Synthesis and Biological Activities of Topoisomerase I Inhibitors, 6-Arylmethylamino Analogues of Edotecarin

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The replacement of 1,3-dihydroxy-2-propylamino moiety at the N6-position of edotecarin (**1**) by arylmethylamino groups yielded a number of more potent topoisomerase I inhibitors with better cytotoxic (CTX) activities in vitro than edotecarin. Among them, the three most potent pyridylmethyl analogues, compounds **22g**, **22m**, and **23c**, showed better antitumor activities against MKN-45 human stomach cancer or MX-1 human breast cancer xenografted mice than those of edotecarin. Furthermore, compounds **22m** and **23c** exhibited complete response against MX-1 cells implanted in mice.

Introduction

Topoisomerase I inhibitors have a wide range of antitumor activities and are among the most widely used anticancer drugs clinically.[1−4] Camptothecin was the first specific topoisomerase I inhibitor to be used in clinical applications.[4−8] Camptothecin analogues such as irinotecan,[1−4,9−17] topotecan,[1−4,16−20] and 9-aminocamptothecin (9-AC[a]),[9,17,21−23] which have superior antitumor activities with acceptable toxicities, have shown significant clinical activity. Their success has stimulated intensive efforts in the improvement of the limitation of these camptothecines, including chemical modifications to stabilize the chemically unstable E-ring lactone moieties at phyiological condition and/or to provide persistent accumulation in target cells. The homocamptothecines,[24−36] which were obtained by expanding the E-ring from a six-membered α-hydroxylactone ring to a seven-membered *β*-hydroxylactone ring, showed enhanced plasma stability with increased topoisomerase I inhibitory activity compared to camptothecines. Diflomotecan[28−32] is the most advanced analogue of this series and now in phase II clinical trial. On the other hand, introduction of lipophilic substituents to the 7-position of camptothecine achieved the rapid uptake, enhanced intracellular accumulation and prolongation/stabilization of the topoisomerase I−DNA−drug ternary complex (cleavable complex) compared with conventional camptothecines.[37−41] Gimatecan,[2−4,37−41] an orally active 7-substituted camptothecin analogue with promising antitumor effect in vivo, has undergone phase II clinical study in advanced epithelial ovarian, fallopian tube, and peritoneal cancers, advanced breast cancer, malignant glioma,and metastatic colorectal cancer.[2−4]

Because of the recognition of topoisomerase I inhibitors as a valuable target for the efficient antitumor agents, extensive effort for the development of structurally diverse topoisomerase I inhbitors has also been made. Among them, indenoisoquinolines[42−49] are chemically stable and form much more stable cleavable complexes than those formed by camptothecines. NSC-725776, the most advanced analogue of this series, has been selected for clinical development.

Edotecarin (**1**) is a potent and specific indolocarbazole topoisomerase I inhibitor[2−4,42,50−52] developed by the chemical derivatization[53,54] of NB-506 (**2**).[55−57] Like other topoisomerase I inhibitors, the primary mechanism of action of edotecarin is to bind to the topoisomerase I−DNA complex,[58] resulting in prevention of religation of single-strand breaks, which ultimately leads to apoptosis and cell death. The cleavable complex formed by edotecarin is more stable than that formed by camptothecin.[58] As a result, edotecarin showed 8-fold more potent inhibition of topoisomerase I mediated DNA cleavage than camptothecin.[59] In preclinical studies, edotecarin exhibited a different and improved therapeutic index against various human cancer cell lines in vitro compared with camptothecin, adriamycin, etoposide, and cisplatin.[58] Several in vivo studies demonstrated that edotecarin is a potential new antineoplastic agent. Edotecarin, when used as a single agent, produced significant antitumor activities in xenografted nude mice of several different human tumors such as LX-1 lung, PC-3 prostate, and central nervous system tumors.[60] In addition to monotherapy, recent in vivo studies suggested that edotecarin may be useful in combination with established treatment regimens for treating SK-BR-3 human breast[61] and HCT-116 human colon[62] cancers. A phase I study, which was designed to assess the maximum tolerated dose (MTD) and pharmacokinetic (PK) profile, showed a recommended dose of edotecarin for phase II trials with an attractive PK profile.[63,64] The toxicities in this phase I study were similar to those typical of cytotoxic chemotherapy but less severe than those associated with other topoisomerase I inhibitors. In addition, in the first phase I study in the U.S., a confirmed partial response was noted in one patient with bladder cancer refractory to both paclitaxel/gemcitabine and second-line MVAC (methotrexate, vinblastine, doxolubicin, and cisplatin) therapy and 12 other patients showed stabilization of the disease.[63] In a second phase I study in Japan, unconfirmed partial responses were also observed in two patients, one with metastatic gastric carcinoma and one with esophageal cancer.[64] These studies demonstrated that edotecarin is an attractive new anticancer drug. Edotecarin has undergone phase II studies in irinotecan-refractory colorectal cancer and breast cancer and phase III study in glioblastoma multiforne.

Recently, we found that 6-arylmethylamino analogues of edotecarin showed superior in vitro CTX activities against

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[a] Abbreviations: CTX, cytotoxicity; 9-AC, 9-aminocamptothecin; MTD, maximum tolerated dose; PK, pharmacokinetic; MVAC, methotrexate, vinblastine, doxolubicin, and cisplatin; TBDMS, *tert*-butyldimethylsilyl; LAH, lithium aluminum hydride; TFA, trifluoroacetic acid; $BocNHNH_2$, *tert*-butyl carbazate; DIBAL, diisobutyl aluminum hydride; Boc, *tert*-butoxycarbonyl; GID_{75}, 75% growth inhibition dose; LD_{10}, 10% lethal dose.

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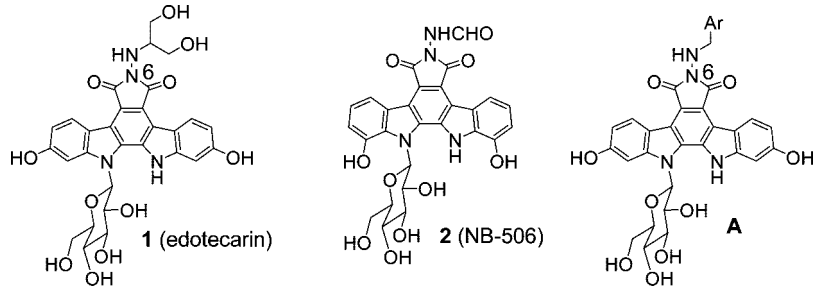
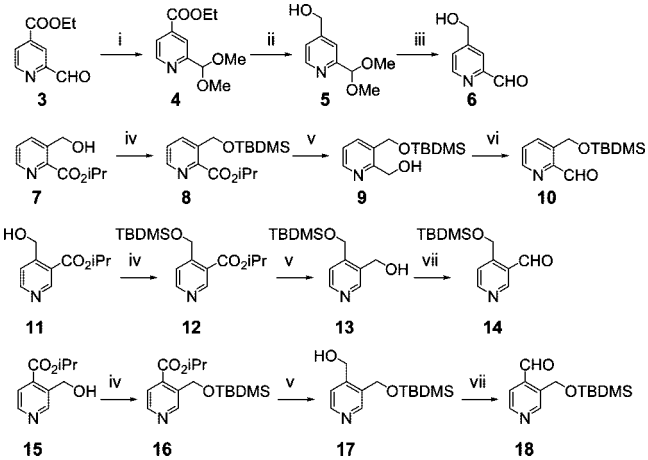


Figure 1

Scheme 1[a]



[a] Reagents and conditions: (i) $(MeO)_3CH$, MeOH, reflux; (ii) LAH, THF, rt; (iii) TFA, 70 °C; (iv) TBDMSCl, imidazole, DMF, rt; (v) $NaBH_4$, $CaCl_2$, EtOH, 0 °C; (vi) MnO_2, CH_2Cl_2, rt; (vii) Dess−Martin periodinate, CH_2Cl_2, rt.

various cell lines and antitumor activities against several human cancer cells implanted in mice. In this paper, we wish to report the synthesis and biological activities of 6-arylmethylamino analogues **A** of edotecarin and discuss the in vivo anticancer effects of several potent analogues (Figure 1).

Chemistry

Three types of methods to synthesize a series of 6-arylmethylamino analogues **A** of edotecarin were employed as described in Schemes 2, 3, and 5, depending on the available starting materials: (i) reaction of 6-amino intermediate **19** with arylaldehydes, followed by reduction of the resultant hydrazones (Scheme 2), (ii) reaction of 6-amino intermediates **19** or **24** with arylmethylbromide (Scheme 3), (iii) Reaction of acid anhydride intermediate **51** with arylmethylhydrazines (Scheme 5).

Pyridine carboaldehydes having *tert*-butyldimethylsilyl (TBDMS) protected (**10**, **14**, and **18**) or unprotected (**6**) hydroxymethyl groups, which were well-designed starting materials used in Scheme 2, were prepared as summarized in Scheme 1. Compound **6** was synthesized starting from ethyl 2-formylpyridine-4-carboxylate **3**.[65] Acetalization with trimethyl-orthoformate followed by reduction with lithium aluminum hydride (LAH) gave acetal **5**, which was converted to compound **6** by hydrolysis with trifluoroacetic acid (TFA). Compounds **10**, **14**, and **18** were prepared in a similar manner from compounds **7**, **11**, and **15**, respectively. After protection of the hydroxymethyl group in compounds **7**, **11**, and **15** with TBDMS groups, reduction of the isopropyl ester group was conducted with $NaBH_4-CaCl_2$ to give compounds **9**, **13**, and **17**. Oxidation of the newly formed hydroxymethyl group with MnO_2 or Dess−Martin periodinate led to compounds **10**, **14**, and **18**.

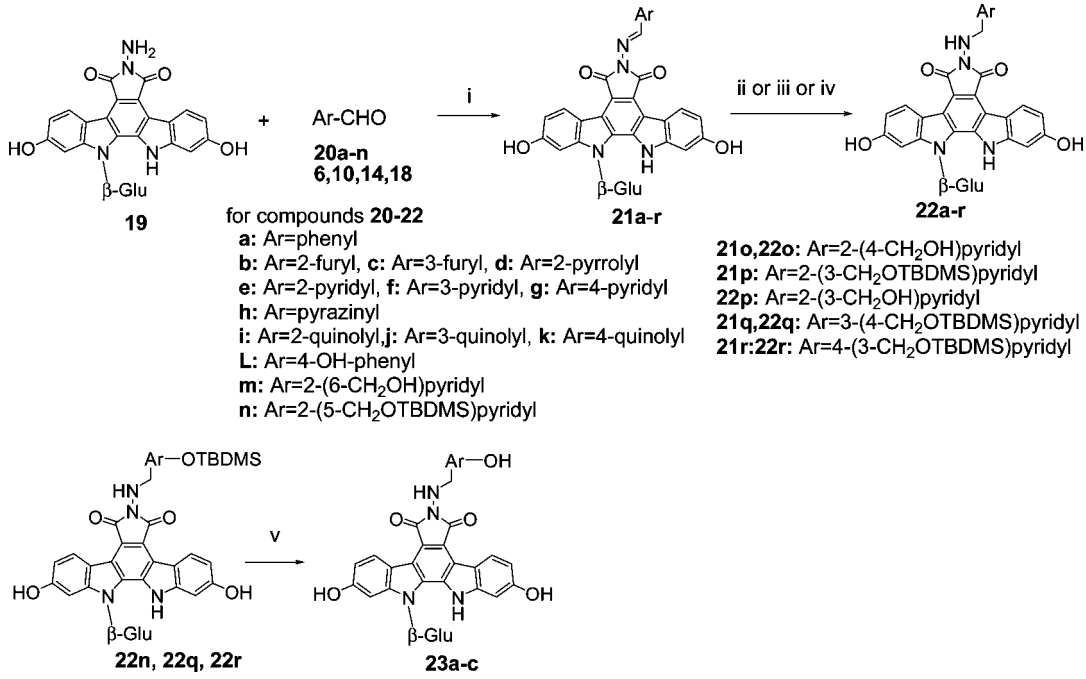
Scheme 2 illustrates the synthesis of the final compounds from 6-amino intermediate **19** and arylaldehydes. Arylaldehydes (**20a**−**k**, **6**, **10**, **14**, and **18**) were reacted with compound **19** in the presence of acetic acid in refluxing MeOH to provide arylmethylhydrazones **21a**−**r**. Three types of reaction conditions were used to reduce the double bond of the hydrazone moieties in **21a**−**r** (conditions ii−iv), depending on the stability of the products (**22a**−**r**) under each reaction condition. In most cases, reduction with $NaBH_3CN$ in the presence of HCl (condition ii) gave the final compounds in the best yields. However, in some cases, because the newly formed benzyl C−N bond was cleaved by the strong acidic media under condition ii, some (un)substituted pyridylmethyl and quinolylmethyl derivatives were prepared by reduction with $NaBH_3CN$-$ZnCl_2$ (condition iii) or catalytic hydrogenation (condition iv).

Compounds **26a**−**e** were readily prepared from 6-amino intermediates **19** or **24** by *N*-alkylation with arylmethylbromides **25a**−**e** in DMF as shown in Scheme 3.

Reaction of arylmethylhydrazines (Ar-CH_2NHNH_2) and acid anhydride intermediate **51** was also an efficient alternative method to provide the final compounds As shown in Scheme 4, arylmethylhydrazines (**32**, **38**, **44**, and **50**) were prepared by reductive amination using *tert*-butyl carbazate ($BocNHNH_2$) and the corresponding aldehydes as a key step. Aldehyde **36** was prepared from commercially available pyridine 3,5-dicarboxylic acid **33**. The two carboxylic acid groups in compound **33** were reduced to hydroxymethyl groups with BH_3-THF complex to give compound **34**. TBDMS protection of one hydroxymethyl group of compound **34** using 1 equiv of TBDMSCl followed by oxidation of the remaining hydroxymethyl group afforded compound **36**. Aldehyde **42** was obtained in a similar manner as the synthesis of compound **6** in Scheme 1 from compound **39**.[66] Aldehyde **47** was synthesized by protection with TBDMSCl followed by diisobutyl aluminum hydride (DIBAL) reduction of the ethyl ester group of compound **45**.[67] Arylaldehydes (**27**, **36**, **42**, and **47**) were reacted with $BocNHNH_2$ in EtOH to give hydrazones (**28**, **37**, **43**, and **48**). Reduction of the double bond of the hydrazone moiety in phenyl derivative **28** was conducted with $NaBH_3CN$ in acidic media to give compound **29**. Transformation of the cyano group to ahydroxymethyl group by consecutive treatment with DIBAL and $NaBH_4$, followed by removal of the *tert*-butoxycarbonyl (Boc) group with TFA, gave the required hydrazine **32**. Double bonds of the hydrazone moieties in pyridyl derivatives **37**, **43**, and **48** were reduced with BH_3-THF complex in THF followed by acid treatment to generate compounds **38**, **44**, and **49**. The Boc group of compound **49** was removed by TFA treatment to yield compound **50**.

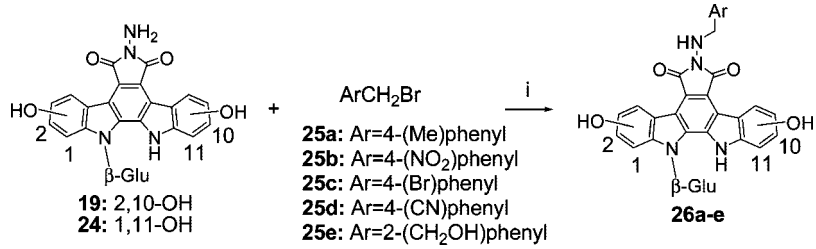
Arylmethylhydrazines (**32**, **38**, **44**, **50**, and **52a**−**b**) were reacted with acid anhydride intermediate **51** in the presence of

Scheme 2[a]



[a] Reagents and conditions: (i) AcOH, MeOH, reflux; (ii) $NaBH_3CN$, 10% HCl/MeOH, rt; (iii) $NaBH_3CN$, $ZnCl_2$, THF, rt; (iv) H_2/5% Pd−C, MeOH/THF, rt; (v) TBAF, THF, rt.

Scheme 3[a]



[a] Reagents and conditions: (i) DMF, rt.

saturated aqueous $NaHCO_3$ solution in DMF to provide final compounds (**53**−**58**) as shown in Scheme 5.

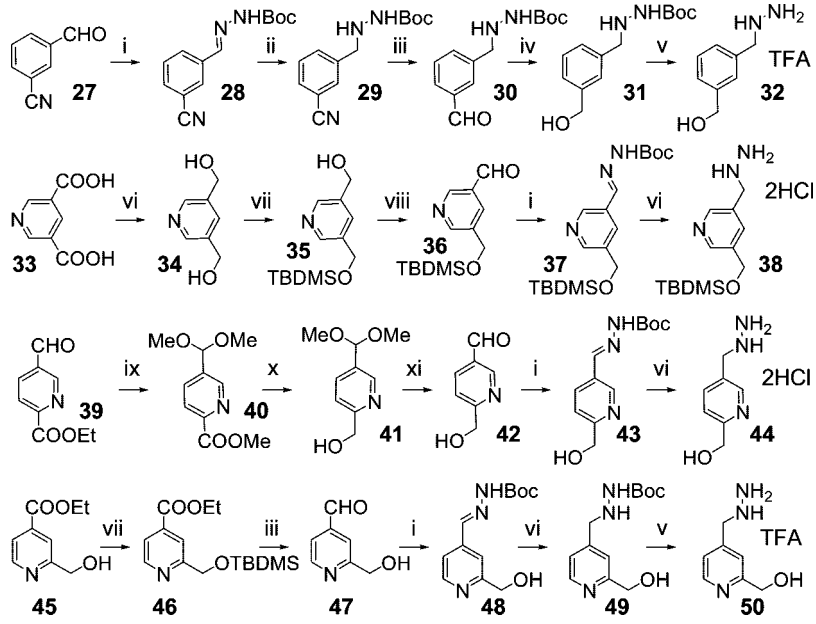
Results and Discussion

At first, some unsubstituted aromatic rings were explored to find potent aromatic moieties for the N6-position. The results are shown in Table 1. Introduction of a phenyl group (**22a**) slightly increased the topoisomerase I inhibition both in enzyme and cells compared to edotecarin (**1**). Compound **22a** also showed comparable CTXs in P388, MKN-45, DLD-1, and HeLa cells to those of edotecarin. Encouraged by this result, some heteroaryl rings were investigated (compounds **22b**−**k**). Most of the heteroaryl rings showed better in vitro potencies than edotecarin. Five-membered heteroaryl groups such as 2-furanyl (**22b**), 3-furanyl (**22c**), and 2-pyrrolyl (**22d**) rings conferred 2- to 5-fold stronger topoisomerase I inhibitory activities with almost equipotent CTX activities compared to edotecarin. On the other hand, introduction of nitrogen-containing six-membered ring such as pyridine and pyrazine rings resulted in substantial expansion of CTX windows compared to edotecarin. In particular, pyridyl derivatives **22e**−**g** dramatically improved CTX in DLD-1 and HeLa cells, which were resistant to edotecarin. Topoisomerase I inhibitory activity and CTX activity of fused heteroaryl derivatives **22i**−**k** were similar to those of edotecarin.

Inhibitory activities of topoisomerase I in P388/S cells of compounds **22a**−**22k** (K^+/SDS assays in P388/S cells) were almost correlated with the CTX activities against P388/S cells and comparable to that of edotecarin, suggesting that the CTX activities of these analogues were observed mainly due to the topoisomerase I inhibition. However, some data (compounds **22d** and **22f**) were deviated from the correlation. It may be due to solid deposition of the compounds in the assay conditions and enough concentration of the compounds to show activities against the K^+/SDS assay were not achieved within the short incubation time (1 h). On the other hand, long incubation time (72 h) for CTX assay enables the precipitation to dissolve in the assay medium to exhibit strong CTX activities.

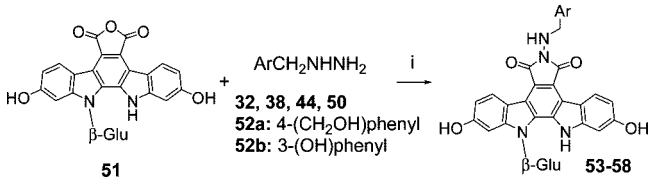
Once we identified the potent aromatic rings, we then focused on the substituents on the rings. In this study, for quick exploration, the phenyl ring was chosen because of its synthetic feasibility. The results are shown in Table 2. It was clear that substituents containing hydroxyl groups played an important role in enhancing activities not only for topoisomerase I but also CTXs. As demonstrated by compounds **22l**, **53**−**55**, and **26e**, introduction of a hydroxyl group or a hydroxymethyl group on any position of the phenyl ring resulted in not only maintenace of the significant CTX activity of unsubstituted derivative **22a** against P388/S and MKN-45 cells but also great improvement of those against DLD-1 and HeLa cells, which

Scheme 4[a]



[a] Reagents and conditions: (i) $BocNHNH_2$, EtOH, 60 °C; (ii) $NaBH_3CN$, 4 N HCl/MeOH, rt; (iii) DIBAL; (iv) $NaBH_4$, MeOH, rt; (v) TFA, CH_2Cl_2, rt; (vi) BH_3-THF complex, THF, 0 °C then 6 N HCl, rt; (vii) TBDMSCl, imidazole, DMF, rt; (viii) MnO_2, $CHCl_3$, rt; (ix) $(MeO)_3CH$, MeOH, reflux; (x) LAH, THF, rt; (xi) TFA, rt.

Scheme 5[a]



[a] Reagents and conditions: (i) sat. $NaHCO_3$, DMF, 80 °C.

were resistant to compound **22a**. Conversely, other substituents such as methyl (**26a**), nitro (**26b**), bromo (**26c**), and cyano (**26d**) groups on the 4-position of the phenyl ring caused significant reduction of not only topoisomerase I inhibitory activity but also CTX activity.

Because potent aromatic rings (Table 1) and substituents on the ring (Table 2) were identified individually, we then synthesized and evaluated (hydroxymethyl)pyridyl derivatives, which were compounds that combined the best results in Tables 1 and 2. The results are summarized in Table 3 As expected, most compounds showed potent activities for both topoisomerase I and CTX and also the reasonable topoisomerase I inhibitions in cells except for compound **23b**. In particular, compound **23c**, a 4-(2-hydroxymethyl)pyridyl derivative, demonstrated the most potent CTX activity in all four cancer cell lines. When compared to edotecarin, compound **23c** exhibited not only 10-fold stronger CTX activities in P388 and MKN-45 cells but also 77-fold and 210-fold higher activities in DLD-1 and HeLa cells, respectively.

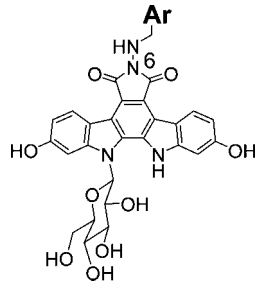
Docking study of compound **23c** with human topoisomerase I−DNA covalent complex was performed[68] to provide the rational molecular basis for enhancement of the topoisomerase I inhibitory activity achieved by introduction of (hydroxylmethyl)pyridyl moiety on N6-position. Among the all possible binding mode generated by the docking study, the most similar one to the experimentally observed binding mode, which was shown by Stacker et al.,[69] was selected. Because of the N6-substituent, a translational movement of molecule was needed compared to the binding mode of N6-unsubstituted indolocarbazole. The structural model suggested that one of two carbonyl oxygens of maleimide ring, hydroxyl group of β-glucose, and phenol group on the glycosylated indole ring, interacted with enzyme (Figure 2a). The pyridine ring on N6-position might occupy space that was created by topoisomerase I and DNA. The hydroxymethyl moiety might interact with Asp533 (Figure 2b). These newly formed interactions would greatly contribute to the significant topoisomerase I inhibitory activity of compound **23c**.

Some analogues that showed potent inhibition of topoisomerase I and CTX activities in vitro were tested for anticancer effects in mice.

Table 4 shows the results of the in vivo anticancer effects of these three compounds. Compound **22m** exhibited an anticancer effect (GID_{75}) comparable to edotecarin (**1**) against MKN-45 xenografted mice when administered by the same schedule as that of edotecarin (5 times/week). On the other hand, compounds **22g** and **23c** showed better antitumor effects than edotecarin, although the administration number was reduced to twice/week. All three compounds also showed much stronger antitumor activity against MX-1 xenografted mice than edotecarin in spite of the smaller administration number than edotecarin. Although these compounds showed higher toxicity than edotecarin in some cases, their safety margins were comparable to that of edotecarin due to their potent antitumor activities, except for the case of compound **22m** against MKN-45 xenografted mice (safety margin: >1.2-fold). In addition, these three compounds showed good selectivities over protein kinase C (PKC) and EGF receptor kinase (EGFRK) comparable to edotecarin.

Parts a and b of Figure 3 show the tumor growth and inhibition of compounds **22g**, **22m**, and **23c** against MKN-45 and MX-1 xenografted nude mice, respectively. As summarized in Figure 3a, tumor regression was observed by administration of compounds **22g**, **22m**, and **23c** against MKN-45 xenografted nude mice. Furthermore, Figure 3b shows that treatments of MX-1 xenografted nude mice by compounds **22m** and **23c**

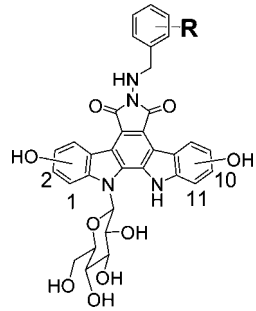
Table 1. In Vitro Activities of Unsubstituted Aryl Derivatives



	Ar	Topo I[a] cleavege EC_{50} (nM)	K^+/SDS[b] (P388/S) EC_{200} (nM)	CTX[c] P388/S IC_{50} (nM)	CTX[d] MKN-45 IC_{50} (nM)	CTX[e] DLD-1 IC_{50} (nM)	CTX[f] HeLa IC_{50} (nM)
22a	phenyl	25	80	1.8	3.4	100	1300
22b	2-furanyl	38	180	1.5	5.0	130	3200
22c	3-furanyl	30	470	1.3	2.8	130	1400
22d	2-pyrrolyl	30	6500	0.77	20	67	1100
22e	2-pyridyl	30	800	0.59	1.4	18	140
22f	3-pyridyl	23	>10000	0.48	0.95	9.3	57
22g	4-pyridyl	22	420	0.48	0.71	3.3	24
22h	pyrazinyl	35	210	0.60	2.8	36	300
22i	2-quinolyl	17	1800	4.8	14	60	110
22j	3-quinolyl	30	>10000	5.7	23	290	77
22k	4-quinolyl	20	10000	1.8	1.4	83	120
1 (edotecarin)	51	100	1.5	4.8	70	840	

[a] Topoisomerase-mediated DNA cleavage assays were carried out using supercoiled pBR322 plasmid DNA.[56] [b] Effects on the formation of protein−DNA complex in P388 cells were investigated by the K^+/SDS method.[56] [c] CTX against murine leukemia cells (P388) was measured by the colormetric tetrazolium−formazan method.[56] [d] CTX against human stomach cancer cells (MKN-45) was measured by the colormetric tetrazolium−formazan method and the sulforhodamine B dye-staining method.[56] [e] CTX against human colon cancer cells (DLD-1). [f] CTX against human uterine−cervix cells (HeLa).

Table 2. In Vitro Activities of Substituents on Phenyl Ring



	R[a]	Topo I cleavegeEC$_{50}$ (nM)	K^+/SDS (P388/S) EC_{200} (nM)	CTX P388/S IC_{50} (nM)	CTX MKN-45 IC_{50} (nM)	CTX DLD-1 IC_{50} (nM)	CTX HeLa IC_{50} (nM)
22a	H (2,10)	25	80	1.8	3.4	100	1300
26a	4-Me (1,11)	800	>10000	58	390	2300	4200
26b	4-NO2 (1,11)	>3000	6000	21	210	2100	2700
26c	4-Br (1,11)	NT[b]	10000	190	1800	13000	>50000
26d	4-CN (1,11)	300	1700	14	85	940	650
22L	4-OH (2,10)	12	120	1.1	3.0	18	21
53	4-CH_2OH (2,10)	34	600	0.55	2.7	21	21
54	3-OH (2,10)	20	23	0.90	4.5	21	46
55	3-CH_2OH (2,10)	22	220	1.2	11	36	21
26e	2-CH_2OH (2,10)	16	24	1.3	0.86	2.6	40

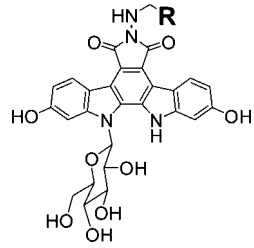
[a] The positions of the two phenol groups are shown in parentheses. [b] NT: not tested.

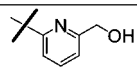
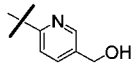
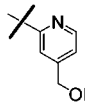
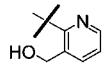
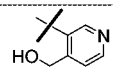
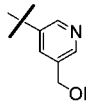
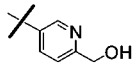
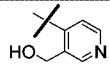
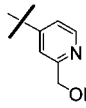
resulted in complete responses at doses of 15 and 3 mg/m^3, respectively.

Conclusion

A number of 6-arylmethylamino derivatives of edotecarin (**1**) were identified as potent topoisomerase I inhibitors, with better CTX activities in vitro than edotecarin. Among them, the three most potent pyridylmethyl analogues, compounds **22**g, **22m**, and **23c**, showed better antitumor activities against MKN-45 human stomach cancer or MX-1 human breast cancer xenografted mice than edotecarin. Furthermore, compounds **22m** and **23c** exhibited complete responses against MX-1 cells

Table 3. In Vitro Activities of (Hydroxymethyl)pyridyl Derivatives



	R	Topo I cleavege EC_{50} (nM)	K^+/SDS (P388/S) EC200 (nM)	CTX P388/S IC_{50} (nM)	CTX MKN-45 IC_{50} (nM)	CTX DLD-1 IC_{50} (nM)	CTX HeLa IC_{50} (nM)
22m		3.3	73	0.50	2.1	4.3	11
23a		27	120	1.10	6.0	9.9	13
22o		22	160	0.46	1.6	9.4	34
22p		19	400	0.40	0.70	2.6	36
23b		25	>10,000	0.33	0.62	3.9	8.9
56		35	90	0.98	2.6	6.2	32
57		30	130	0.49	1.6	25	11
23c		29	130	0.28	0.35	0.91	4.0
58		31	100	0.49	0.80	6.8	39

implanted in mice. The strategy to introduce arylmethyl groups at the 6-position successfully achieved both more potent in vitro activity and in vivo efficacy than edotecarin, and these compounds may be important for future cancer therapy.

Experimental Section

^{1}H NMR spectra were recorded on Varian VXR-300 or Varian MERCURY 400 spectrometers with tetramethylsilane (TMS) as an internal standard. Specific rotation was measured with a Jasco DIP-370 polarimeter. Mass spectra (MS) were measured on a JEOL JMS-SX102A (FAB) or Waters ZQ2000 (ESI) with binary HPLC pump Waters 1525, or micromass Quattoro II with HP 1100 series HPLC system. HRMS were measured on a LC (Waters CapLC)−MS (micromass Q-Tof-2) system (ESI). Analytical HPLC of the products was recorded on the Waters LC-MS 2690 separations module system (MS; micromass ZMD (ESI)) using a Waters ACQUITY UPLC HSS T3 (S-1.8 μm) 2.1 mm × 100 mm column (gradient as follows: 9−95% acetonitrile (0.1% formic acid)/water (0.1% formic acid), 0.4 mL/min for 20 min). All tested compounds possess a purity of at least 95%. Especially, the purities of compounds **22g**, **22m**, and **23c**, which were tested in vivo antitumor effect, were >99%.

Representative Procedure for the Reduction of Hydrazone. Procedure 1. 6-(Benzylamino)-2,10-dihydroxy-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22a). First, 10% HCl/MeOH (3 mL) was added dropwise to the mixture of compound **21a** (25 mg, 0.04 mmol) and sodium cyanoborohydride (7.6 mg, 0.12 mmol) in MeOH/THF (1:2, 4.5 mL), and after addition, the mixture was stirred at room temperature for 1 h. The resulting reaction mixture was concentrated under reduced pressure, and the residue was purified by LH-20 eluting with MeOH to give compound **22a** (18.4 mg, 74%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.18 (1H, s), 9.76 (1H, s), 9.72 (1H, s), 8.86 (1H, d, J = 8.4 Hz), 8.78 (1H, d, J = 8.4 Hz), 7.49 (2H, d, J = 7.2 Hz), 7.30 (2H, t, J = 6.9 Hz), 7.23 (1H, td, J = 5.1, 2.1 Hz), 7.17 (1H, d, J = 2.1 Hz), 6.97 (1H, d, J = 2.4 Hz), 6.82 (2H, td, J = 8.4, 2.4 Hz), 6.07 (1H, t, J = 4.8 Hz), 5.96 (1H, d, J = 8.1 Hz), 5.84 (1H, t, J = 3.3 Hz), 5.30 (1H, d, J = 5.1 Hz), 5.09 (1H, d, J

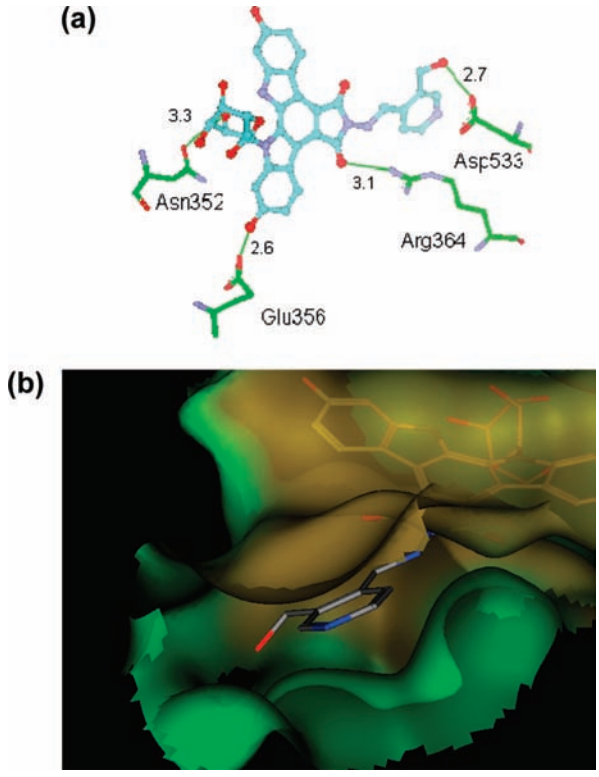


Figure 2. (a) Predicted binding mode of compound **23c** (blue) in the topoisomerase I−DNA covalent complex. Side chains of amino acids which could interact with compound are diagrammed, and important hydrogen bonds are indicated with green lines. (b) Surface of topoisomerase I (green)−DNA (gold) covalent complex, showing pyridine ring on N6-position of compound **23c** occupied the space created by topoisomerase I and DNA.

= 3.3 Hz), 4.90 (1H, d, *J* = 3.3 Hz), 4.26 (2H, d, *J* = 4.8 Hz), 4.02 (1H, dd, *J* = 11.7, 4.2 Hz), 3.96−3.86 (2H, m), 3.78 (1H, dd, *J* = 8.1, 4.2 Hz), 3.53−3.46 (2H, m).

Representative Procedure for the Reduction of Hydrazone. Procedure 2. 2,10-Dihydroxy-6-[(pyridin-2-ylmethyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22e). The mixture of compound **21e** (20 mg, 0.032 mmol), sodium cyanoborohydride (80 mg, 1.3 mmol), and zinc chloride (1.0 M in diethyl ether, 0.64 mL) in THF (5 mL) was stirred at room temperature for 18 h. The resulting reaction mixture was concentrated under reduced pressure, and the residue was purified by LH-20 eluting with MeOH to give compound **22e** (10.5 mg, 53%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.19 (1H, s), 9.78 (2H, brs), 8.85 (1H, d, *J* = 8.5 Hz), 8.76 (1H, d, *J* = 8.5 Hz), 8.41 (1H, d, *J* = 4.4 Hz), 7.82−7.75 (2H, m), 7.26−7.20 (2H, m), 7.17 (1H, d, *J* = 2.1 Hz), 6.98 (1H, d, *J* = 2.1 Hz), 6.80 (2H, td, *J* = 8.6, 2.1 Hz), 6.25 (1H, t, *J* = 4.5 Hz), 5.96 (1H, d, *J* = 8.1 Hz), 5.86 (1H, s), 5.38 (1H, brs), 5.15 (1H, brs), 4.91 (1H, brs), 4.36 (2H, d, *J* = 4.8 Hz), 4.00 (1H, d, *J* = 10.5 Hz), 3.95−3.84 (2H, m), 3.78 (1H, d, *J* = 10.5 Hz), 3.55−3.46 (2H, m).

Representative Procedure for the Reduction of Hydrazone. Procedure 3. 2,10-Dihydroxy-6-[(pyridin-3-ylmethyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22f). The mixture of compound **21f** (25 mg, 0.040 mmol) and 5% palladium on carbon (10 mg) in MeOH/THF (1:1, 5 mL) was equipped with a balloon of hydrogen gas and stirred at room temperature. After the reaction was over, the mixture was filtered through a pack of celite. The filtrate was concentrated under reduced pressure, and the residue was purified by LH-20 eluting with MeOH to give compound **22f** (3.5 mg, 14%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.16 (1H, s), 9.85 (2H, brs), 8.83 (1H, d, *J* = 8.5 Hz), 8.75 (1H, d, *J* = 8.5 Hz), 8.62 (1H, d, *J* = 2.4 Hz), 8.41 (1H, dd, *J* = 5.1, 1.8 Hz), 7.92 (1H, td, *J* = 8.1, 2.4 Hz), 7.33 (1H, dd, *J* = 8.1, 5.1 Hz), 7.15 (1H, d, *J* = 1.5 Hz), 6.96 (1H, d, *J* = 1.5 Hz), 6.80 (2H, td, *J* = 8.6, 1.5 Hz), 6.26 (1H, t, *J* = 4.2 Hz), 5.95 (1H, d, *J* = 8.1 Hz), 5.88 (1H, brs), 5.38 (1H, brs), 5.16 (1H, brs), 4.92 (1H, brs), 4.29 (1H, d, *J* = 4.5 Hz), 4.02 (1H, d, *J* = 10.8 Hz), 3.97−3.85 (2H, m), 3.76 (1H, d, *J* = 10.8 Hz), 3.52−3.44 (2H, m).

6-[(2-Furylmethyl)amino]-2,10-dihydroxy-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22b). Reduction of compound **21b** using procedure 1 gave compound **22b** (53%). ^{1}H NMR (300 MHz, DMSO-d_6) δ11.10 (1H, brs), 10.50−9.20 (2H, brs), 8.82 (1H, d, *J* = 8.5 Hz), 8.73 (1H, d, *J* = 8.5 Hz), 7.55 (1H, s), 7.13 (1H, s), 6.95 (1H, d, *J* = 2.0 Hz), 6.78−6.74 (3H, m), 6.35−6.31 (2H, m), 6.05 (1H, t, *J* = 5.1 Hz), 5.94 (1H, d, *J* = 8.3 Hz), 5.82 (1H, brs), 5.50−4.83 (3H, brs), 4.00 (2H, d, *J* = 10.0 Hz), 3.95−3.85 (2H, m), 3.76 (1H, d, *J* = 10.0 Hz), 3.50 (2H, d, *J* = 9.2 Hz).

6-[(3-Furylmethyl)amino]-2,10-dihydroxy-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22c). Reduction of compound **21c** using procedure 1 gave compound **22c** (77%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.18 (1H, s), 10.89 (2H, brs), 8.85 (1H, d, *J* = 8.5 Hz), 8.77 (1H, d, *J* = 8.5 Hz), 7.62 (1H, s), 7.56 (1H, t, *J* = 1.0 Hz), 7.16 (1H, s), 6.97 (1H, d, *J* = 1.9 Hz), 6.80 (2H, td, *J* = 8.9, 1.9 Hz), 6.57 (1H, s), 5.99−5.94 (2H, m), 5.86 (1H, brs), 5.34 (1H, brs), 5.12 (1H, brs), 4.91 (1H, brs), 4.11 (2H, d, *J* = 4.6 Hz), 4.01 (1H, d, *J* = 10.2 Hz), 3.95−3.87 (2H, m), 3.78 (1H, d, *J* = 10.2 Hz), 3.50 (2H, brs).

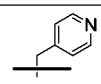
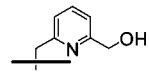
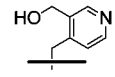
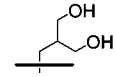
2,10-Dihydroxy-6-[(1*H*-pyrrol-2-ylmethyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22d). Reduction of compound **21d** using procedure 1 gave compound **22d** (43%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.17 (1H, s), 10.73 (1H, s), 9.79 (2H, brs), 8.85 (1H, d, *J* = 8.5 Hz), 8.77 (1H, d, *J* = 8.5 Hz), 7.16 (1H, s), 6.97 (1H, d, *J* = 1.9 Hz), 6.80 (2H, td, *J* = 8.9, 1.9 Hz), 6.64 (1H, s), 5.97−5.80 (4H, m), 5.38 (1H, brs), 5.15 (1H, brs), 4.91 (1H, brs), 4.17 (2H, d, *J* = 5.1 Hz), 4.12−3.75 (5H, m), 3.52−3.49 (2H, m).

2,10-Dihydroxy-6-[(pyridin-4-ylmethyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22g). Reduction of compound **21g** using procedure 3 gave compound **22g** (14%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.16 (1H, s), 9.85 (2H, brs), 8.83 (1H, d, *J* = 8.5 Hz), 8.75 (1H, d, *J* = 8.5 Hz), 8.626 (1H, d, *J* = 2.4 Hz),8.41 (1H, dd, *J* = 5.1, 1.8 Hz), 7.92 (1H, td, *J* = 8.1, 2.4 Hz), 7.33 (1H, dd, *J* = 8.1, 5.1 Hz), 7.15 (1H, d, *J* = 1.5 Hz), 6.96 (1H, d, *J* = 1.5 Hz), 6.80 (2H, td, *J* = 8.6, 1.5 Hz), 6.26 (1H, t, *J* = 4.2 Hz), 5.95 (1H, d, *J* = 8.1 Hz), 5.88 (1H, brs), 5.38 (1H, brs), 5.16 (1H, brs), 4.92 (1H, brs), 4.29 (2H, d, *J* = 4.5 Hz), 4.02 (1H, d, *J* = 10.8 Hz), 3.97−3.85 (2H, m), 3.76 (1H, d, *J* = 10.8 Hz), 3.52−3.44 (2H, m); $[\alpha]^{26}_D$ 163.9 (*c* 1.02, DMSO). Anal. ($C_{32}H_{27}N_5O_9 \cdot H_2O$) C, H, N.

2,10-Dihydroxy-6-[(pyrazin-2-ylmethyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22h). Reduction of compound **21h** using procedure 3 gave compound **22h** (35%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.16 (1H, s), 9.85 (2H, brs), 8.83 (1H, d, *J* = 8.5 Hz), 8.75 (1H, d, *J* = 8.5 Hz), 8.62−8.58 (2H, m), 8.41 (1H, d, *J* = 5.1 Hz), 7.15 (1H, d, *J* = 1.5 Hz), 6.96 (1H, d, *J* = 1.5 Hz), 6.80 (2H, td, *J* = 8.6, 1.5 Hz), 6.26 (1H, t, *J* = 4.2 Hz), 5.95 (1H, d, *J* = 8.1 Hz), 5.88 (1H, brs), 5.38 (1H, brs), 5.16 (1H, brs), 4.92 (1H, brs), 4.29 (2H, d, *J* = 4.5 Hz), 4.02 (1H, d, *J* = 10.8 Hz), 3.97−3.85 (2H, m), 3.76 (1H, d, *J* = 10.8 Hz), 3.52−3.44 (2H, m).

2,10-Dihydroxy-6-[(quinolin-2-ylmethyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22i). Reduction of compound **21i** using procedure 2 gave compound **22i** (25%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.18 (1H, s), 9.78 (1H, s), 9.75 (1H, s), 8.83 (1H, d, *J* = 8.5 Hz), 8.75 (1H, d, *J* = 8.5 Hz), 8.39 (1H, d, *J* = 8.6 Hz), 7.99 (1H, d, *J* = 8.6 Hz), 7.93 (1H, d, *J* = 8.6 Hz), 7.87 (1H, d, *J* = 8.6 Hz), 7.65 (1H, t, *J* = 8.6 Hz), 7.52 (1H, t, *J* = 8.6 Hz), 7.17 (1H, d, *J* = 1.5 Hz), 6.96 (1H, d, *J* = 1.5 Hz), 6.80 (2H, td, *J* = 8.6, 1.5 Hz), 6.41 (1H, t, *J* = 4.8 Hz), 5.95 (1H, d, *J* = 8.7 Hz), 5.86 (1H, t, *J* = 3.9 Hz), 5.33 (1H, d, *J* = 3.9 Hz), 5.10 (1H, d, *J* = 3.9 Hz),

Table 4. Biological Profiles of Compounds **22g**, **22m**, **23c**, and Edotecarin

		22g	**22m**	**23c**	**1**
Head structure					
Topo I cleavege: EC_{50} (nM)		22	3.3	29	51
PKC: IC_{50} (nM)[a]		2,200	9,500	6,800	18,000
EGFRK:IC_{50} (nM)[b]		>200,000	>200,000	>200,000	>200,000
MKN-45	CTX: IC_{50} (nM)	0.71	2.1	0.35	4.8
	GID_{75}[c] (mg/m^2)	12[f]	<90[g]	<30[f]	45[g]
	LD_{10}[d] (mg/m^2)	705	110	>300	>1,600
	safety margin: LD_{10}/GID_{75}[e]	59	>1.2	>10	>36
MX-1	GID_{75}[c] (mg/m^2)	27[f]	<4.5[f]	<12[f]	160[g]
	LD_{10}[d] (mg/m^2)	>800	>60	>120	>2,000
	safety margin: LD_{10}/GID_{75}[e]	>30	>13	>10	>13

[a] The Histon II−As was used as a substrate for protein kinase C.[56] [b] Poly(Glu_4Tyr_1) was used as a substrate for EGF receptor kinase.[56] [c] GID_{75}: approximate 75% growth inhibition dose reflects the anticancer effect on MKN-45 or MX-1 (human breast cancer) cells implanted subcutaneously into a side flank of nude mice. [d] LD_{10}: approximate 10% lethal dose at the treatment schedule. [e] Safety margin: the ratio LD_{10}/GID_{75}. [f] Compounds were injected iv twice/week for two consecutive weeks, and treatment was initiated when tumors grew to 0.2 cm^3 or larger. These compounds showed wider safety margins by intermitted administration (twice/week) than by consecutive administration (five times/week). [g] Compounds were injected iv Five times/week for two consecutive weeks, and treatment was initiated when tumors grew to 0.2 cm^3 or larger.

4.88 (1H, d, J = 3.9 Hz), 4.56 (2H, d, J = 4.8 Hz), 4.02−3.98 (1H, m), 3.93−3.85 (2H, m), 3.80−3.72 (1H, m), 3.52−3.45 (2H, m).

2,10-Dihydroxy-6-[(quinolin-3-ylmethyl)amino]-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22j). Reduction of compound **21j** using procedure 2 gave compound **22j** (33%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.16 (1H, s), 9.75 (2H, brs), 9.08 (1H, d, J = 1.9 Hz), 8.82 (1H, d, J = 8.6 Hz), 8.74 (1H, d, J = 8.6 Hz), 8.37 (1H, d, J = 1.9 Hz), 7.97 (1H, d, J = 8.7 Hz), 7.91 (1H, d, J = 8.7 Hz), 7.68 (1H, t, J = 8.7 Hz), 7.54 (1H, t, J = 8.7 Hz), 7.15 (1H, d, J = 2.1 Hz), 6.96 (1H, d, J = 2.1 Hz), 6.80 (2H, td, J = 8.6, 2.1 Hz), 6.37 (1H, t, J = 4.2 Hz), 5.94 (1H, d, J = 8.1 Hz), 5.86 (1H, brs), 5.34 (1H, brs), 5.11 (1H, brs), 4.88 (1H, brs), 4.48 (2H, d, J = 4.2 Hz), 4.01 (1H, d, J = 10.3 Hz), 3.89 (2H, brs), 3.76 (1H, d, J = 10.3 Hz), 3.52−3.45 (2H, m).

2,10-Dihydroxy-6-[(quinolin-4-ylmethyl)amino]-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22k). Reduction of compound **21k** using procedure 3 gave compound **22k** (35%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.13 (1H, brs), 8.86 (1H, d, J = 4.2 Hz), 8.85 (1H, d, J = 8.7 Hz), 8.76 (1H, d, J = 8.7 Hz), 8.50 (1H, d, J = 8.1 Hz), 8.03 (1H, d, J = 8.1 Hz), 7.79 (1H, d, J = 4.2 Hz), 7.76 (1H, t, J = 8.1 Hz), 7.69 (1H, t, J = 8.1 Hz), 7.12 (1H, d, J = 2.1 Hz), 6.93 (1H, d, J = 2.1 Hz), 6.78 (2H, td, J = 8.6, 2.1 Hz), 6.33 (1H, t, J = 4.2 Hz), 5.92 (1H, d, J = 8.5 Hz), 5.40−4.95 (3H, brs), 4.76 (2H, d, J = 4.2 Hz), 4.02 (1H, d, J = 10.3 Hz), 3.95−3.86 (2H, m), 3.77 (1H, d, J = 10.3 Hz), 3.52−3.45 (2H, m).

2,10-Dihydroxy-6-[(4-hydroxybenzyl)amino]-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22l). Reduction of compound **21l** using procedure 1 gave compound **22l** (86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.17 (1H, s), 9.77 (1H, s), 9.74 (1H, s), 9.24 (1H, s), 8.86 (1H, d, J = 8.4 Hz), 8.78 (1H, d, J = 8.4 Hz), 7.25 (2H, d, J = 8.7 Hz), 7.17 (2H, d, J = 1.8 Hz), 6.97 (1H, d, J = 1.8 Hz), 6.83(1H, td, J = 8.7, 1.8 Hz), 6.67 (2H, d, J = 8.7 Hz), 5.96 (1H, d, J = 8.1 Hz), 5.87 (1H, t, J = 4.8 Hz), 5.85 (1H, t, J = 3.9 Hz), 5.32 (1H, d, J = 4.5 Hz), 5.10 (1H, d, J = 4.8 Hz), 4.91 (1H, d, J = 4.8 Hz), 4.12 (2H, d, J = 4.8 Hz), 4.07−3.95 (1H, m), 3.91 (2H, s), 3.82−3.75 (1H, m), 3.55−3.46 (2H, m).

2,10-Dihydroxy-6-({[6-(hydroxymethyl)pyridin-2-yl]methyl}amino)-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22m). Reduction of compound **21m** using procedure 2 gave compound **22m** (40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.19 (1H, s), 9.80 (2H, brs), 8.85 (1H, d, J = 8.3 Hz), 8.77 (1H, d, J = 8.3 Hz), 7.80 (1H, t, J = 7.6 Hz), 7.64 (1H, d, J = 7.6 Hz), 7.31 (1H, d, J = 7.6 Hz), 7.17 (1H, d, J = 2.1 Hz), 6.98 (1H, d, J = 2.1 Hz), 6.82 (2H, td, J = 8.3, 2.1 Hz), 6.22 (1H, t, J = 5.1 Hz), 5.97 (1H, d, J = 8.3 Hz), 5.88 (1H, brs), 5.35 (1H, brs), 5.30 (1H, brs), 5.13 (1H, brs), 4.91 (1H, d, J = 3.4 Hz), 4.43 (2H, s), 4.34 (2H, d, J = 5.1 Hz), 4.01 (1H, d, J = 10.2 Hz), 3.94−3.88 (2H, m), 3.78 (1H, d, J = 10.2 Hz), 3.53−3.45 (2H, m); $[\alpha]^{26}_D$ 151.1 (*c* 0.92, DMSO). Anal. ($C_{33}H_{29}N_5O_{10}\cdot 2H_2O\cdot$ MeOH) C, H, N.

6-({[5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-2-yl]methyl}amino)-2,10-dihydroxy-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22n). Reduction of compound **21n** using procedure 2 gave compound **22n**, which was used for the next reaction without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.18 (1H, s), 9.78 (2H, brs), 8.82 (1H, d, J = 8.3 Hz), 8.76 (1H, d, J = 8.3 Hz), 8.27 (1H, s), 7.75 (1H, t, J = 5.2 Hz), 7.70 (1H, d, J = 5.2 Hz), 7.17 (1H, d, J = 2.1 Hz), 6.98 (1H, d, J = 2.1 Hz), 6.80 (3H, td, J = 8.3, 2.1 Hz), 6.21 (1H, t, J = 4.9 Hz), 5.95 (1H, d, J = 8.3 Hz), 5.90 (1H, brs), 5.38 (1H, brs), 5.12 (1H, brs), 4.90 (1H, brs), 4.68 (2H, s), 4.35 (1H, d, J = 4.9 Hz), 4.08 (1H, brs), 4.01 (1H, d, J = 10.2 Hz), 3.89 (2H, s), 3.78 (1H, d, J = 10.2 Hz), 3.49 (2H, brs), 0.82 (9H, s), 0.03 (6H, s). MS (FAB) *m*/*z* 766 $(M + H)^+$.

Representative Procedure for the Removal of *tert*-butyl(dimethyl)silyl group. Procedure 4. 2,10-Dihydroxy-6-({[5-(hydroxymethyl)pyridin-2-yl]methyl}amino)-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (23a). A mixture of compound **22n** (15.3 mg) and tetrabutylammonium fluoride (1.0 M in THF, 1 mL, 1 mmol) in THF (2 mL) was stirred

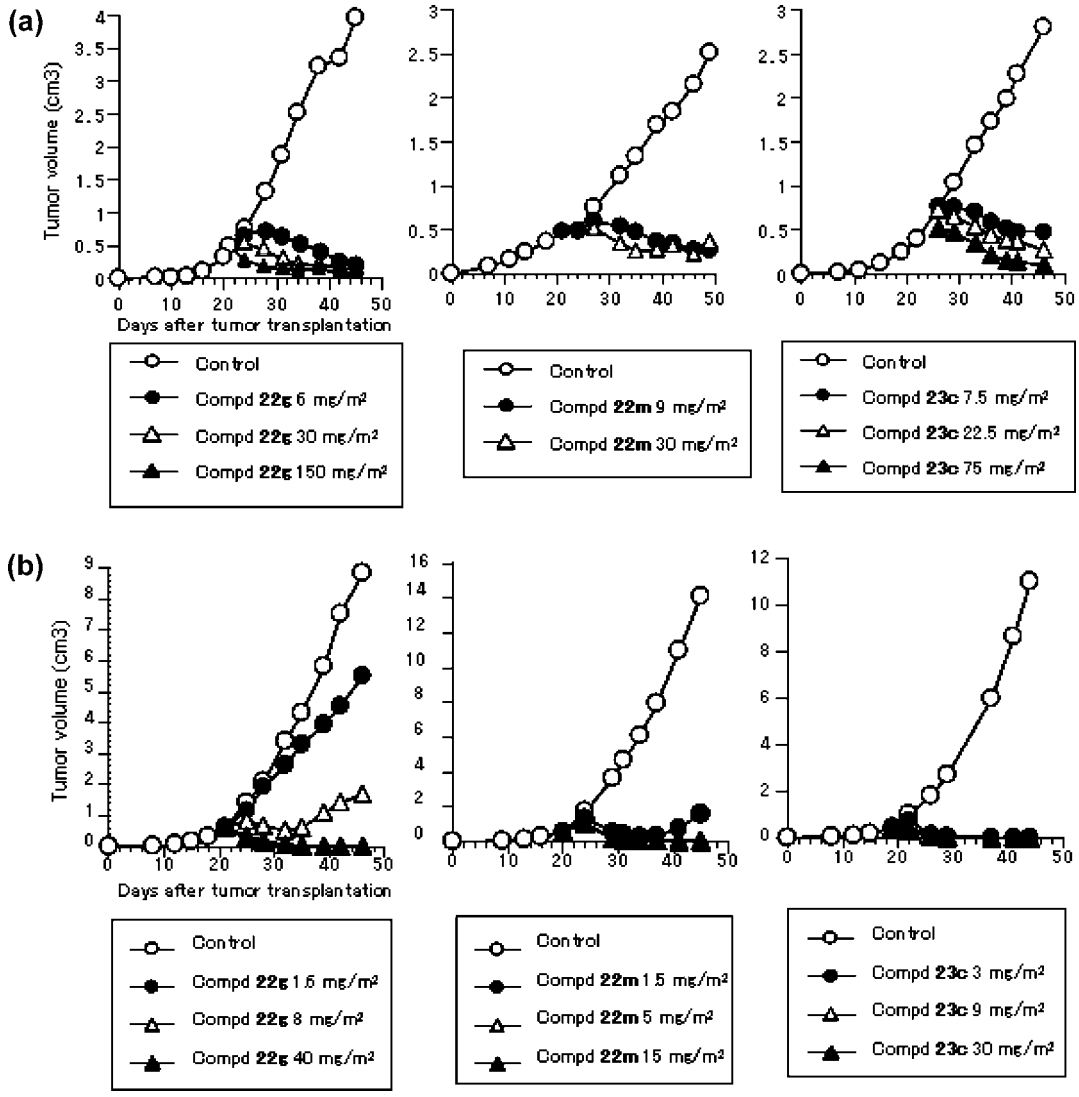


Figure 3. (a) Tumor growth and inhibition of compounds **22g**, **22m**, and **23c** against MKN-45 xenografted nude mice. (b) Tumor growth and inhibition of compounds **22g**, **22m**, and **23c** against MX-1 xenografted nude mice.

at room temperature for 1.0 h. The mixture was concentrated under reduced pressure, and the residue was purified by LC-20 eluting with MeOH to give compound **23a** (31%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.18 (1H, s), 9.77 (2H, brs), 8.84 (1H, d, J = 8.5 Hz), 8.76 (1H, d, J = 8.5 Hz), 8.35 (1H, d, J = 1.7 Hz), 7.72 (2H, s), 7.17 (1H, d, J = 1.7 Hz), 6.98 (1H, d, J = 1.7 Hz), 6.80 (3H, td, J = 8.3, 1.7 Hz), 6.22 (1H, t, J = 4.6 Hz), 5.96 (1H, d, J = 8.9 Hz), 5.87 (1H, brs), 5.35 (1H, brs), 5.22 (1H, t, J = 4.6 Hz), 5.11(1H, brs), 4.91 (1H, s), 4.46 (2H, d, J = 4.6 Hz), 4.35 (1H, d, J = 4.6 Hz), 4.01 (1H, d, J = 10.2 Hz), 3.91 (2H, s), 3.78 (1H, d, J = 10.2 Hz), 3.49 (2H, brs).

2,10-Dihydroxy-6-({[4-(hydroxymethyl)pyridin-2-yl]methyl}amino)-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22o). Reduction of compound **21o** using procedure 2 gave compound **22o** (51%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.19 (1H, s), 9.78 (1H, s), 9.75 (1H, s), 8.85 (1H, d, J = 8.5 Hz), 8.77 (1H, d, J = 8.5 Hz), 8.36 (1H, d, J = 5.0 Hz), 7.67 (1H, s), 7.19 (1H, d, J = 5.0 Hz), 7.18 (1H, s), 6.98 (1H,s), 6.81 (2H, t, J = 8.5 Hz), 6.18 (1H, t, J = 4.8 Hz), 5.97 (1H, d, J = 8.1 Hz), 5.87 (1H, t, J = 4.0 Hz), 5.44 (1H, t, J = 5.6 Hz), 5.34 (1H, d, J = 3.5 Hz), 5.12 (1H, d, J = 4.3 Hz), 4.92 (1H, d, J = 4.3 Hz), 4.56 (2H, d, J = 5.6 Hz), 4.33 (2H, d, J = 4.8 Hz), 4.06−3.71 (4H, m), 3.58−3.46 (2H, m).

2,10-Dihydroxy-6-({[3-(hydroxymethyl)pyridin-2-yl]methyl}amino)-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22p). Reduction of compound **21p** using procedure 1 gave compound **22p** (68%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.18 (1H, s), 9.77 (2H, brs), 8.82 (1H, d, J = 9.0 Hz), 8.74 (1H, d, J = 9.0 Hz), 8.27 (1H, dd, J = 5.1, 1.8 Hz), 7.83 (1H, d, J = 8.1 Hz), 7.28 (1H, dd, J = 8.1, 5.1 Hz), 7.17 (1H, d, J = 1.8 Hz), 6.98 (1H, d, J = 1.8 Hz), 6.81 (2H, td, J = 8.5, 1.8 Hz), 6.15 (1H, t, J = 5.1 Hz), 5.97 (1H, d, J = 8.1 Hz), 5.86 (1H, t, J = 4.2 Hz), 5.33 (1H, d, J = 5.4 Hz), 5.31 (1H, d, J = 5.4 Hz), 5.12 (1H, d, J = 4.8 Hz), 4.93(1H, d, J = 4.2 Hz), 4.87 (2H, d, J = 5.1 Hz), 4.33 (2H, d, J = 4.2 Hz), 4.05−3.96 (1H, m), 3.91 (2H, s), 3.82−3.75 (1H, m), 3.58−3.46 (2H, m).

6-({[4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-3-yl]methyl}amino)-2,10-dihydroxy-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22q). Reduction of compound **21q** using procedure 3 gave compound **22q**, which was used for the next reaction without further purification. This product was characterized by MS analysis. MS (FAB) *m*/*z* 766 $(M + H)^+$.

2,10-Dihydroxy-6-({[4-(hydroxymethyl)pyridin-3-yl]methyl}amino)-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (23b). Removal of TBDMS group of compound **22q** using procedure 4 gave compound **23b** (25%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.18 (1H, s), 9.75 (1H, s), 9.73 (1H, s), 8.84 (1H, d, J = 8.4 Hz), 8.77 (1H, d, J = 8.4 Hz), 8.42 (1H, d, J = 4.9 Hz), 8.39 (1H, s), 8.48 (1H, d, J = 4.9 Hz), 7.17 (1H, d, J = 1.7 Hz), 6.97 (1H, d, J = 1.8 Hz), 6.81 (2H, td, J = 8.5, 1.7 Hz), 6.09 (1H, t, J = 4.7 Hz), 5.97 (1H, d, J = 8.1 Hz), 5.84 (1H, t, J = 3.8 Hz), 5.39 (1H, t, J = 5.8 Hz), 5.30 (1H, d, J = 4.8 Hz), 5.09 (1H, d, J = 4.2 Hz), 4.95 (2H, d, J = 5.8 Hz),

4.90 (1H, d, J = 3.3 Hz), 4.27 (2H, d, J = 4.7 Hz), 4.03−3.75 (4H, m), 3.52−3.47 (2H, m).

6-({[3-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-4-yl]methyl}amino)-2,10-dihydroxy-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22r). Reduction of compound **21r** using procedure 3 gave compound **22r**, which was used for the next reaction without further purification. ^{1}H NMR (300 MHz, CD_3OD) δ: 8.93 (1H, d, J = 8.3 Hz), 8.79 (1H, d, J = 8.3 Hz), 8.52 (1H, s), 8.37 (1H, t, J = 5.1 Hz), 7.62 (1H, d, J = 5.1 Hz), 7.09 (1H, d, J = 2.1 Hz), 6.98 (1H, d, J = 2.1 Hz), 6.78 (3H, td, J = 8.3, 2.1 Hz), 5.94 (1H, d, J = 8.3 Hz), 5.10 (2H, s), 4.40 (2H, s), 4.25−4.17 (2H, m), 4.01−3.90 (2H, m), 3.85−3.67 (2H, m), 0.93 (9H, s), 0.18 (6H, s); MS (FAB) m/z 766 $(M + H)^+$.

2,10-Dihydroxy-6-({[3-(hydroxymethyl)pyridin-4-yl]methyl}amino)-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (23c). Removal of TBDMS group of compound **22r** using procedure 4 gave compound **23c** (49%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.19 (1H, s), 9.78 (1H, s), 9.75 (1H, s), 8.85 (1H, d, J = 9.1 Hz), 8.77 (1H, d, J = 9.1 Hz), 8.51 (1H, s), 8.11 (1H, d, J = 5.1 Hz), 7.59 (1H, d, J = 5.1 Hz), 7.17 (1H, d, J = 2.1 Hz), 6.97 (1H, d, J = 2.1 Hz), 6.81 (2H, td, J = 8.8, 2.1 Hz), 6.25 (1H, t, J = 4.9 Hz), 5.98 (1H, d, J = 8.6 Hz), 5.86 (1H, t, J = 4.0 Hz), 5.32 (1H, d, J = 4.4 Hz), 5.23 (1H, t, J = 5.6 Hz), 5.11 (1H, d, J = 4.4 Hz), 4.91 (1H, d, J = 4.4 Hz), 4.74 (2H, d, J = 5.6 Hz), 4.35 (2H, d, J = 4.9 Hz), 4.01(1H, d, J = 10.6 Hz), 3.96 −3.85 (2H, m), 3.77 (1H, d, J = 10.6 Hz), 3.54−3.46 (2H, m); $[\alpha]^{26}_D$ 141.5 (c 0.86, DMSO). Anal. ($C_{33}H_{29}N_5O_{10}\cdot 2H_2O\cdot$MeOH) C, H, N.

Representative Procedure for the Reaction of Compounds 19 or 24 with Arylmethylbromides. 2,10-Dihydroxy-6-[(4-methylbenzyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (26a). A mixture of compound **24** (50 mg, 0.094 mmol) and 4-methylbenzylbromide (200 mg, 1.09 mmol) in DMF (1 mL) was stirred at room temperature for 3 h. The resulting reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by LH-20 eluting with MeOH to give compound **26a** (14 mg, 23%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.90 (1H, s), 10.36 (1H, s), 9.96 (1H, s), 8.70 (1H, d, J = 7.8 Hz), 8.53 (1H, d, J = 7.8 Hz), 7.40 (2H, d, J = 8.1 Hz), 7.20 (2H, td, J = 7.8, 1.5 Hz), 7.15 (2H, d, J = 9.6 Hz), 7.19−6.98 (1H,m), 7.03 (2H, td, J = 7.8, 1.5 Hz), 6.08 (1H, brs), 5.51−5.36 (2H, m), 4.88 (1H, brs), 4.24 (2H, s), 4.10−3.96 (2H, m), 3.80−3.57 (3H, m), 3.50−3.40 (2H, m), 2.25 (3H, s).

2,10-Dihydroxy-6-[(4-nitrolbenzyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (26b). Compound **26b** was prepared in a similar manner to the synthesis of compound **26a** by substituting 4-nitrobenzylbromide **25b** with compound **25a** in 30% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (1H, s), 10.34 (1H, s), 9.95 (1H, s), 8.68 (1H, d, J = 7.8 Hz), 8.50 (1H, d, J = 7.8 Hz), 8.18 (2H, d, J = 9.0 Hz), 7.84 (2H, d, J = 9.0 Hz), 7.18 (2H, t, J = 8.1 Hz), 7.00 (2H, t, J = 8.1 Hz), 6.42 (1H, brs), 5.45 −5.33 (2H, m), 5.23 (1H, brs), 4.87 (1H, d, J = 5.4 Hz), 4.45 (2H, s), 4.10−3.96 (2H, m), 3.80−3.56 (3H, m), 3.50−3.40 (2H, m).

2,10-Dihydroxy-6-[(4-bromobenzyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (26c). Compound **26c** was prepared in a similar manner to the synthesis of compound **26a** by substituting 4-bromobenzylbromide **25c** with compound **25a** in 18% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (1H, s), 10.34 (1H, s), 9.95 (1H, s), 8.68 (1H, d, J = 7.8 Hz), 8.50 (1H, d, J = 7.8 Hz), 7.49 (4H, s), 7.18 (2H, t, J = 7.8 Hz), 7.00 (2H, t, J = 7.8 Hz), 6.22 (1H, t, J = 3.5 Hz), 5.39 (1H, d, J = 6.0 Hz), 5.32 (1H, t, J = 5.4 Hz), 5.18 (1H, d, J = 4.8 Hz), 4.85 (1H, d, J = 5.7 Hz), 4.25 (2H, s), 4.10−3.90 (2H, m), 3.78−3.52 (3H, m), 3.68 −3.55 (2H, m).

2,10-Dihydroxy-6-[(4-cyanobenzyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (26d). Compound **26d** was prepared in a similar manner to the synthesis of compound **26a** by substituting 4-cyanobenzylbromide **25d** with compound **25a** in 30% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (1H, s), 10.35 (1H, s), 9.95 (1H, s), 8.68 (1H, d, J = 8.0 Hz), 8.50 (1H, d, J = 8.0 Hz), 7.81−7.74 (4H, m), 7.16 (2H, t, J = 8.0 Hz), 7.18−6.98 (2H, m), 6.36 (1H, t, J = 3.5 Hz), 5.40 (1H, d, J = 5.6 Hz), 5.32 (1H, t, J = 5.4 Hz), 5.19 (1H, d, J = 5.3 Hz), 4.85 (1H, d, J = 5.4 Hz), 4.39 (2H, s), 4.10−3.90 (2H, m), 3.77−3.51 (3H, m), 3.41 −3.2 5 (2H, m).

2,10-Dihydroxy-6-{[2-(hydroxymethyl)benzyl]amino}-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (26e). Compound **26e** was prepared in a similar manner to the synthesis of compound **26a** by substituting 2-(hydroxymethyl)benzylbromide **25e** and compound **19** with compounds **25a** and **24** in 12% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.19 (1H, s), 9.78 (1H, s), 9.75 (1H, s), 8.85 (1H, d, J = 8.6 Hz), 8.78 (1H, d, J = 8.6 Hz), 7.44 (1H, d, J = 7.2 Hz), 7.37 (1H, d, J = 7.2 Hz), 7.23 (1H, t, J = 7.2 Hz), 7.18 (2H, s), 6.98 (1H, s), 6.81 (2H, d, J = 8.8 Hz), 6.05−5.95 (2H, m), 5.85 (1H, brs), 5.33 (1H, d, J = 3.2 Hz), 5.17−5.10 (2H, m), 4.92 (1H, d, J = 4.0 Hz), 4.82 (2H, d, J = 5.2 Hz), 4.28 (2H, d, J = 5.2 Hz), 4.10−3.72 (4H, m), 3.55−3.45 (2H, m).

Representative Procedure for the Reaction of Compound 51 with Arylmethylhydrazines. 2,10-Dihydroxy-6-{[4-(hydroxymethyl)benzyl]amino}-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (53). A mixture of compound **51** (100 mg, 0.19 mmol), [4-(hydrazinomethyl)phenyl-]methanol hydrochloride **52a** (100 mg, 0.53 mmol) in DMF (5 mL), and sat. aqueous $NaHCO_3$ solution (1 mL) was stirred at 80 °C for 0.5 h. The resulting reaction mixture was partitioned between methyl ethyl ketone and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by LH-20 eluting with MeOH to give compound **53** (33 mg, 27%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.10 (1H, brs), 8.83 (1H, d, J = 8.4 Hz), 8.73 (1H, d, J = 8.4 Hz), 7.44 (2H, d, J = 8.4 Hz), 7.23 (2H, d, J = 8.4 Hz), 7.11 (1H, s), 6.94 (1H, s), 6.78 (2H, td, J = 9.0, 2.1 Hz), 6.02 (1H, t, J = 4.8 Hz), 5.92 (1H, d, J = 8.1 Hz), 5.23−4.80 (2H, m), 4.43 (2H, s), 4.24 (2H, s), 3.71−4.06 (4H, m), 3.54−3.45 (2H, m), 3.42 −3.25 (3H, m).

2,10-Dihydroxy-6-[(3-hydroxybenzyl)amino]-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (54). Compound **54** was prepared in a similar manner to the synthesis of compound **53** by substituting 3-(hydrazinomethyl)phenol hydrochloride **52b** with compound **52a** in 37% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.18 (1H, s), 9.76 (1H, s), 9.73 (1H, s), 9.28 (1H, s), 8.87 (1H, d, J = 8.5 Hz), 8.79 (1H, d, J = 8.5 Hz), 7.17 (1H, s), 7.08 (1H, dd, J = 8.0, 7.5 Hz), 6.97 (1H, d, J = 2.3 Hz), 6.93−6.78 (4H, m), 6.60 (1H, dd, J = 8.0, 1.5 Hz), 5.98−5.95 (2H, m), 5.87−5.81 (1H, m), 5.30 (1H, d, J = 4.2 Hz), 5.09 (1H, d, J = 4.9 Hz), 4.90 (1H, d, J = 5.1 Hz), 4.15 (2H, s), 4.05−3.72 (4H, m), 3.51−3.47 (2H, m).

2,10-Dihydroxy-6-{[3-(hydroxymethyl)benzyl]amino}-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (55). Compound **55** was prepared in a similar manner to the synthesis of compound **53** by substituting [3-(hydrazinomethyl)phenyl]methanol hydrochloride **32** with compound **52a** in 37% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.18 (1H, s), 8.86 (1H, d, J = 8.4 Hz), 8.78 (1H, d, J = 8.4 Hz), 7.42 (1H, s), 7.40 (1H, d, J = 7.5 Hz), 7.27 (1H, t, J = 7.5 Hz), 7.18 (1H, d, J = 7.5 Hz), 7.20−7.15 (1H, m), 6.98 (1H, d, J = 1.8 Hz), 6.81 (2H, td, J = 8.1, 1.8 Hz), 6.00 (1H, t, J = 4.8 Hz), 5.95 (1H, d, J = 8.7 Hz), 5.43 (1H, brs), 5.16 (2H, brs), 4.93 (1H, brs), 4.47 (2H, s), 4.25 (2H, d, J = 4.8 Hz), 4.10 (1H, brs), 4.02 (1H, d, J = 10.8 Hz), 4.00−3.85 (2H, m), 3.85−3.70 (1H, m), 3.50−3.40 (2H, m).

2,10-Dihydroxy-6-({[5-(hydroxymethyl)pyridin-3-yl]methyl}amino)-13-(β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (56). Compound **56** was prepared in a similar manner to the synthesis of compound **53** by substituting **38**

with compound **52a** in 15% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.18 (1H, brs), 9.82 (2H, brs), 8.84 (1H, d, J = 8.5 Hz), 8.76 (1H, d, J = 8.5 Hz), 8.55 (1H, s), 8.37 (1H, s), 7.84 (1H, s), 7.16 (1H, s), 7.00 (1H, s), 6.79 (2H, t, J = 8.1 Hz), 6.21 (1H, t, J = 4.7 Hz), 5.95 (1H, d, J = 7.8 Hz), 5.93 (1H, brs), 5.40 (1H, brs), 5.30 (1H, brs), 5.19 (1H, brs), 4.93 (1H, brs), 4.50 (2H, s), 4.56 (2H, d, J = 4.7 Hz), 4.02 (1H, d, J = 10.8 Hz), 4.00−3.85 (2H, m), 3.77 (1H, d, J = 10.8 Hz), 3.54−3.40 (2H, m).

2,10-Dihydroxy-6-({[6-(hydroxymethyl)pyridin-3-yl]methyl}amino)-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (57). Compound **57** was prepared in a similar manner to the synthesis of compound **53** by substituting **44** with compound **52a** in 19% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.12 (1H, brs), 9.82 (2H, brs), 8.81 (1H, d, J = 8.7 Hz), 8.73 (1H, d, J = 8.7 Hz), 8.51 (1H, s), 7.90 (1H, d, J = 7.9 Hz), 7.39 (1H, d, J = 7.9 Hz), 7.11 (1H, s), 6.93 (1H, s), 6.77 (2H, t, J = 8.7 Hz), 6.21 (1H, t, J = 3.8 Hz), 5.92 (1H, d, J = 7.9 Hz), 5.50−4.85 (5H, m), 4.48 (2H, s), 4.27 (2H, d, J = 3.8 Hz), 4.01 (1H, d, J = 10.8 Hz), 3.97−3.85 (2H, m), 3.77 (1H, d, J = 10.8 Hz), 3.54−3.40 (2H, m).

2,10-Dihydroxy-6-({[2-(hydroxymethyl)pyridin-4-yl]methyl}amino)-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (58). Compound **58** was prepared in a similar manner to the synthesis of compound **53** by substituting **50** with compound **52a** in 20% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.17 (1H, brs), 9.82 (2H, brs), 8.85 (1H, d, J = 8.4 Hz), 8.77 (1H, d, J = 8.4 Hz), 8.41 (1H, d, J = 5.1 Hz), 7.56 (1H, s), 7.47 (1H, d, J = 5.1 Hz), 7.15 (1H, s), 6.96 (1H, s), 6.81 (2H, t, J = 8.7 Hz), 6.26 (1H, t, J = 4.9 Hz), 5.94 (1H, d, J = 8.6 Hz), 5.92 (1H, brs), 5.36 (2H, brs), 5.18 (1H, brs), 4.91 (1H, brs), 4.51 (2H, d, J = 1.8 Hz), 4.32 (2H, d, J = 4.9 Hz), 4.03 (1H, d, J = 10.5 Hz), 3.97−3.86 (2H, m), 3.77 (1H, d, J = 10.5 Hz), 3.52−3.45 (2H, m).

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Supporting Information Available: Additional experimental procedures and characterizarion for compounds **4**−**18**, **21a**−**r**, **28**−**50**; HPLC and HRMS analyses data of all tested compounds; Combustion analysis data of in vivo tested compounds **22g**, **22m**, **23c**; Topoisomerase I-mediated DNA cleavages induced by compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Rothenberg, M. L. Topoisomerase I inhibitors: review and update. *Ann. Oncol.* **1997**, *8*, 837–855.
(2) Beretta, G. L.; Perego, P.; Zunino, F. Targeting topoisomerase I: molecular mechanisms and cellular determinants of responce to topoisomerase I inhibitors. *Expert Opin. Ther. Targets* **2008**, *12*, 1243–1256.
(3) Teicher, B. A. Next generation topoisomerase I inhibitors: rationale and biomarker strategies. *Biochem. Pharmacol.* **2008**, *75*, 1262–1271.
(4) Pommier, Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat. Rev. Cancer* **2006**, *6*, 789–802.
(5) Potmesil, M. Camptothecin: from the bench research to the hospital wards. *Cancer Res.* **1994**, *54*, 1431–1439.
(6) Wall, M. E.; Wani, M. C. Camptothecin and taxol: discovery to clinic—thirteenth Bruce F. Cain memorial award lecture. *Cancer Res.* **1995**, *55*, 753–760.
(7) Goldwasser, F.; Bae, I.; Valenti, M.; Torres, K.; Pommier, Y. Topoisomerase I-related parameters and camptothecin activity in the colon carcinoma cell lines from the National Cancer Research Institute anticancer screen. *Cancer Res.* **1995**, *55*, 2116–2121.
(8) Oberlies, N. H.; Kroll, D. Camptothecin and taxol: historic achievements in natural products research. *J. Nat. Prod.* **2004**, *67*, 127–135.
(9) Pizzolato, J. F.; Saltz, L. B. The camptothecins. *Lancet* **2003**, *361*, 2235–2242.
(10) Douillard, J. Y.; Cunningham, D.; Roth, A. D.; Navarro, M.; James, R. D.; Karasek, P.; Jandik, P.; Iveson, T.; Carmichael, J.; Alakl, M.; Gruia, G.; Awad, L.; Rougier, P. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* **2000**, *355*, 1041–1047.
(11) Cunningham, D.; Pyrhonen, S.; James, R. D.; Punt, C. J. A.; Hickish, T. F.; Heikkila, R.; Johannesen, T. B.; Starkhammar, H.; Topham, C. A.; Awad, L.; Jacques, C.; Herait, P. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. *Lancet* **1998**, *352*, 1413–1418.
(12) Saltz, L. B.; Cox, J. V.; Blanke, C.; Rosen, L. S.; Fehrenbacher, L.; Moore, M. J.; Maroun, J. A.; Ackland, S. P.; Locker, P. K.; Pirotta, N.; Elfring, G. L.; Miller, L. L. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med.* **2000**, *343*, 905–914.
(13) Noda, K.; Nishiwaki, N.; Kawahara, M.; Negoro, S.; Sugiura, T.; Yokoyama, A.; Fukuoka, M.; Mori, K.; Watanabe, K.; Tamura, T.; Yamamoto, S.; saijo, N. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N. Engl. J. Med* **2002**, *346*, 85–91.
(14) Enzinger, P. C.; Kulke, M. H.; Clark, J. W.; Ryan, D. P.; Kim, H.; Earle, C. C.; Vincitore, M. M.; Michelini, A. L.; Mayer, R. J.; Fuchs, C. S. A phase II trial of irinotecan in patients with previously untreated advanced esophageal and gastric adenocarcinoma. *Dig. Dis. Sci.* **2005**, *50*, 2218–2223.
(15) Vallbohmer, D.; Iqbal, S.; Yang, D. Y.; Rhodes, K. E.; Zhang, W.; Gordon, M.; Fazzone, W.; Schultheis, A. M.; Sherrod, A. E.; Danenberg, K. D.; lenz, H.-J. Molecular determinants of irinotecan efficacy. *Int. J. Cancer* **2006**, *119*, 2435–2442.
(16) Zunino, F.; Pratesi, G. Camptothecins in clinical development. *Expert Opin. Invest. Drugs* **2004**, *13*, 269–284.
(17) Garcia-Carbonero, R.; Supko, J. G. Current perspective on the clinical experience, pharmacology, and continued development of the camptothecins. *Clin. Cancer Res.* **2002**, *8*, 641–661.
(18) Kollmannsberger, C.; Mross, K.; Jakob, A.; Kanz, L.; Bokemeyer, C. Topotecan, a novel topoisomerase I inhibitor: pharmacology and clinical experience. *Oncology* **1999**, *56*, 1–12.
(19) Stewart, D. J. Topotecan in the first-line treatment of small cell lung cancer. *Oncologist* **2004**, *9*, 33–42.
(20) Markman, M. New, expanded, and modified use of approved antineoplastic agents in ovarian cancer. *Oncologist* **2007**, *12*, 186–190.
(21) Sugimori, M.; Ejima, A.; Ohsuki, S.; Uoto, K.; Mitsui, I.; Kawato, Y.; Hirota, Y.; Sato, K.; Terasawa, H. Synthesis and antitumor activity of ring A- and F-modified hexacyclic camptothecin analogues. *J. Med. Chem.* **1998**, *41*, 2308–2318.
(22) Saltz, L. B.; Kemeny, N. E.; Tong, W.; Harrison, J.; Berkery, R.; Kelsen, D. P. 9-Aminocamptothecin by 72 h continuous intravenous infusion is inactive in the treatment of patients with 5-fluorouracil-refractory colorectal carcinoma. *Cancer* **1997**, *80*, 1727–1732.
(23) Kindler, H. L.; Avadhani, A.; Wade-Oliver, K.; Karrison, T.; Mani, S.; Vokes, E. E. 9-Aminocamptothecin (9-AC) given as a 120 h continuous infusion in patients with advanced adenocarcinomas of the stomach and gastroesophageal junction: a phase II trial of the University of Chicago phase II consortium. *Invest. New Drugs* **2004**, *22*, 323–327.
(24) Bailly, C.; Lanciaux, A.; Dassonneville, L.; Demarquay, D.; Coulomb, H.; Huchet, M.; Lavergne, O.; Bigg, D. C. H. Homocamptothecin, an E-ring-modified camptothecin analogue, generates new topoisomerase I-mediated DNA breaks. *Biochemistry* **1999**, *38*, 15556–15563.
(25) Lesueur-Ginot, L.; Demarquay, D.; Kiss, R.; Kasprzyk, P. G.; Dassonneville, L.; Bailly, C.; Camara, J.; Lavergne, O.; Bigg, D. C. H. Homocamptothecin, an E-ring-modified camptothecin with enhanced lactone stability, retains topoisomerase I-targeted activity and antitumor properties. *Cancer Res.* **1999**, *59*, 2939–2943.
(26) Bailly, C.; Laine, W.; Baldeyrou, B.; Huchet, M.; Coulomb, H.; Lanco, C.; Lavergne, O.; Bigg, D. C. A novel B-ring modified homocamptothecin, 12-Cl-hCPT, showing antiproliferative and topoisomerase I inhibitory activities superior to SN-38. *Anti-Cancer Drug Des.* **2001**, *16*, 27–36.
(27) Lavergne, O.; Lesueur-Ginot, L.; Rodas, Francesc, P.; Kasprzyk, P. G.; Pommier, J.; Demarquay, D.; Prevost, G.; Ulibarri, G.; Rolland, A.; Schiano-Liberatore, A.-M.; Harnett, J.; Pons, D.; Camara, J.; Bigg, D. C. H. Homocamptothecins: synthesis and antitumor activity of novel E-ring-modified camptothecin analogs. *J. Med. Chem.* **1998**, *41*, 5410–5419.
(28) Lavergne, O.; Demarquay, D.; Bailly, C.; Lanco, C.; Rolland, A.; Huchet, M.; Coulomb, H.; Muller, N.; Baroggi, N.; Camara, J.; Le Breton, C.; Manginot, E.; Cazaux, J.-B.; Bigg, D. C. H. Topoisomerase I-mediated antiproliferative activity of enantiomerically pure fluorinated homocamptothecins. *J. Med. Chem.* **2000**, *43*, 2285–2289.
(29) Gelderblom, H.; Salazar, R.; Verweij, J.; Pentheroudakis, G.; de Jonge, M. J. A.; Devlin, M.; van Hooije, C.; Seguy, F.; Obach, R.; Prunonosa, J.; Principe, P.; Twelves, C. Phase I pharmacological and bioavailability study of oral diflomotecan (BN80915), a novel E-ring-modified

camptothecin analogue in adults with solid tumors. *Clin. Cancer Res.* **2003**, *9*, 4101–4107.
(30) Lansiaux, A.; Facompre, M.; Wattez, N.; Hildebrand, M.-P.; Bal, C.; Demarquay, D.; Lavergne, O.; Bigg, D. C. H.; Bailly, C. Apoptosis induced by the homocamptothecin anticancer drug BN80915 in HL-60 cells. *Mol. Pharmacol.* **2001**, *60*, 450–461.
(31) Larsen, A. K.; Gilbert, C.; Chyzak, G.; Plisov, S. Y.; Naguibneva, I.; Lavergne, O.; Lesueur-Ginot, L.; Bigg, D. C. H. Unusual potency of BN 80915, a novel fluorinated E-ring modified camptothecin, toward human colon carcinoma cells. *Cancer Res.* **2001**, *61*, 2961–2967.
(32) Troconiz, I. F.; Garrido, M. J.; Segura, C.; Cendros, J.-M.; Principe, P.; Peraire, C.; Obach, R. Phase I dose-finding study and a pharmacokinetic/pharmacodynamic analysis of the neutropenic response of intravenous diflomotecan in patients with advanced malignant tumours. *Cancer Chemother. Pharmacol.* **2006**, *57*, 727–735.
(33) Demarquay, D.; Coulomb, H.; Huchet, M.; Lesueur-Ginot, L.; Camara, J.; Lavergne, O.; Bigg, D. C. H. The homocamptothecin, BN 80927, is a potent topoisomerase I poison and topoisomerase II catalytic inhibitor. *Ann. N.Y. Acad. Sci.* **2000**, *922*, 301–302.
(34) Huchet, M.; Demarquay, D.; Coulomb, H.; Kasprzyk, P.; Carlson, M.; Lauer, J.; Lavergne, O.; Bigg, D. C. H. The dual topoisomerase inhibitor, BN 80927, is highly potent against cell proliferation and tumor growth. *Ann. N.Y. Acad. Sci.* **2000**, *922*, 303–305.
(35) Lavergne, O.; Harnett, J.; Rolland, A.; Lanco, C.; Lesueur-Ginot, L.; Demarquay, D.; Huchet, M.; Coulomb, H.; Bigg, D. C. H. BN 80927: a novel homocamptothecin with inhibitory activities on both topoisomerase I and topoisomerase II. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2599–2602.
(36) Demarquay, D.; Huchet, M.; Coulomb, H.; Lesueur-Ginot, L.; Lavergne, O.; Camara, J.; Kasprzyk, P. G.; Prevost, G.; Bigg, D. C. H. BN80927: A novel homocamptothecin that inhibits proliferation of human tumor cells in vitro and in vivo. *Cancer Res.* **2004**, *64*, 4942–4949.
(37) Perego, P.; Ciusani, E.; Gatti, L.; Carenini, N.; Corna, E.; Zunino, F. Sensitization to gimatecan-induced apoptosis by tumor necrosis factor-related apoptosis inducing ligand in prostate carcinoma cells. *Biochem. Pharmacol.* **2006**, *71*, 791–798.
(38) Di Francesco, A. M.; Riccardi, A. S.; Barone, G.; Rutella, S.; Meco, D.; Frapolli, R.; Zucchetti, M.; D'Incalci, M.; Pisano, C.; Carminati, P.; Riccardi, R. The novel lipophilic camptothecin analogue gimatecan is very active in vitro in human neuroblastoma: A comparative study with SN38 and topotecan. *Biochem. Pharmacol.* **2005**, *70*, 1125–1136.
(39) De Cesare, M.; Pratesi, G.; Veneroni, S.; Bergottini, R.; Zunino, F. Efficacy of the novel camptothecin gimatecan against orthotopic and metastatic human tumor xenograft models. *Clin. Cancer Res.* **2004**, *10*, 7357–7364.
(40) De Cesare, M.; Pratesi, G.; Perego, P.; Carenini, N.; Tinelli, S.; Merlini, L.; Penco, S.; Pisano, C.; Bucci, F.; Vesci, L.; Pace, S.; Capocasa, F.; Carminati, P.; Zunino, F. Potent antitumor activity and improved pharmacological profile of ST1481, a novel 7-substituted camptothecin. *Cancer Res.* **2001**, *61*, 7189–7195.
(41) Perego, P.; De Cesare, M.; De Isabella, P.; Carenini, N.; Beggiolin, G.; Pezzoni, G.; Palumbo, M.; Tartaglia, L.; Pratesi, G.; Pisano, C.; Carminati, P.; Scheffer, G. L.; Zunino, F. A novel 7-modified camptothecin analog overcomes breast cancer resistance protein-associated resistance in a mitoxantrone-selected colon carcinoma cell line. *Cancer Res.* **2001**, *61*, 6034–6037.
(42) Long, B. H.; Balasubramanian, B. N. Non-camptothecin topoisomerase I active compounds as potential anticancer agents. *Expert Opin. Ther. Pat.* **2000**, *10*, 635–666.
(43) Marchand, C.; Antony, S.; Kohn, K. W.; Cushman, M.; Ioanoviciu, A.; Staker, B. L.; Burgin, A. B.; Stewart, L.; Pommier, Y. A novel norindenoisoquinoline structure reveals a common interfacial inhibitor paradigm for ternary trapping of the topoisomerase I−DNA covalent complex. *Mol. Cancer Ther.* **2006**, *5*, 287–295.
(44) Nagarajan, M.; Morrell, A.; Antony, S.; Kohlhagen, G.; Agama, K.; Pommier, Y.; Ragazzon, P. A.; Garbett, N. C.; Chaires, J. B.; Hollingshead, M.; Cushman, M. Synthesis and Biological Evaluation of Bisindenoisoquinolines as Topoisomerase I Inhibitors. *J. Med. Chem.* **2006**, *49*, 5129–5140.
(45) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. A systematic study of nitrated indenoisoquinolines reveals a potent topoisomerase I inhibitor. *J. Med. Chem.* **2006**, *49*, 7740–7753.
(46) Morrell, A.; Placzek, M. S.; Steffen, J. D.; Antony, S.; Agama, K.; Pommier, Y.; Cushman, M. Investigation of the lactam side chain length necessary for optimal indenoisoquinoline topoisomerase I inhibition and cytotoxicity in human cancer cell cultures. *J. Med. Chem.* **2007**, *50*, 2040–2048.
(47) Morrell, A.; Placzek, M.; Parmley, S.; Grella, B.; Antony, S.; Pommier, Y.; Cushman, M. Optimization of the indenone ring of indenoisoquinoline topoisomerase I inhibitors. *J. Med. Chem.* **2007**, *50*, 4388–4404.
(48) Morrell, A.; Placzek, M.; Parmley, S.; Antony, S.; Dexheimer, T. S.; Pommier, Y.; Cushman, M. Nitrated indenoisoquinolines as topoisomerase I inhibitors: a systematic study and optimization. *J. Med. Chem.* **2007**, *50*, 4419–4430.
(49) Antony, S.; Agama, K. K.; Miao, Z.-H.; Hollingshead, M.; Holbeck, S. L.; Wright, M. H.; Varticovski, L.; Nagarajan, M.; Morrell, A.; Cushman, M.; Pommier, Y. Bisindenoisoquinoline bis-1,3-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propylamino}propane bis(trifluoroacetate) (NSC 727357), a DNA intercalator and topoisomerase inhibitor with antitumor activity. *Mol. Pharmacol.* **2006**, *70*, 1109–1120.
(50) Saif, M. W.; Diasio, R. B. Edotecarin: a novel topoisomerase I inhibitor. *Clin. Colorectal Cancer* **2005**, *5*, 27–36.
(51) Long, B. H.; Rose, W. C.; Vyas, D. M.; Matson, J. A.; Forenza, S. Discovery of antitumor indolocarbazoles: rebeccamycin, NSC 655649, and fluoroindolocarbazoles. *Curr. Med. Chem.: Anti-Cancer Agents* **2002**, *2*, 255–266.
(52) Yamashita, Y.; Fujii, N.; Murakata, C.; Ashizawa, T.; Okabe, M.; Nakano, H. Induction of mammalian DNA topoisomerase I mediated DNA cleavage by antitumor indolocarbazole derivatives. *Biochemistry* **1992**, *31*, 12069–12075.
(53) Ohkubo, M.; Kojiri, K.; Kondo, H.; Tanaka, S.; Kawamoto, H.; Nishimura, T.; Nishimura, I.; Yoshinari, T.; Arakawa, H.; Suda, H.; Morishima, H.; Nishimura, S. Synthesis and biological activities of topoisomerase I inhibitors, 6-*N*-amino analogues of NB-506. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1219–1224.
(54) Ohkubo, M.; Nishimura, T.; Kawamoto, H.; Nakano, M.; Honma, T.; Yoshinari, T.; Arakawa, H.; Suda, H.; Morishima, H.; Nishimura, S. Synthesis and biological activities of NB-506 analogues modified at the glucose group. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 419–422.
(55) Arakawa, H.; Iguchi, T.; Morita, M.; Yoshinari, T.; Kojiri, K.; Suda, H.; Okura, A.; Nishimura, S. Novel indolocarbazole compound 6-*N*-formylamino-12,13-dihydro-1,11-dihydroxy-13-(β-D-glucopyranosyl)-5*H*-indolo[2,3-α]pyrrolo-[3,4-*c*]carbazole-5,7(6*H*)-dione (NB-506): its potent antitumor activities in mice. *Cancer Res.* **1995**, *55*, 1316–1320.
(56) Yoshinari, T.; Matsumoto, M.; Arakawa, H.; Okada, H.; Noguchi, K.; Suda, H.; Okura, A.; Nishimura, S. Novel antitumor indolocarbazole compound 6-*N*-formylamino-12,13-dihydro-1,11-dihydroxy-13-(β-D-glucopyranosyl)-5*H*-indolo[2,3-α]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (NB-506): induction of topoisomerase I-mediated DNA cleavage and mechanisms of cell line-selective cytotoxicity. *Cancer Res.* **1995**, *55*, 1310–1315.
(57) Fukasawa, K.; Kimatani, H.; Haya, Y.; Suda, H.; Okura, A.; Nishimura, S.; Yoshinari, T. Sequence-selective DNA cleavage by a topoisomerase I poison, NB-506. *Int. J. Cancer* **1998**, *75*, 145–150.
(58) Yoshinari, T.; Ohkubo, M.; Fukasawa, K.; Egashira, S.; Haya, Y.; Matsumoto, M.; Nakai, K.; Arakawa, H.; Morishima, H.; Nishimura, S. Mode of action of a new indolocarbazole anticancer agent, J-107088, targeting topoisomerase I. *Cancer Res.* **1999**, *59*, 4271–4275.
(59) Arakawa, H.; Morita, M.; Kodera, T.; Okura, A.; Ohkubo, M.; Morishima, H.; Nishimura, S. In vivo anti-tumor activity of a novel indolocarbazole compound, J-107088, on murine and human tumors transplanted into mice. *Jpn. J. Cancer* **1999**, *90*, 1163–1170.
(60) Meng, L.; Liao, Z.; Pommier, Y. Non-camptothecin DNA topoisomerase I inhibitors in cancer therapy. *Curr. Top. Med. Chem.* **2003**, *3*, 305–320.
(61) Ciomei, M.; Croci, V.; Stellari, F.; Amboldi, N.; Giavarini, R.; Pesenti, E. Antitumor activity of edotecarin in breast carcinoma models. *Cancer Chemother. Pharmacol.* **2007**, *60*, 229–235.
(62) Ciomei, M.; Croci, V.; Ciavolella, A.; Ballinari, D.; Pesenti, E. Antitumor efficacy of edotecarin as a single agent and in combination with chemotherapy agents in a xenograft model. *Clin. Cancer Res.* **2006**, *12*, 2856–2861.
(63) Yamada, Y.; Tamura, T.; Yamamoto, N.; Shimoyama, T.; Ueda, Y.; Murakami, H.; Kusaba, H.; Kamiya, Y.; Saka, H.; Tanigawara, Y.; McGovren, J. P.; Natsumeda, Y. Phase I and pharmacokinetic study of edotecarin, a novel topoisomerase I inhibitor, administered once every 3 weeks in patients with solid tumors. *Cancer Chemother. Pharmacol.* **2006**, *58*, 173–182.
(64) Hurwitz, H. I.; Cohen, R. B.; McGovren, J. P.; Hirawat, S.; Petros, W. P.; Natsumeda, Y.; Yoshinari, T. A phase I study of the safety and pharmacokinetics of edotecarin (J-107088), a novel topoisomerase I inhibitor, in patients with advanced solid tumors. *Cancer Chemother. Pharmacol.* **2007**, *59*, 139–147.
(65) Queguiner, G.; Pastour, P. Synthesis in the pyridine series. V. Study of the reduction of ethyl pyridinedicarboxylates. *Bull. Soc. Chim. Fr.* **1969**, *10*, 3678–3683.
(66) Dawson, M. I.; Chan, R.; Hobbs, P. D.; Chao, W.; Schiff, L. J. Aromatic retinoic acid analogs. 2.Synthesis and pharmacological activity. *J. Med. Chem.* **1983**, *26*, 1282–1293.
(67) Kojima, T.; Hayashi, K.; Iizuka, S.; Tani, F.; Naruta, Y.; Kawano, M.; Ohashi, Y.; Hirai, Y.; Ohkubo, K.; Matsuda, Y.; Fukuzumi, S.

Synthesis and characterization of mononuclear ruthenium(III) pyridylamine complexes and mechanistic insights into their catalytic alkane functionalization with *m*-chloroperbenzoic acid. *Chem.—Eur. J.* **2007**, *13*, 8212–8222.

(68) Docking study was performed using the *Molecular Operating Environment (MOE), version* 2008.10; Chemical Computing Group: Montreal, 2006) suite using 1T8I (PDB ID) as a receptor. During the docking process, a rotamer exploration (implemented in the MOE suite) of all side chains involved in the inhibitor binding was carried out. Among the all possible binding mode generated by the docking study, the most similar one to the experimentally observed binding mode of indrocarbazole scaffold in topoisomerase I mutant (1SEU (PDB ID)) was selected.

(69) Staker, B. L.; Feese, M. D.; Cushman, M.; Pommier, Y.; Zembower, D.; Stewart, L.; Burgin, A. B. Structures of three classes of anticancer agents boiund to the human topoisomerase I—DNA covalent complex. *J. Med. Chem.* **2005**, *48*, 2336–2345.

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