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Synthesis and biological evaluation of rebeccamycin analogues modified at the imide moiety

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ABSTRACT

Glycosylated indolocarbazoles related to the antibiotic rebeccamycin represent an important class of antitumour drugs. In the course of our structure–activity relationship studies, new rebeccamycin analogues modified at the imide moiety were synthesised. The antiproliferative activity of the compounds was evaluated on three human cancer cell lines, A2780 (ovarian cancer), H460 (lung cancer), and GLC4 (small-cell lung cancer). The in vitro cytotoxicity of compounds **2** and **4**, characterised respectively by a 1,3-dioxolan and (1,3-dioxolan-4-yl)methylene groups linked to the imide moiety, was higher than the reference compound, edotecarin. The effect of compound **2** in inducing tumour regression in the A2780 xenograft model was also investigated.

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Glycosylated [2,3-a] indolocarbazoles have attracted attention for their antitumour properties.[1] The microbial metabolite rebeccamycin has shown remarkable activity in the poisoning of topoisomerase I, a nuclear enzyme that resolves topological problems arising from numerous vital nuclear processes including DNA replication, transcription, recombination, and chromatin remodelling.[2–4] The crystal structure of reconstituted human topoisomerase I in covalent and noncovalent complexes with 22-base pair DNA duplexes was published[5,6] and a model for the interaction of this complex with the known topoisomerase I inhibitor, camptothecin,[7] and with other classes of inhibitors, indenoisoquinolines, indolocarbazoles and phenantridines,[8] proposed. Since the isolation of rebeccamycin, a large number of analogues has been designed to elucidate structure–activity relationships and to obtain compounds with better pharmacological properties.[9,10] In addition, the DNA-binding properties and the DNA sequence selectivity of this class of compounds were investigated.[11,12] The sugar moiety has been revealed to be fundamental for the biological activity, the indolocarbazole aglycone being completely devoid of activity; a β configuration at the glycosidic linkage is mandatory for topoisomerase I inhibition, as the α-*N*-glycosidic analogues do not behave as intercalating agents and have much less effect on enzyme inhibition; the nature and position of the substituents on the indolo rings influenced target interaction, as derivatives with hydroxy groups in the 2 and 10 positions showed a more favourable DNA intercalation and enzyme inhibition than those with chlorine atoms in the position 1 and 11; substitution with a methyl group on the indole nitrogen abolished both topoisomerase I inhibition and DNA intercalation. A few synthetic compounds structurally related to rebeccamycin, like NCS655649,[13] edotecarin[14] and BMS-250749[15] (Fig. 1) have entered clinical trials.

On the basis of these studies, a model for the drug-topoisomerase I-DNA ternary complex has been proposed in which the planar indolocarbazole residue is inserted between two consecutive base pairs disposing the sugar moiety into the major groove of the double helix. The substituent on the imide nitrogen is supposed to protrude toward the opposite groove where it can interact with topoisomerase I.[16] Later, an X-ray crystal structure of the indolocarbazole monosaccharide SA315F bound to a topoisomerase I-DNA complex, confirmed the intercalative binding mode of this class of compounds.[17] The solved structure revealed that the pyranosyl substiutent lay on the major groove side of the binding site while the maleimmide ring protruded into the minor groove side of the intercalation binding pocket with the substituted nitrogen, presumably protonated, positioned approximately 4 Å from Arg364, a residue that if mutated, confers resistance to different classes of topoisomerase I inhibitors.

Our interest in this class of compounds prompted us to synthesise indolocarbazole analogues modified both at the carbohydrate moiety—results on this work have been already reported[18]—and on the residue linked to the imide nitrogen, (the subject of this study), in order to evaluate the influence of these structural modifications on the biological activity of the new compounds.

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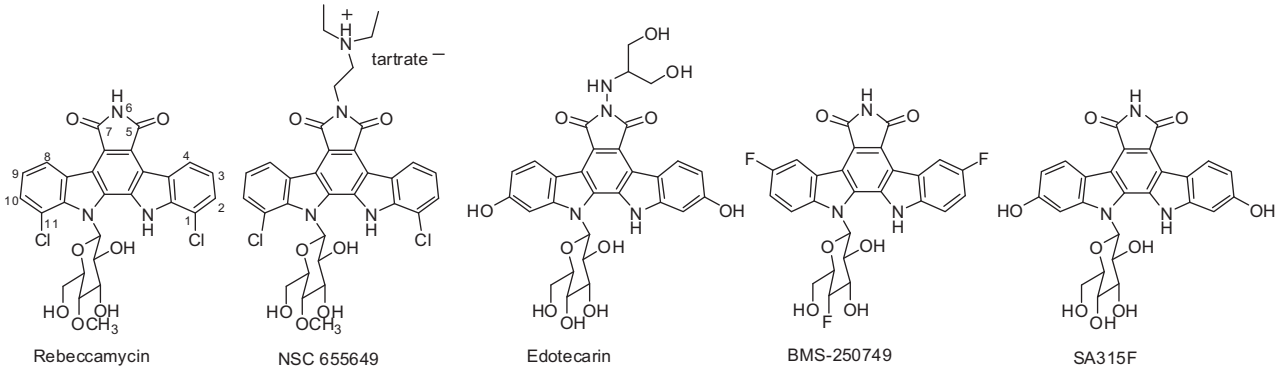
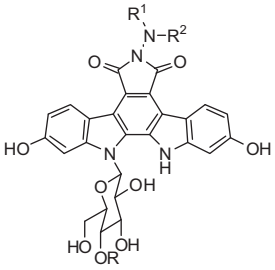


Figure 1. Chemical structures of some natural (rebeccamycin) and synthetic glycosylated indolocarbazole compounds.



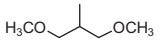
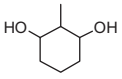
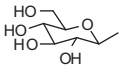
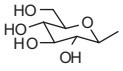
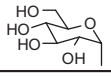
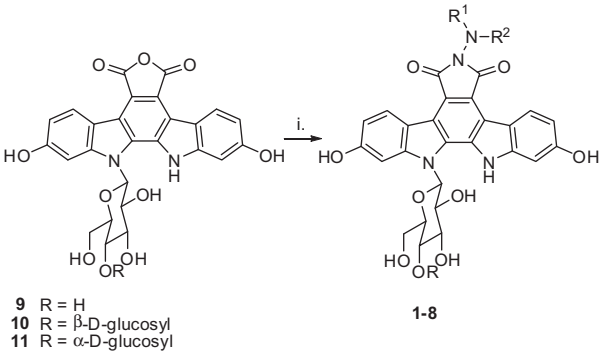
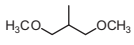
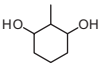
Compounds	R	R^1	R^2
1	H		H
2	H		H
3	H		-
4	H		H
5	H		H
6			H
7			H
8			H

Figure 2. Structures of the compounds **1**–**8**.



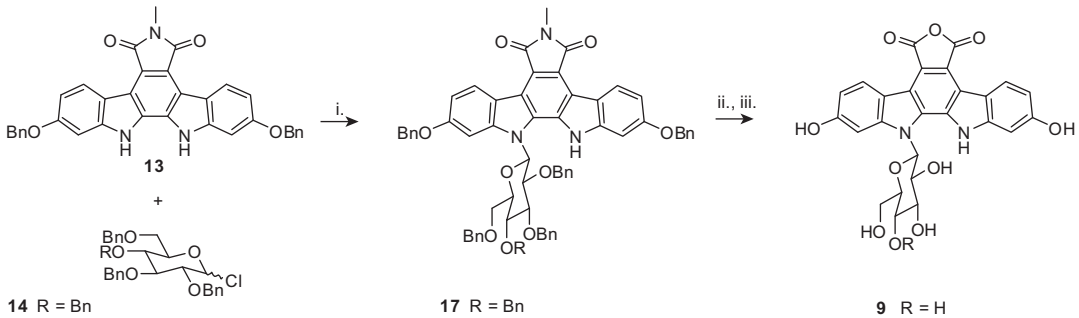
$R^1R^2NNH_2$	R^1	R^2
12a		H
12b		H
12c		-
12d		H
12e		H

Scheme 1. General synthetic scheme for compounds **1**–**8**.

Substitutions on the original unsubstituted imide group of rebeccamycin, and their influence on the biological activity of indolocarbazole derivatives, have been reviewed.[9,10,19] Analogues with amino, hydroxy, formylamino, methyl and imidazolylpropyl[20] groups introduced in the rebeccamycin or in the dechlorinated series, in most cases maintained the enzyme inhibition and the cytotoxic activity. In compounds like edotecarin and the rebeccamycin analogue NCS655649, in which respectively, a 2-hydroxy-1-(hydroxy-methyl)ethyl and a diethylaminoethyl group, are linked to the imide function, an increased potency with respect to the parent compounds, was observed. On the other hand, as previously noted, a methyl group on the imide nitrogen inhibited topoisomerase I inhibition while the anhydride group left the enzyme activity unaffected.

The new derivatives are listed in Figure 2. They all share the 2,10-dihydroxy indolocarbazole framework found in edotecarin. Compounds **1**–**5** bear the same β-linked glucosyl residue also present in edotecarin, while compounds **6**–**8** have either a β-glucosyl-β-glucose (**6**, **7**) or an α-glucosyl-β-glucose (**8**) disaccharide moiety linked to the indolocarbazole aglycone. The main concern in the selection of the residues linked to the imide function, was the retention of the hydrophilic character of these groups. Polar groups were introduced in more constrained structures with respect to edotecarin, with the aim of preserving the solubility of the new derivatives, and to evaluate how these modifications would affect the interactions in the binding pocket and hence the biological activity of these derivatives.

The synthesis of compounds **1**–**8** is outlined in Scheme 1. The key precursors in this synthetic sequence were the anhydrides



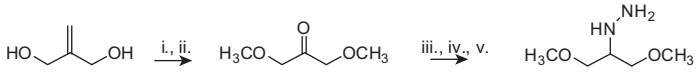
i. KOH, Na_2SO_4, CH_3CN, r.t.; ii. 10% Pd/C, H_2, CH_2Cl_2/CH_3OH 2/1, r.t.; iii. 2M aq. KOH, r.t.

Scheme 2. General synthetic scheme for anhydrides **9**–**11**.

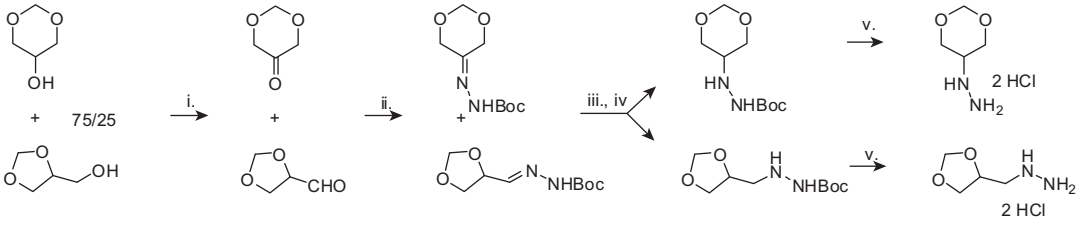
9–**11** bearing either the mono or disaccharide moiety. These were reacted with hydrazines **12a–e** in DMF at 60–70 °C. After standard work-up (dilution with water, extraction of the product in ethyl acetate/methylethylketone and silica gel chromatography) the final compounds **1**–**8** were obtained in yields ranging from 70% to 80%.

Anhydrides **9**–**11** were obtained following a convergent synthetic strategy in which the indolopyrrolocarbazole moiety **13**, easily obtained using the method of Ohkubo and co-workers[21] was coupled with different mono- and disaccharides (Scheme 2). *N*-Glycosylation of aglycone **13** was achieved using 1-chloro glycosides as glycosyl donors in heterogeneous basic media.[22] This glycosylation protocol is reported to promote almost exclusively the formation of the β-glycoside (ratio α/β anomer = 1/10). Glycosyl donor **14** was quantitatively obtained from commercially available tetra-*O*-benzyl-D-glucose by treatment with oxalyl chloride under standard conditions. 1-Chloro disaccharides **15** and **16** were obtained by elaboration of D-glucose and tetra-*O*-benzyl-D-glucose as already reported.[18] Deprotection of the benzyl groups, followed by standard elaboration of the *N*-methyl imide function, gave the corresponding glycosylated anhydrides.

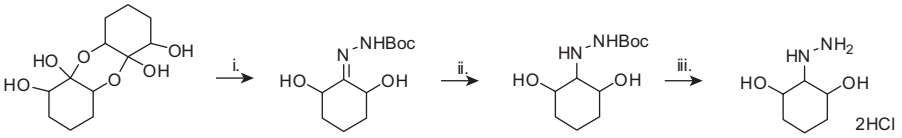
The hydrazines **12a–e** were either purchased (**12c**) or synthesised from commercially available precursors following synthetic procedures reported in the literature (Scheme 3). 2-Methylene-1,3-propandiol was elaborated to (1,3-dimethoxypropan-2-yl)hydrazine **12a**, by methylation of the hydroxyl groups, double bond oxidation and treatment of the resulting ketone with *tert*-butylhydrazine carboxylate. A mixture of 1,3-dioxan-5-ol and (1,3-dioxolan-4-yl)methanol was oxidised with oxalyl chloride, DMSO and triethylamine to the corresponding carbonyl com-



i. KOH, MeI, DMSO, r.t., 3h; ii. $KMnO_4$, Al_2O_3, H_2O, CH_2Cl_2, r.t.; iii. $BocNHNH_2$, EtOH, r.t., 12h; iv. $NaCNBH_3$, 6M HCl, MeOH, 5h; v. 6M HCl, H_2O, 4h r.t.



i. $(COCl)_2$, DMSO, Et_3N, r.t.; ii. $BocNHNH_2$, EtOH, r.t., 4h; iii. BH_3.THF,THF, r.t; iv. Flash chromatography (SiO_2, toluene/AcOEt 6/4); v. AcCl, MeOH, r.t., 1.5h



i.$BocNHNH_2$, EtOH, r.t., 4h; ii. $NaCNBH_3$, 6M HCl, MeOH, 5h; iii. 2M HCl

Scheme 3. General synthetic scheme for hydrazides **12a**–**e**.

pounds. Their reaction with *tert*-butoxycarbonylhydrazine gave the corresponding hydrazones, which were reduced and separated by flash chromatography. Deprotection of the *tert*-butoxycarbonyl group afforded hydrazides **12b** and **12e**. In an analogous fashion, the reaction of 2-hydroxycyclohexanone dimer with *tert*-butoxycarbonylhydrazine and the reduction of the resulting hydrazone with sodium cyanoborohydride gave, after deprotection of the Boc group, hydrazine **12d**.

In order to verify drug target inhibition, a preliminary evaluation of the new compounds was performed in agarose gels using a DNA cleavage assay based on human recombinant topoisomerase I (data not shown). Next, the in vitro antiproliferative activity of compounds **1**–**8** was determined against a panel of three human cancer cell lines—A2780 (ovarian cancer), H460 (lung cancer) and GLC-4 (small-cell lung cancer)- using edotecarin and camptothecin as reference compounds. The Sulforhodamine B (SRB) assay[23] was used to determine the activity of the new indolocarbazole derivatives and the reference compounds. All compounds were dissolved in sterile DMSO and diluted with saline (0.9% NaCl) immediately before use. A2780 (2000 cells/well), H460 (1500 cells/well) and GLC-4 (1500 cells/well) cell lines were seeded in 96-well microtiter plates and incubated for 24 h at 37 °C in a 5% CO_2 incubator. Drugs were then added to the wells to achieve final drug concentrations ranging from 0.001 to 10 μM. After 24 h of drug exposure, cells were washed twice with phosphate buffer saline (PBS) and incubated in drug-free medium for about three doubling times (72 h), at which point the cellular viability was measured using the SRB assay. The IC_{50} (the concentration achieving 50% cellular mortality compared to untreated control) was evaluated by a curve in which the surviving percentage of cells was reported as a function of the drug concentration. The H460 cell line responded weakly to the tested compounds, while in the A2780 and GLC4 cell lines the new compounds showed similar cytotoxicity profiles (Table 1).

Among the monosaccharides derivatives, compounds **2** and **4**, displayed a higher cytotoxicity with respect to edotecarin. Compound **2**, characterised by a 1,3-dioxolan moiety linked to the imide nitrogen, was 10-fold more potent than edotecarin (IC_{50} 0.7 nM and IC_{50} 6.5 respectively) in the A2780 cell line and equipotent in the GLC4 cell line (IC_{50} 0.7 nM and IC_{50} 0.8 nM respectively), while compound **4**, bearing a (1,3-dioxolan-4-yl)methylene group, was 3-fold more potent (IC_{50} 1.7 nM) in the A2780 cell line and equipotent in the GLC4 cell line (IC_{50} 0.8 nM). The other monosaccharide analogues **1**, **3**, and **5**, exhibited reduced cytotoxicity in comparison to edotecarin (respectively 2-fold, 10-fold and 20-fold less than the reference compound in the A2780 cell line).

When the promising 1,3-dioxolan and the (1,3-dioxolan-4-yl)methylene groups were introduced in anhydrides **10**–**11** bearing disaccharide residues, the resulting compounds **6**–**8** did not maintain the activity of the corresponding monosaccharide analogues. Compound **6**, characterised by a 1,3-dioxolan moiety and a β-D-glucosyl-β-D-glucose linked to the indolocarbazole core, exhibited a considerable loss of activity both in the A2780 (IC_{50} 127 nM) and in the GLC4 cell line (IC_{50} 70 nM). Derivatives **7** and **8**, bearing the (1,3-dioxolan-4-yl)methylene group and a β-D-glucosyl-β-D-glucose or an α-D-glucosyl-β-D-glucose respectively, showed decreased activity in both the cell lines used. It is noteworthy that, the α-1,3 configuration between the two glucosyl moieties (**8**) seemed slightly more favourable than the β-1,3 one (**7**) in both the cell lines (**7**: IC_{50} 55 nM in A2780 cell line; IC_{50} 74 nM in GLC4 cell line; **8**: IC_{50} 11.7 nM in A2780 cell line, IC_{50} 5.3 nM in GLC4 cell line).

Table 1
Cytotoxic activities of compounds **1**–**8** (IC_{50}, in nM)

Compound	A2780	H460	GLC4
1	12.7 ± 7.3	>125	3.8 ± 0.8
2	0.7 ± 0.1	>250	0.7 ± 0.3
3	54 ± 9.5	>1000	90 ± 9.5
4	1.7 ± 1.1	>1000	0.8 ± 0.4
5	90 ± 9	>250	101 ± 19
6	127 ± 28	700 ± 50	70 ± 16
7	55 ± 22	113 ± 8	74 ± 3
8	11.7 ± 6.3	>250	5.3 ± 1.3
Edotecarin	6.5 ± 0.4	193 ± 145	0.8 ± 0.1
Cpt[a]	7 ± 6	5 ± 4	10 ± 8

[a] Cpt = Camptothecin.

Table 2
Antitumour efficacy of compound **2** on A 2780 xenograft model

Compound	Dose ($mgKg^{-1}$)	Schedule, Route	TVI[a] (%)	Deaths/ Total[b]
2	90	q4dx4, i.v.	99.7	0/5
Edotecarin	90	q4dx4, i.v	99.8	0/5

[a] Tumour volume inhibition in treated versus control mice determined at the nadir of tumour volume in the treated group.
[b] Number of deaths out of the total number of mice.

Compound **2** was evaluated in a preliminary experiment for its effect in inducing tumour regression in the A2780 human ovarian carcinoma xenograft model. Human tumour cell lines A2780 (ECACC) were originated from subcutaneous (s.c.) in vivo injection of tumour cells (10×10^6 cells/flank/0.2 ml). Tumour cells were suspended in a 0.9% NaCl sterile solution and 0.2 ml of tumour cells suspension was injected into the right flank of female nude mice. Female athymic nude mice, 6–8 weeks old, were purchased from Harlan Italy, maintained in microisolator cages and supplied with sterile materials under standard conditions, according to UKCCCR guideline.[24] For anti-tumour activity experiments, each experimental group included five mice bearing a subcutaneous tumour in the right flank. Tumour growth was followed by calibre measurement of length and width at predetermined (weekly or twice weekly) times. Tumour volume (TV) was calculated using the formula: volume in mm^3 = $width^2 \times length/2$ (from Ref. 25). The compound was dissolved in sterile water for injection and diluted with saline (0.9% NaCl) immediately before use. It was administrated i.v. with a twice weekly schedule (q4dx4) at the same dose as edotecarin and at a dose volume of 10 ml/kg (Table 2). Drug treatment started when the tumours were approximately 50 mm^3 in volume.

Compound **2** showed an efficacy comparable to that of edotecarin in terms of tumour volume inhibition, and no significant toxicity was observed at the schedule and dosage used.

In summary, a series of indolocarbazole glycosides (**1–8**)[26] modified in the upper heterocycle and bearing both mono- and disaccharide residues were synthesised and their biological activity was investigated on a panel of tumour cell lines. The preservation of the hydrophilic character was the main concern in the choice of the groups linked to the imide nitrogen. Two compounds (**2** and **4**), bearing respectively the 1,3-dioxolan and the (1,3-dioxolan-4-yl)methylene groups linked at the imide nitrogen and the same glucose residue as edotecarin, exhibited a higher in vitro activity with respect to this reference compound. One of them (**2**) was also evaluated on an A2780 xenograft model. These results showed that introduction of hydrophilic substituents at position 6 on the nitrogen imide may represent a profitable route for the design of a new class of anticancer compounds. The planned studies on the binding mode of these new indolocarbazole glycosides could aid in making further improvements.

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References and notes

1. Prudhomme, M. *Curr. Pharm. Des.* **1997**, *3*, 265.
2. Wang, J. C. *Annu. Rev. Biochem.* **1996**, *65*, 635.
3. Pommier, Y.; Pourquier, P.; Fan, Y.; Strumberg, D. *Biochim. Biophys. Acta* **1998**, *1400*, 83.
4. Capranico, G.; Marinello, J.; Baranello, L. *Biochim. Biophys. Acta* **2010**, *1806*, 240.
5. Redimbo, M. R.; Stewart, L.; Kuhn, P.; Champoux, J. J.; Hol, W. G. J. *Science* **1998**, *279*, 1504.
6. Redimbo, M. R.; Champoux, J. J.; Hol, W. G. J. *Biochemistry* **2000**, *39*, 6832.
7. Kerrigan, J. E.; Pilch, D. S. *Biochemistry* **2001**, *40*, 9792.
8. Pommier, Y. *Chem. Rev.* **2009**, *109*, 2894.
9. Prudhomme, M. *Eur. J. Med. Chem.* **2003**, *38*, 123.
10. Prudhomme, M. *Curr. Med. Chem. Anticancer Agents* **2004**, *4*, 509.
11. Bailly, C.; Colson, P.; Houssier, C.; Rodrigues-Pereira, E.; Prudhomme, M.; Waring, M. J. *Mol. Pharmacol.* **1998**, *53*, 77.
12. Bailly, C.; Riou, J.-F.; Colson, P.; Houssier, C.; Rodrigues-Pereira, E.; Prudhomme, M. *Biochemistry* **1997**, *36*, 3917.
13. Dowlati, A. et al *J. Clin. Oncol.* **2001**, *19*, 2309.
14. Danny, W. *IDrugs* **2004**, *7*, 137.
15. Saulnier, M. G.; Balasubramanian, B. N.; Long, B. H., et al *J. Med. Chem.* **2005**, *48*, 2258.
16. Bailly, C.; Carrasco, C.; Hamy, F.; Vezin, H.; Prudhomme, M.; Saleem, A.; Rubin, E. *Biochemistry* **1999**, *38*, 8605.
17. Staker, B. L.; Feese, M. D.; Cushman, M.; Pommier, Y.; Zembo-wer, D.; Stewart, L.; Burgin, A. B. *J. Med. Chem.* **2005**, *48*, 2336.
18. Animati, F.; Berettoni, M.; Bigioni, M.; Binaschi, M.; Felicetti, P.; Gontrani, L.; Incani, O.; Madami, A.; Monteagudo, E.; Olivieri, L.; Resta, S.; Rossi, C.; Cipollone, A. *Chem. Med. Chem.* **2008**, *3*, 266.
19. Prudhomme, M. *Curr. Med. Chem.* **2000**, *7*, 1189.
20. Zhang, G.; Shen, J.; Cheng, H.; Zhu, L.; Fang, L.; Luo, S.; Muller, M.; Lee, G. E.; Du, Y.; Sun, D.; Wang, P. G. *J. Med. Chem.* **2005**, *48*, 2600.
21. Okhubo, M.; Nishimura, T.; Jona, H.; Honma, T.; Morishima, H. *Tetrahedron* **1996**, *52*, 8099.
22. Okhubo, M.; Kawamoto, H.; Ohno, T.; Nakano, M.; Morishima, H. *Tetrahedron* **1997**, *53*, 585.
23. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesh, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
24. United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia, 2nd Ed., *Br. J. Cancer* **1998**, *77*, 1.
25. Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1.
26. *Data for compounds **1**–**8**:* 6-(1,3-Dimethoxypropan-2-ylamino)-2,10-dihydroxy-12-β-D-glucopyranosyl-12,13-dihydro-5*H*-indolo-[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6*H*)-dione (**1**): ^{1}H NMR (300 MHz, DMSO-d_6) 11.20 (1H, s), 9.77 (2H, br s), 8.89 (1H, d, *J* = 6 Hz), 8.81(1H, d, *J* = 6 Hz), 7.18 (1H, s), 7.00 (1H, s), 6.82 (2H, dt), 5.98 (1H, d, *J* = 6 Hz), 5.89 (1H, br s), 5.54(1H, s), 5.37 (1H, br s), 5.14 (1H, br s), 4.03 (1H, d, *J* = 6 Hz), 3.98–3.88 (2H, m), 3.79 (1H, d, *J* = 6 Hz), 3.67–3.60 (2H, m), 3.56–3.50 (2H, br s), 3.44 (4H, d, *J* = 6 Hz), 3.24 (6H, s); ESI-MS (*m*/*z*): ES$^-$ 635.2 [M-H]$^-$, (Calcd 636.21). 6-(1,3-Dioxan-5-ylamino)-2,10-di-hydroxy-12-β-D-glucopyranosyl-12,13-dihydro-5*H*-indolo[2,3-a]-pyrrolo-[3,4-c]carbazole-5,7(6*H*)-dione (**2**): ^{1}H NMR (300 MHz, DMSO-d_6) 11.21 (1H, s), 9.79 (2H, br s), 8.88 (1H, d, *J* = 6 Hz), 8.81(1H, d, *J* = 6 Hz), 7.19 (1H, s), 7.01 (1H, s), 6.83 (2H, dt), 5.98 (1H, d, *J* = 6 Hz), 5.90 (1H, br s), 5.85 (1H, d, *J* = 3 Hz), 5.37 (1H, br s), 5.14 (1H, br s), 4.92 (1H, s), 4.83(1H, d, *J* = 6 Hz), 4.66 (1H, d, *J* = 6 Hz), 4.11 (1H, dd), 4.06–4.01 (2H, m), 3.98–3.90 (2H, m), 3.79 (1H, d, *J* = 6 Hz), 3.72–3.64 (1H, dd), 3.57–3.48 (2H, m); ESI-MS (*m*/*z*): ES$^-$ 619.2 [M-H]$^-$, (Calcd 620.18). 2,10-Dihydroxy-6-morpholino-12-β-D-glucopyranosyl-12,13-dihydro-5*H*-indolo-[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6*H*)-dione (**3**): ^{1}H NMR (300 MHz, DMSO-d_6) 11.24 (1H, s), 9.84–9.72 (2H, br s), 8.88 (1H, d, *J* = 6 Hz), 8.78 (1H, d, *J* = 6 Hz), 7.20 (1H, s), 7.00 (1H, s), 6.82 (2H, t like), 5.98 (1H, d, *J* = 6 Hz), 5.89 (1H, br s), 5.34(1H, br s), 5.12 (1H, br s), 4.90 (1H, br s), 4.08–3.99 (1H, m), 3.97–3.89 (2H, br s), 3.81–3.73 (4H, br s), 3.54–3.27 (7H, m); ESI-MS (*m*/*z*): ES+ 605.2 [M+H]$^+$, ES$^-$ 603.1 [M−H]$^-$, (Calcd 604.18). 6-((1,3-dioxolan-4-yl)methylamino)-2,10-di-hydroxy-12-β-D-glucopyra-nosyl-12,13-dihydro-5*H*-indolo[2,3-a]-pyrrolo[3,4-c]carbazole-5,7(6*H*)-dione (**4**): ^{1}H NMR (300 MHz, DMSO-d_6) 11.21 (1H, s), 9.84 (2H, br s), 8.88 (1H, d, *J* = 6 Hz), 8.79 (1H, d, *J* = 6 Hz), 7.18 (1H, s), 7.00 (1H, s), 6.82 (2H, dt), 5.97 (1H, d, *J* = 6 Hz), 5.91 (1H, t), 5.47 (1H, br s), 5.23 (1H, br s), 4.99 (1H, br s), 4.89 (2H, s), 4.75 (2H, s), 4.18 (1H, p), 4.03–3.99 (2H, m), 3.97–3.87 (2H, m), 3.77 (1H, d like), 3.74–3.71 (4H, dd, *J* = 6 Hz), 3.56–3.46 (2H, m), 3.10–3.03 (1H, m); ESI-MS (*m*/*z*): ES$^-$ 619.2 [M−H]$^-$, (Calcd 620.18). 6-(2,6-Dihydroxycyclo-hexylamino)-2,10-dihydroxy-12-β-D-glucopyranosyl-12,13-dihy-dro-5*H*-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6*H*)-dione (**5**): ^{1}H NMR (300 MHz, DMSO-d_6) 11.23 (1H, s), 9.88–9.69 (4H, m), 8.93–8.74 (4H, m), 7.20 (2H, s), 7.00 (2H, s), 6.88–6.77 (4H, m), 6.02–5.84 (4H, m), 5.67 (1H, m), 5.40–5.29 (2H, m), 5.12 (1H, br s), 4.96–4.88 (1H, m), 4.87–4.82 (1H, m), 4.73–4.65 (1H, m), 4.60–4.52(1H, m), 4.07–3.98 (1H, m), 3.88–3.76 (2H, m), 3.75–3.57 (1H, m), 3.01–2.92 (2H, m), 1.99–1.51 (4H, m); ESI-MS (*m*/*z*): ES$^-$ 647.2 [M−H]$^-$, (Calcd 648.62). 6-(1,3-Dioxan-5-ylami-no)-12-[3′-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-2,10-di-hydroxy-12,13-dihydro-5*H*-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6*H*)-dione (**6**): ^{1}H NMR (300 MHz, DMSO-d_6) 11.18 (1H, s), 9.83 (2H, br s), 8.87 (1H, d, *J* = 6 Hz), 8.79 (1H, d, *J* = 6 Hz), 7.21 (1H, s), 7.00 (1H, s), 6.84 (2H, t), 6.16 (1H, d, *J* = 6.0 Hz), 6.05 (1H, br s), 5.87 (1H, d, *J* = 3.0 Hz), 5.76 (2H, s), 5.05–4.97 (4H, m), 4.88–4.82 (2H, m), 4.67–4.62 (2H, m), 4.32 (1H, d, *J* = 6 Hz), 4.12–3.98 (6H, m), 3.88–3.81 (1H, m), 3.80–3.63 (6H, m), 3.45–3.36 (1H, m), 3.24–3.17 (1H, t), 3.15–2.93 (3H, m); ESI-MS (*m*/*z*): ES$^-$ 781.3 [M−H]$^-$, (Calcd 782.23). 6-((1,3-Dioxolan-4-yl)-methyl-amino)-12-[3′-*O*-(β-D-glucopyrano-syl)-β-D-glucopyranosyl]-2,10-dihydroxy-12,13-dihydro-5*H*-indolo[2,3-a]pyrrolo-[3,4-c]-carbazole-5,7(6*H*)-dione (**7**): ^{1}H NMR (300 MHz, DMSO-d_6) 11.18 (1H, s), 9.83 (2H, br s), 8.89 (1H, d, *J* = 6 Hz), 8.80 (1H, d, *J* = 6 Hz), 7.21 (1H, s), 7.00 (1H, s), 6.84 (2H, t), 6.15 (1H, d, *J* = 6.9 Hz), 6.05 (1H, br s), 5.89 (1H, t), 5.06–4.92 (4H, m), 4.89 (1H, s), 4.83 (1H, br s), 4.76 (1H, s), 4.63 (1H, br s), 4.33 (1H, d, *J* = 6 Hz), 4.19 (1H, p), 4.04–3.98 (3H, m), 3.85–3.65 (4H, m), 3.46–3.32 (1H, m), 3.24–3.17 (1H, m), 3.14–2.93 (4H, m); ESI-MS (*m*/*z*): ES$^-$ 781.2 [M−H]$^-$, (Calcd 782.23). 6-((1,3-Dioxolan-4-yl)-methylamino)-12-[3′-*O*-(α-D-glucopyranosyl)-β-D-glucopyrano-syl]-2,10-dihydroxy-12,13-dihydro-5*H*-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6*H*)-dione (**8**): ^{1}H NMR (300 MHz, DMSO-d_6) 11.19 (1H, s), 9.88 (2H, br s), 8.88 (1H, d, *J* = 6 Hz), 8.80 (1H, d, *J* = 6 Hz), 7.21 (1H, s), 7.02 (1H, s), 6.83 (2H, t), 6.11 (4H, d, *J* = 6 Hz), 5.91 (1H, t), 5.71 (1H, br s), 5.33 (1H, br s), 4.98 (1H, d, *J* = 3 Hz), 4.88 (1H, s), 4.75 (1H, s), 4.62 (1H, m), 4.25–4.12 (2H, m), 4.06–3.98 (3H, m), 3.84–3.69 (2H, m), 3.62–3.54 (1H, m), 3.11–2.97 (2H, m); ESI-MS (*m*/*z*): ES$^-$ 781.3 [M−H]$^-$, (Calcd 782.23).