.1, 104.8, 104.8, 107.4, 112.2, 116.3, 117.9, 120.8, 122.1, 127.8, 132.6, 133.5, 139.1, 145.2, 145.5, 148.1, 149.7, 159.2. HRDARTMS (*m/z*) Calcd for $C_{30}H_{34}ClN_2O_5$ [$(M+H)^+$]: 537.2156. Found: 537.2159.

13-[3-(Dimethylamino)propyl]-3,10-diisopropoxy-2,11-dimethoxy-5,13-dihydro-6*H*-benzo[6,7]-indolo[3,2-*c*]quinolin-6-one (24). Under an argon atmosphere, a mixture of **23** (30.0 mg, 55.9 μmol), a THF solution of dimethylamine (2.0 M, 280 μL, 0.559 mmol), KI (46.4 mg, 0.280 mmol), and DMSO (5.0 mL) was heated in a sealed tube at 80 °C. After stirring for 20 h at 80 °C, the mixture was cooled to rt. The volatiles were removed by bulb-to-bulb distillation. To the residue was added CH_2Cl_2 and water and the two phases were separated. The aqueous phase was extracted with CH_2Cl_2 and the combined extracts were washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography over silica gel 60N (EtOAc to CH_2Cl_2–MeOH = 10:1) to give **24** as a brown powder (15.7 mg, 51%). Recrystallization from CH_2Cl_2 gave a colorless powder. Mp 289–290.5 °C. IR (KBr): 1653, 1500, 1436, 1257, 1113 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.50 (d, J = 6.1 Hz, 6H), 1.55 (d, J = 5.1 Hz, 6H), 2.26 (s, 6H), 2.37–2.50 (m, 4H), 4.01 (s, 3H), 4.06 (s, 3H), 4.77 (sep, J = 6.1 Hz, 1H), 4.82 (br s, 1H), 5.03 (br s, 2H), 7.26 (s, 1H), 7.39 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.64 (s, 1H), 7.78 (s, 1H), 8.59 (d, J = 8.4 Hz, 1H), 12.24 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 22.1, 22.1, 29.1, 45.7, 46.9, 56.1, 56.6, 56.9, 71.1, 71.5, 102.0, 102.6, 105.3, 105.8, 107.8, 112.7, 116.8, 119.0, 121.4, 122.2, 128.5, 133.2, 133.8, 140.1, 145.9, 146.0, 149.1, 150.0, 161.5. HRDARTMS (*m/z*) Calcd for $C_{32}H_{40}N_3O_5$ [$(M+H)^+$]: 546.2968. Found: 546.2981.

Trifluoroacetic acid salt of 13-[3-(dimethylamino)propyl]-3,10-dihydroxy-2,11-dimethoxy-5,13-dihydro-6*H*-benzo[6,7]indolo[3,2-*c*]quinolin-6-one (25). Under an argon atmosphere, a nitrobenzene solution of $AlCl_3$ (1.0 M, 235 μL, 0.235 mmol) was added dropwise to a solution of **24** (20.0 mg, 36.7 μmol) in CH_2Cl_2 (8.0 mL) at rt. After stirring for 72 h at rt, a solution of $NaHCO_3$ (59.3 mg, 0.706 mmol) and Rochelle salt (199 mg, 0.705 mmol) in water (3.6 mL) was added. After stirring for 1 h, the mixture was evaporated. The nitrobenzene was removed azeotropically with water under reduced pressure. To the residue was added CH_2Cl_2 (1.5 mL) and TFA (1.5 mL) and then the mixture was evaporated. The residue was purified by column chromatography over Sephadex LH-20 using following solvent systems (water containing 0.1% TFA, water–MeOH = 1:1 containing 0.1% TFA, and MeOH containing 0.1% TFA) to give **28** as a brown powder (19.8 mg, 94%). Mp 292–293.5 °C (sealed capillary). IR (KBr): 3412, 1683, 1203, 1144 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 2.50–2.58 (m, 2H), 2.79 (s, 3H), 2.79 (s, 3H), 3.21–3.29 (m, 2H), 3.99 (s, 3H), 4.09 (s, 3H), 5.16 (t, J = 7.9 Hz, 2H), 7.03 (s, 1H), 7.38 (s, 1H), 7.53 (s, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.69 (s, 1H), 8.30 (d, J = 8.5 Hz, 1H), 9.58 (br s, 1H), 9.77 (br s, 1H), 9.94 (br s, 1H), 11.37 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 25.4, 42.5, 45.1, 53.8, 55.6, 56.1, 101.5, 102.9, 104.1, 104.7, 107.4, 112.5, 115.8, 117.9, 120.5, 121.6, 128.1, 132.7, 133.8, 139.4, 143.9, 145.5, 148.4, 148.6, 159.4. HRFABMS (*m/z*) Calcd for $C_{26}H_{28}N_3O_5$ [$(M-CF_3COO)^+$]: 462.2029. Found: 462.2000.

Topoisomerase I inhibitory assay. The topoisomerase I relaxation assay was performed according to previous reports.[24,25] In brief, 2U of DNA topoisomerase I isolated from calf thymus (TaKaRa Bio) was mixed with 1 μg of supercoiled DNA pBR322 (TaKaRa Bio) in 20 μL of a reaction buffer (35 mM Tris–HCl, pH 8, 72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine, 0.01% BSA) in the presence or absence of the test drugs previously dissolved in DMSO. The mixture was incubated at 37 °C for 30 min and then the reaction was terminated by adding 2 μL of 10% SDS solution. After digestion of the enzyme by adding 2 μL of 0.6 μg/mL of proteinase K and incubating at 37 °C for 30 min, excess compounds were removed by extraction with $CHCl_3$/isoamyl alcohol (24:1). The reaction product was subjected to 1% agarose gel electrophoresis and the gel was stained with 0.5 μg/mL ethidium bromide (EtBr).

Antiproliferative activity against 39 human cancer cell lines (JFCR39). This experiment was carried out at the Screening Committee of Anticancer Drugs according to the standard protocol used by the Committee. Inhibition of cell growth was assessed by measuring the changes in the total cellular protein levels following 48 h treatment with a given test compound, using the sulforhodamine B colorimetric assay. The molar concentration of a test compound required for 50% growth inhibition (GI_{50}) of cells was calculated as reported previously.[11]

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