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. Diflomotecan²⁸⁻³² 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 inhibitors has also been made. Among them, indenoisoquino-lines^{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 breast⁶¹ and HCT-116 human colon⁶² 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; *tert*-butyl carbazate; DIBAL, diisobutyl aluminum hydride; Boc, *tert*-butoxycarbonyl; GID₇₅, 75% growth inhibition dose; LD₁₀, 10% lethal dose.



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) NaBH₄, CaCl₂, EtOH, 0 °C; (vi) MnO₂, CH₂Cl₂, rt; (vii) Dess–Martin periodinate, CH₂Cl₂, 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 \mathbf{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 (TB-DMS) 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 NaBH₄-CaCl₂ to give compounds 9, 13, and 17. Oxidation of the newly formed hydroxymethyl group with MnO₂ 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 NaBH₃CN 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 NaBH₃CN-ZnCl₂ (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-CH2NHNH2) 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 (BocNHNH₂) 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 BH₃-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 TBDM-SCl 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 BocNHNH₂ 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 NaBH₃CN in acidic media to give compound 29. Transformation of the cyano group to ahydroxymethyl group by consecutive treatment with DIBAL and NaBH₄, 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 BH₃-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) NaBH₃CN, 10% HCl/MeOH, rt; (iii) NaBH₃CN, ZnCl₂, THF, rt; (iv) H₂/5% Pd-C, MeOH/THF, rt; (v) TBAF, THF, rt.

Scheme 3^a



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

saturated aqueous NaHCO₃ 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 2to 5-fold stronger topoisomerase I inhibitory activities with almost equipotent CTX activities compared to edotecarin. On the other hand, introduction of nitrogen-containing sixmembered 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 **221**, **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) BocNHNH₂, EtOH, 60 °C; (ii) NaBH₃CN, 4 N HCl/MeOH, rt; (iii) DIBAL; (iv) NaBH₄, MeOH, rt; (v) TFA, CH₂Cl₂, rt; (vi) BH₃-THF complex, THF, 0 °C then 6 N HCl, rt; (vii) TBDMSCl, imidazole, DMF, rt; (viii) MnO₂, CHCl₃, rt; (ix) (MeO)₃CH, MeOH, reflux; (x) LAH, THF, rt; (xi) TFA, rt.

Scheme 5^a



^a Reagents and conditions: (i) sat. NaHCO₃, 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 performed⁶⁸ to provide the rational molecular basis for enhancement of the topoisomerase I inhibitory activity achieved by introduction of (hydroxyl-methyl)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 indolocar-

bazole. 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 (GID₇₅) 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.^{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 ₂ OH (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 ₂ OH (2,10)	22	220	1.2	11	36	21
26e	2-CH ₂ OH (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



	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	Хмон	3.3	73	0.50	2.1	4.3	11
23a	XN, OH	27	120	1.10	6.0	9.9	13
220	Х <mark>л</mark> Он	22	160	0.46	1.6	9.4	34
22p	HO	19	400	0.40	0.70	2.6	36
23b	HO	25	>10,000	0.33	0.62	3.9	8.9
56	Х _{ОН}	35	90	0.98	2.6	6.2	32
57	Клон	30	130	0.49	1.6	25	11
23c	HOLON	29	130	0.28	0.35	0.91	4.0
58	Х _{Сн}	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

¹H 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 μ m) 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-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)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%). ¹H 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-5H-indolo[2,3-a]pyrrolo[3,4c]carbazole-5,7(6H)-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%). ¹H 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 = 8.1Hz), 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%). ¹H 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-FuryImethyI)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%). ¹H 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-FuryImethyI)amino]-2,10-dihydroxy-13-(β-D-glucopyranosyI)-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%). ¹H 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), 3.50 (2H, brs).**

2,10-Dihydroxy-6-[(1*H***-pyrrol-2-ylmethyl)amino]-13-(\beta-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%). ¹H NMR (300 MHz, DMSO-d_6) \delta 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%). ¹H 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); [α]²⁶_D 163.9 (*c* 1.02, DMSO). Anal. (C₃₂H₂₇N₅O₉•H₂O) 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%). ¹H 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%). ¹H 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 (15) 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),

			22g	22m	23c	1
Head structure			× -	ОН	HO	ОН
Topo I cle	eavege: 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	
	CTX: IC50 (nM)		0.71	2.1	0.35	4.8
MKN-	$\text{GID}_{75}^{c} (\text{mg/m}^2)$		12 ^f	<90 ^g	<30 ^f	45 ^g
45	$LD_{10}^{d} (mg/m^2)$		705	110	>300	>1,600
	safety LD ₁₀ /GID ₇₅ e	margin:	59	>1.2	>10	>36
MX-1	$\text{GID}_{75}^{\text{c}} (\text{mg/m}^2)$		27 ^f	<4.5 ^f	<12 ^f	160 ^g
	$LD_{10}^{d} (mg/m^2)$		>800	>60	>120	>2,000
	safety LD ₁₀ /GID ₇₅ e	margin:	>30	>13	>10	>13

^a The Histon II-As was used as a substrate for protein kinase C.^{56 b} Poly(Glu₄Tyr₁) was used as a substrate for EGF receptor kinase.^{56 c} GID₇₅: 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 LD₁₀: approximate 10% lethal dose at the treatment schedule. ^e Safety margin: the ratio LD₁₀/GID₇₅. ^f Compounds were injected iv twice/week for two consecutive weeks, and treatment was initiated when tumors grew to 0.2 cm³ 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 cm³ 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-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione (22j). Reduction of compound 21j using procedure 2 gave compound **22j** (33%). ¹H 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-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione (22k). Reduction of compound 21k using procedure 3 gave compound **22k** (35%). ¹H 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-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)dione (221). Reduction of compound 211 using procedure 1 gave compound **221** (86%). ¹H 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[3,4c]carbazole-5,7(6H)-dione (22m). Reduction of compound 21m using procedure 2 gave compound 22m (40%). ¹H NMR (400 MHz, DMSO- d_6) δ : 11.19 (1H, s), 9.80 (2H, brs), 8.85 (1H, d, J = 8.3Hz), 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, J = 8.3, 2.1 Hz)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); $[\alpha]_{D}^{26}$ 151.1 (*c* 0.92, DMSO). Anal. (C₃₃H₂₉N₅O₁₀·2H₂O· MeOH) C, H, N.

6-({[5-({[tert-Butyl(dimethyl)silyl]oxy}methyl)pyridin-2yl]methyl}amino)-2,10-dihydroxy-13-(β -D-glucopyranosyl)-12,13-dihydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione (22n). Reduction of compound **21n** using procedure 2 gave compound **22n**, which was used for the next reaction without further purification. ¹H 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, 2.1Hz), 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 = 10.2Hz), 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-(\beta-D-glucopyranosyl)-12,13dihydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-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%). ¹H 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 = 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-(\beta-D-glucopyranosyl)-12,13-dihydro-5H-indolo[2,3-*a***]pyrrolo[3,4***c*]**carbazole-5,7(6H)-dione (220).** Reduction of compound **210** using procedure 2 gave compound **220** (51%). ¹H 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-5H-indolo[2,3-a]pyrrolo[3,4*c*]carbazole-5,7(6H)-dione (22p). Reduction of compound 21p using procedure 1 gave compound 22p (68%). ¹H 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), 3.82–3.75 (1H, m), 3.58–3.46 (2H, m).

6-({[4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-3yl]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-(\beta-D-glucopyranosyl)-12,13-dihydro-5H-indolo[2,3-*a***]pyrrolo[3,4***c***]carbazole-5,7(6H)-dione (23b). Removal of TBDMS group of compound 22q** using procedure 4 gave compound **23b** (25%). ¹H 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-({[*I*-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-4yl]methyl}amino)-2,10-dihydroxy-13-(β-D-glucopyranosyl)-12,13dihydro-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. ¹H NMR (300 MHz, CD₃OD) 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 = 8.3Hz), 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-(\$\beta\$-D-glucopyranosyl)-12,13-dihydro-5H-indolo[2,3-a]pyrrolo[3,4*c*]carbazole-5,7(6H)-dione (23c). Removal of TBDMS group of compound **22r** using procedure 4 gave compound **23c** (49%). ¹H 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); $[\alpha]_{^{26}D}^{26}$ 141.5 (*c* 0.86, DMSO). Anal. (C₃₃H₂₉N₅O₁₀·2H₂O·MeOH) C, H, N.

Representative Procedure for the Reaction of Compounds 19 or 24 with Arylmethylbromides. 2,10-Dihydroxy-6-[(4-methylbenzvl)amino]-13-(*β*-D-glucopyranosvl)-12,13-dihydro-5*H*-indolo[2,3a]pyrrolo[3,4-c]carbazole-5,7(6H)-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 Na₂SO₄, and concentrated under reduced pressure. The residue was purified by LH-20 eluting with MeOH to give compound **26a** (14 mg, 23%). ¹H NMR (300 MHz, DMSO- d_6) δ 10.90 (1H, s), 10.36 (1H, s), 9.96 (1H, s), 8.70 (1H, d, J = 7.8Hz), 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. ¹H 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 **25a** by substituting 4-bromobenzylbro-mide **25c** with compound **25a** in 18% yield. ¹H 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 25a in 30% yield. ¹H 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. ¹H 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-(\beta-D-glucopyranosyl)-12,13-dihydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-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 NaHCO₃ 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 Na₂SO₄, and concentrated under reduced pressure. The residue was purified by LH-20 eluting with MeOH to give compound **53** (33 mg, 27%). ¹H NMR (300 MHz, DMSO- d_6) δ : 11.10 (1H, brs), 8.83 (1H, d, J = 8.4 Hz), 8.73 (1H, d, J = 8.4Hz), 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. ¹H 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-(\beta-D-glucopyranosyl)-12,13-dihydro-5*H***-indolo[2,3-***a***]pyrrolo[3,4-***c***]car-bazole-5,7(6***H***)-dione (55). Compound 55 was prepared in a similar manner to the synthesis of compound 53 by substituting [3-(hydrazi-nomethyl)phenyl]methanol hydrochloride 32 with compound 52a in 37% yield. ¹H NMR (300 MHz, DMSO-***d***₆) \delta: 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), 4.02 (1H, brs), 4.47 (2H, s), 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-5H-indolo[2,3-a]pyrrolo[3,4*c*]carbazole-5,7(6H)-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. ¹H NMR (300 MHz, DMSOd₆) 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-(\beta-D-glucopyranosyl)-12,13-dihydro-5H-indolo[2,3-*a***]pyrrolo[3,4***c*]**carbazole-5,7(6H)-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. ¹H 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 = 10.8Hz), 3.54–3.40 (2H, m).

2,10-Dihydroxy-6-({[2-(hydroxymethyl)pyridin-4-yl]methyl}amino)-13-(\beta-D-glucopyranosyl)-12,13-dihydro-5H-indolo[2,3-*a***]pyrrolo[3,4***c*]**carbazole-5,7(6H)-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. ¹H 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 = 10.5Hz), 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.

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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.

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