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Synthesis, cytotoxic activities and cell cycle arrest profiles of naphtho[2,1-α]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,12*H*)-dione glycosides

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ABSTRACT

Naphtho[2,1-α]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,12*H*)-dione (NPCD) is known to be a very potent and selective cyclin D1-CDK4 inhibitors and could induce strong G1 phase arrest in breast tumor cell lines. In this work, the synthesis of five NPCD glycosides and their cytotoxic activities against eight tumor cell lines are presented, as well as the investigation of their cell cycle arrest profiles. The results showed that the introduction of a sugar moiety onto NPCD did not affect much of their cytotoxic activities, while the subtle structure of the sugar moiety affected the underlying mechanism strongly. In addition, NPCD showed distinct cell-cycle arrest profiles in BxPC3 prostate cells and MCF-7 breast cells, while NPCD glycosides shared similar cell cycle arrest profiles in MCF-7 and BxPC3 cells, which also indicated that not only the indolocarbazole framework as well known before but the sugar moiety can have a profound impact on the mechanism of action for these types of compounds.

The glycosylated indolo[2,3-α]carbazole represents an interesting class of compounds that exhibit diverse biological activities.[1] There are two classes of indolocarbazole glycosides, differ both in structure and in mechanism of action (Fig. 1). The staurosporine class containing two glycosidic bonds linked to the indolocarbazole heterocycle is inhibitors of protein kinases.[2] In contrast, the rebeccamycin[3] class of indolocarbazole glycosides has a single glycosidic linkage and has shown remarkable activity in the poisoning of DNA topoisomerase I.[4] And this latter class is currently under evaluation in the clinic for treatment of a variety of malignancies including advanced renal carcinoma, metastatic or locally recurrent colorectal cancer and stage IIIB or IV breast cancer.[5]

Recently, several aryl[α]pyrrolo[3,4-*c*]carbazole analogues have been developed as very potent CDK (cyclin-dependent kinases) inhibitors.[6] For instance, naphtho[2,1-α]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,12*H*)-dione[7] (NPCD, Fig. 1), in which one indole ring is replaced by a naphthyl ring, is a very potent and selective cyclin D1-CDK4 inhibitors. Cyclin-CDK complexes regulate the progression of cells through the cell cycle. To date, strong evidence suggests a G1-phase role for D-type cyclins through associate with CDK4 and CDK6.[8] Our previous work[9] has proved that NPCD can cause long-lasting growth arrest in G1 phase and cell death of breast cancer cell lines at an IC_{50} of 3–8 μM. Decreased phosphorylation of Rb by D1-CDK4/6 and decreased p27kip1 protein level may be part of the underlying mechanism.[9]

With our continuous interest in the effect of sugar attachments to a planar aromatic molecule on the biological activities[10] and in order to search for potential antitumor agents of NPCD class with high developability value, we decide to synthesize and evaluate NPCD glycosides on the tumor cell growth inhibitory activities.

Previous studies[11] have shown that rebeccamycin with just a single *N*-glycosidic bond favors a single conformation in which the pyranose oxygen atom grips the indole NH group through an intramolecular hydrogen bond (Fig. 1). Whereas when the indole NH proton of indolocarbazole glycosides is replaced with a methyl group, which is certain to prevent intramolecular hydrogen bonding, the biological activity is severely compromised in all cases tested.[12] As for NPCD glycoside, since one indole ring is replaced by a naphthyl ring, the oxygen atom in the sugar ring thus has no chance to form an intramolecular hydrogen bond like that it performs in rebeccamycin. However, our present study showed that all of NPCD glycosides tested still displayed tumor cell growth inhibitory activities as strong as NPCD. And interestingly the subsequent cell cycle arrest investigation indicated that the underlying mechanism might be very different from that of NPCD. The results obtained will render new clues to the understanding of the anticancer profile for these types of compounds.

Several typical sugar heads, such as α-L-rhamnopyranosyl, α-D-ribopyranosyl, β-D-glucopyranosyl, β-D-ribofuranosy, 2-deoxy-β-D-ribofuranosyl were introduced onto the indole nitrogen atom of NPCD (**1a**–**e**, Table 1).[13]

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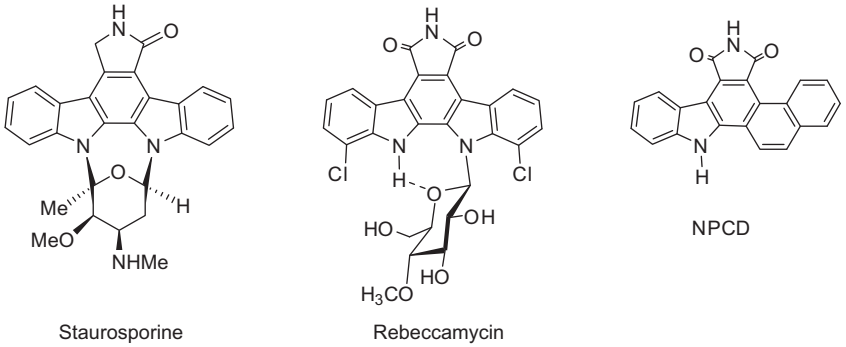
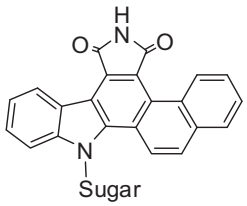


Figure 1. Chemical structures of staurosporine, rebeccamycin and NPCD.

Table 1
Structures of NPCD and its glycosides **1a**–**e**



Compound	R
NPCD	H
1a	α-L-Rhamnopyranosyl
1b	α-D-Ribopyranosyl
1c	β-D-Glucopyranosyl
1d	β-D-Ribofuranosyl
1e	2-Deoxy-β-D-ribofuranosyl

All of the NPCD glycosides were synthesized following an optimized modular synthetic approach developed by Faul et al.[14] (Scheme 1). The key intermediates maleimides **3a**–**e** were prepared in good yields by condensation of (*N*-glycosyl indolyl)-3-glyoxylyl esters **2a**–**e** with the complementary naphthylacetamide in the presence of *t*-BuOK. Deprotection of the PMB groups on the sugar rings of **3a**–**e** provided compounds **4a**–**e**. Cyclizations of **4a**–**e** to the *N*-glycosyl naphthylcarbazoles **1a**–**e** were achieved by irradiation of their dry acetone solution with Osram Hg HP (HQL 125W) lamp for 48 h.

(*N*-Glycosyl indolyl)-3-glyoxylyl esters **2a**–**c** which contain the sugar moiety of pyranosyl configuration were prepared as shown in Scheme 2. Direct coupling of the free sugars with indoline heated at reflux in ethanol furnished the indoline N-glycosides **5a**–**c** (α-glycosidic bond for **5a**, α-glycosidic bond for **5b**, β-glycosidic bond for **5c**). Upon treated by DDQ, crude **5a**–**c** were converted to indolyl N-glycosides **6a**–**c** smoothly, which were then protected on the sugar hydroxyls by PMB groups to provide **7a**–**c**. Compounds **2a**–**c** were then prepared in good yields by treatment of indolyl N-glycosides **7a**–**c** in Et_2O with oxalyl chloride, followed by sodium methoxide at a temperature of −60 °C.

The synthesis of (*N*-glycosyl indolyl)-3-glyoxylyl esters **2d** and **2e**, which both contain the sugar moiety of furanosyl configuration, were described in Scheme 3. Ribofuranosyl per-acetate **8** was employed as the glycosyl donor to be coupled with indoline to form the *N*-(β-D-ribofuranosyl) indoline **9**, which was then treated with DDQ to convert the indoline moiety to an indole ring (compound **10**). While for the preparation of *N*-(2-deoxy-β-D-ribofuranosyl) indole **12**, *p*-methyl benzoyl protected 2-deoxy-α-D-ribofuranosyl chloride **11** was employed as the donor to carry out the glycosylation with indole directly in the presence of NaH. Compounds **10** and **12** were deesterified and the resulting intermediates were then protected by PMB groups on the sugar hydroxyls to form compounds **13** and **14**, respectively. Upon treated with oxalyl chloride and sodium methoxide at a low temperature (−60 °C), compounds **13** and **14** were converted to **2d** and **2e** smoothly.

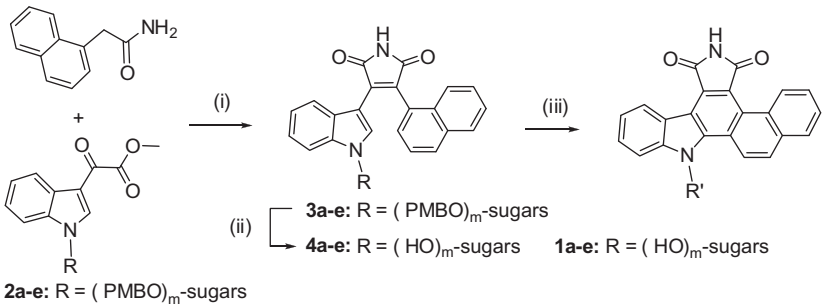
The new NPCD glycosides were screened for their tumor cell growth inhibitory activities by 74 h drug exposure. The IC_{50} values of NPCD glycosides **1a**–**e** along with NPCD for comparison against eight tumor cell lines are presented in Table 2. All the NPCD glycosides displayed good antitumor cell growth activities as strong as NPCD. These results indicated that introduction of a sugar moiety onto NPCD did not affect much of the cytotoxic activities.

Analysis of cell cycle profile with FACS revealed that NPCD treatment of MCF-7 breast cells at a concentration of 10 μM for 24 h significantly arrested the cells at G1 phase, as evidenced by an increased percentage of the cells at G1 phase and a corresponding decrease in the percentage of the cells at S phase (Fig. 2). However, NPCD glycosides **1a**–**e** did not show any G1-phase arrest, instead slight to strong S phase arrests were observed at a concentration of 10 μM (Fig. 2). These results suggested the underlying mechanisms of NPCD glycosides might be very different from that of NPCD. In addition, the different cell cycle arrest profiles of NPCD glycosides between themselves revealed that the subtle structure of sugar moiety in these compounds affected the underlying mechanism strongly.

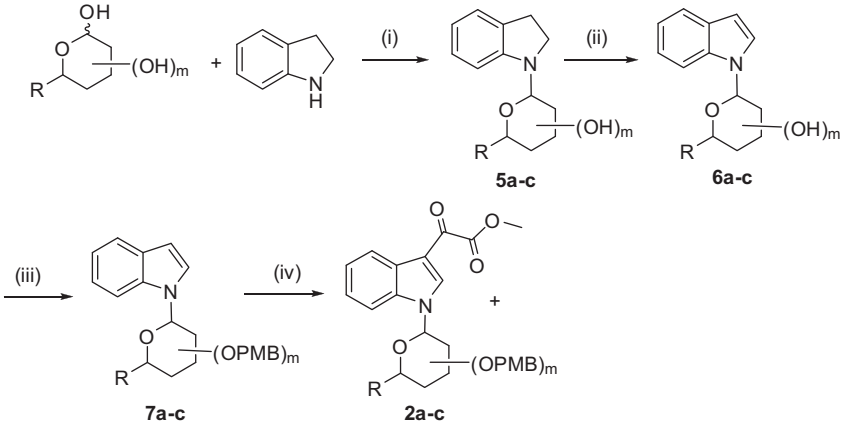
Although we have proved before that NPCD targeted D1-CDK4/6 and thus induced G1-phase arrest in several breast tumor cell lines including MCF-7[9], this has not been proved in other gland cell lines before. But interestingly, when BxPC3 prostate cells were treated with NPCD (10 μM), a significant increased percentage of the cells at G2/M phase was observed (Fig. 3), which suggested that NPCD might target the critical regulators of G2/M progression[8] of BxPC3 cells. The different cell-cycle arrest profiles of NPCD in MCF-7 and BxPC3 cells make it an interesting molecule for biological research.

In contrast, the NPCD glycosides, especially for **1c**–**e**, which displayed clear S phase arrest effect in MCF-7 cells, still induced strong S phase arrest in BxPC3 cells. The results suggested that these NPCD glycosides probably targeted the same cell-cycle regulators in BxPC3 cells as those in MCF-7 cells, respectively, which indicated the sugar moieties played a more important role here than their aglycon part (NPCD part) during the interaction with their targets, otherwise they might change much of the cell cycle arrest profiles as NPCD did. The detailed mechanisms of NPCD and its glycosides on the cell cycles both in MCF-7 and BxPC3 cells were under investigation.

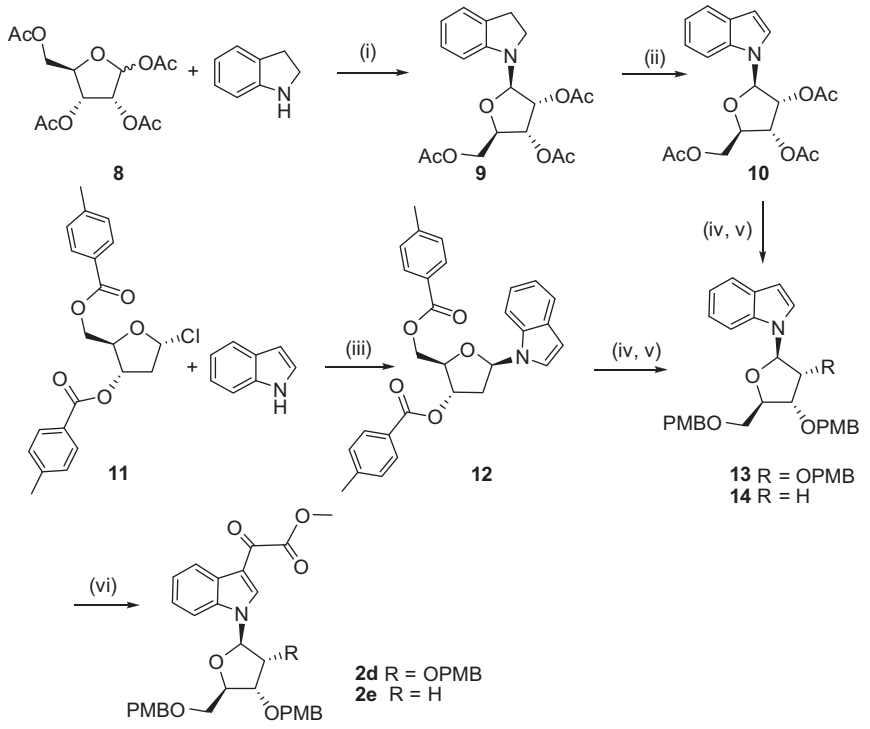
In conclusion, we have conducted the synthesis and evaluation of a panel of NPCD glycosides on their cytotoxic activities as well as cell cycle arrest profiles in MCF-7 and BxPC3 cells. Glycosides **1a**-**e** shared a same planar aromatic aglycon (NPCD) and showed almost



Scheme 1. General route to the synthesis of NPCD glycosides. Reagents and conditions: (i) *t*-BuOK, THF, 42% for **3a**, 46% for **3b**, 58% for **3d**, 73% for **3e**; (ii) DDQ, CH_2Cl_2/H_2O (v/v = 10:1), 51% for **4a**, 60% for **4b**, 31% for **4c** over two steps, 58% for **4d**, 39% for **4e**; (iii) *hv*, acetone, 60% for **1a**, 75% for **1b**, 72% for **1c**, 64% for **1d**, 50% for **1e**.



Scheme 2. General route to the synthesis of **2a**–**c**. Reagents and conditions: (i) EtOH, reflux; (ii) DDQ, 1,4-dioxane, 38% for **6a**, 60% for **6b**, 47% for **6c**; (iii) NaH, PMBCl, THF, 44% for **7a**, 33% for **7b**, 85% for **7c**; (iv) $(COCl)_2$, MeONa, MeOH, THF 88% for **2a**, 75% for **2b**, 82% for **2c**.



Scheme 3. General route to the synthesis of **2d**–**e**. Reagents and conditions: (i) EtOH, H_2O, 80 °C, 52%; (ii) DDQ, 1,4-dioxane, 73%; (iii) NaH, CH_3CN, 0 °C, 73%; (iv) MeONa, MeOH; (v) NaH, PMBCl, THF, 88% for **13** over two steps, 34% for **14**, over 2 steps; (vi) $(COCl)_2$, MeONa, MeOH, THF 88% for **2d**, 62% for **2e**.

Table 2
Cytotoxic activities of NPCD and its glycosides on eight tumor cell lines (IC_{50}, in μM)

	BxPC3	PC3	MCF-7	MDA-MB-435	MDA-MB-231	MDA-MB-468	A549	HCT116
1a	7.3 ± 2.9	2.7 ± 0.2	4.5 ± 1.0	3.6 ± 0.1	4.7 ± 0.8	5.0	2.8	3.1 ± 0.8
1b	6.6 ± 1.7	2.1 ± 0.6	4.8 ± 2.3	3.3 ± 0.3	3.3 ± 0.2	3.4	2.0	6.0 ± 0.7
1c	5.4 ± 1.8	2.3 ± 0.9	6.1 ± 3.5	3.5 ± 0.2	2.7 ± 0.2	6.1	2.8	5.1 ± 0.0
1d	5.3 ± 2.1	2.4 ± 0.9	5.3 ± 3.0	3.2 ± 0.1	2.4 ± 0.3	4.4	2.1	3.8 ± 0.3
1e	7.4 ± 3.1	2.6 ± 0.8	7.3 ± 3.5	4.1 ± 0.5	3.3 ± 0.0	9.1	3.4	8.7 ± 1.0
NPCD	8.3 ± 2.5	5.9 ± 1.3	8.3 ± 4.2	5.7 ± 1.5	8.1 ± 0.6	>10	2.6	5.7 ± 1.3

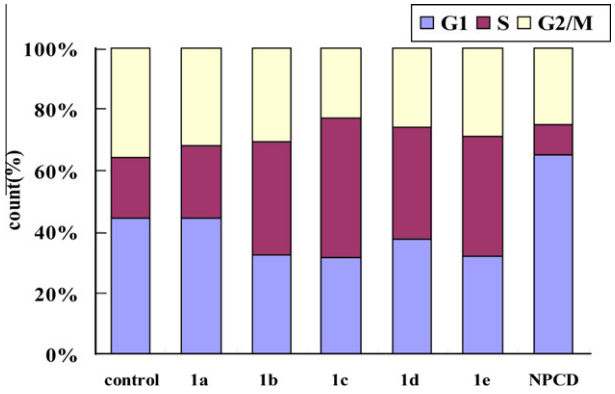


Figure 2. MCF-7 Cell-cycle arrest profile of compounds **1a–e** and NPCD (10 μM for each compound).

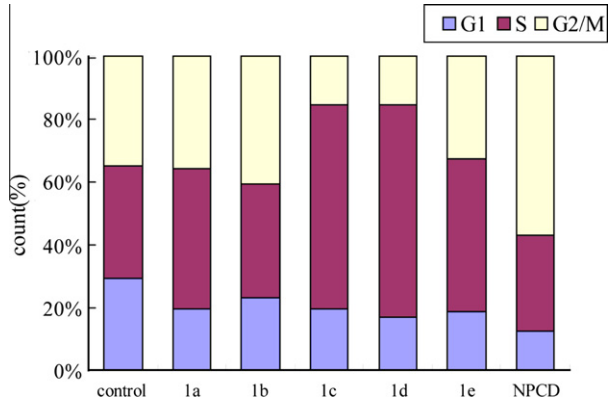


Figure 3. BxPC3 cell-cycle arrest profile of compounds **1a–e** and NPCD (10 μM for each compound).

the same tumor cell growth inhibitory activities as that of NPCD in general, but they displayed different cell cycle arrest profiles from NPCD and each other both in MCF-7 and BxPC3 cells. In addition, NPCD showed distinct cell cycle arrest profile in BxPC3 prostate cells from that in MCF-7 breast cells. However, NPCD glycosides shared similar cell cycle arrest profiles in MCF-7 to those in BxPC3 cells. These results indicated that not only the indolocarbazole framework as well known before[1,6,15] but the sugar moiety can have a profound impact on the mechanism of action. The results obtained will render new clues to the understanding of the anti-cancer profile for these types of compounds.

Acknowledgments

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13. *Data for* **1a–e**: 14-(α-L-Rhamnopyranosyl)-naphtho[2,1-α]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,12*H*)-dione (**1a**): ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.41 (s,1H, –NH), 9.37 (d, 1H, *J* = 8.4 Hz), 9.26 (d, 1H, *J* = 8.0 Hz), 8.47 (d, 1H, *J* = 8.8 Hz), 8.41 (d, 1H, *J* = 9.2 Hz), 8.10 (d, 1H, *J* = 9.2 Hz), 8.07 (d, 1H, *J* = 7.7 Hz), 7.75 (t like, 1H, *J* = 7.0, 7.7 Hz), 7.67 (t like, 1H, *J* = 7.0, 8.4 Hz), 7.53 (t like, 1H, *J* = 7.0, 8.4 Hz), 7.40 (t like, 1H, *J* = 7.0, 7.3 Hz), 6.39 (s, 1H), 5.73 (d, 1H, *J* = 5.1 Hz, –OH), 5.11 (d, 1H, *J* = 5.5 Hz, –OH), 5.09 (d, 1H, *J* = 5.9 Hz, –OH), 4.70 (s, 1H), 3.84 (t, 1H, *J* = 5.0, 5.9 Hz), 3.57 (td, 1H, *J* = 5.1, 9.5 Hz), 3.42–3.47 (m, 1H), 1.17 (d, 3H, *J* = 5.8 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.3, 169.8, 142.3, 141.3, 131.9, 130.7, 128.1, 128.1, 128.0, 127.9, 127.0, 126.4, 125.5, 124.3, 122.7, 122.2, 121.6, 121.3, 121.2, 118.1, 117.0, 87.6, 75.4, 73.0, 72.2, 71.4, 17.9; ESI–MS (m/z): 483.1 [M+H]$^+$, (Calcd 483.15). 14-(β-D-ribopyranosyl)-naphtho[2,1-α]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,12*H*)-dione (**1b**): ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.41 (s,1H, –NH), 9.53 (d, 1H, *J* = 8.4 Hz), 9.24 (d, 1H, *J* = 8.1 Hz), 8.56 (d, 1H, *J* = 9.1 Hz), 8.10 (dd, 2H, *J* = 7.7, 9.1 Hz), 8.02 (d, 1H, *J* = 8.4 Hz), 7.76 (t like, 1H, *J* = 7.3, 7.7 Hz), 7.71 (t like, 1H, *J* = 7.3, 8.4 Hz), 7.59 (t like, 1H, *J* = 7.3, 7.7 Hz), 7.46 (t like, 1H, *J* = 7.3, 7.7 Hz), 6.34 (d, 1H, *J* = 9.5 Hz, H-1), 5.12 (d, 1H, *J* = 3.0 Hz, –OH), 5.00 (d, 1H, *J* = 6.6 Hz, –OH), 4.91 (d, 1H, *J* = 6.5 Hz, –OH), 4.48 (ddd, 1H, *J* = 2.6, 6.6, 9.5 Hz), 3.99–4.05 (m, 2H), 3.84–3.88 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 169.8, 143.0, 141.6, 132.1, 130.5, 128.4, 128.3, 128.1, 128.1, 127.4, 127.3, 126.9, 125.8, 125.3, 122.5, 122.3, 121.8, 121.7, 121.5, 116.6, 115.3, 85.6, 71.7, 67.2, 66.6, 65.9; ESI–MS (m/z): 483.1 [M+H]$^+$, (Calcd 483.15). 14-(β-D-glucopyranosyl)-naphtho[2,1-α]pyrrolo[3,4-*c*]carbazole-5,7(6H, 12H)-dione (**1c**): ^{1}H NMR (400 MHz,

DMSO-d_6) δ: 11.45 (s, 1H), 9.54 (d, J = 9.0 Hz, 2H), 9.46 (d, J = 8.3 Hz, 1H), 9.29 (d, J = 7.9 Hz, 1H), 9.24 (d, J = 8.0 Hz, 1H), 8.53 (d, J = 9.2 Hz, 1H), 8.18–8.03 (m, 4H), 7.98 (dd, J = 8.8, 4.5 Hz, 2H), 7.84 – 7.57 (m, 6H), 7.47 (dd, J = 14.1, 7.1 Hz, 2H), 6.02 (d, J = 9.2 Hz, 1H), 5.98 (d, J = 9.2 Hz, 1H), 5.27 – 5.17 (m, 3H), 5.15 (d, J = 4.9 Hz, 1H), 5.10 (d, J = 5.8 Hz, 1H), 4.96 (t, J = 5.5 Hz, 1H), 4.80 (t, J = 5.7 Hz, 1H), 4.69 (d, J = 5.7 Hz, 1H), 4.17 (m, 1H), 3.99–3.79 (m, 4H), 3.78–3.52 (m, 3H), 3.51–3.39 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 170.4, 169.9, 169.8, 144.9, 142.8, 141.5, 132.3, 132.2, 130.7, 130.6, 128.4, 128.2, 128.2, 128.1, 127.7, 127.5, 127.3, 127.1, 127.0, 125.9, 125.4, 125.2, 124.3, 122.5, 122.4, 122.3, 122.0, 121.9, 121.7, 121.4, 120.6, 118.2, 116.7, 115.3, 112.0, 88.9, 86.5, 80.6, 79.2, 77.8, 77.6, 70.4, 69.8, 69.5, 68.8, 61.0, 60.5; ESI–MS (m/z): 499.1 [M+H]$^+$, (Calcd 499.15). 14-(β-D-ribofuranosyl)-naphtho[2,1-α]pyrrolo[3,4-c] carbazole-5,7 (6*H*,12*H*)-dione (**1d**): ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.43 (s,1H, –NH), 9.56 (d, 1H, J = 8.4 Hz), 9.25 (d, 1H, J = 8.0 Hz), 8.63 (d, 1H, J = 8.4 Hz), 8.17 (d, 1H, J = 8.0 Hz), 8.10 (dd, 2H, J = 7.7, 9.1 Hz), 7.77 (t like, 1H, J = 7.0, 7.7 Hz), 7.71 (t like, 1H, J = 7.3, 8.1 Hz), 7.60 (t like, 1H, J = 7.3, 8.1 Hz), 7.49 (t like, 1H, J = 7.3, 7.7 Hz), 6.40 (d, 1H, J = 7.0 Hz, H-1), 5.29 (t like, 1H, J = 4.7, 5.2 Hz, –OH), 5.19 (d, 1H, J = 4.8Hz, –OH), 5.17 (d, 1H, J = 5.5 Hz, –OH), 4.65–4.68 (m, 1H), 4.00–4.18 (m, 2H), 3.80–3.91 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 169.7, 142.6, 132.2, 130.6, 128.4, 128.4, 128.3, 128.2, 127.5, 127.4, 127.3, 126.0, 125.4, 122.8, 122.3, 122.2, 117.1, 92.4, 70.0, 69.2, 61.2, 48.6; ESI–MS (m/z): 483.1 [M+H]$^+$, (Calcd 483.15). 14-(β-D-ribofuranosyl)-naphtho[2,1-α]pyrrolo[3,4-c]carbazole-5,7(6*H*,12*H*)-dione (**1e**): ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.4 (s, 1H), 9.52 (d, 1H, J = 8.4 Hz), 9.25 (d, 1H, J = 8.0 Hz), 8.58 (d, 1H, J = 7.3 Hz), 8.15 (d, 1H, J = 9.2 Hz), 8.11 (d, 1H, J = 7.3 Hz), 8.04 (d, 1H, J = 8.0 Hz), 7.77 (td, 1H, J = 1.1, 7.7 Hz), 7.71 (td, 1H, J = 1.4, 8.4 Hz), 7.62 (td, 1H, J = 1.0, 8.0 Hz), 7.47 (tlike, 1H, J = 7.3, 7.7 Hz), 6.54 (dd, 1H, J = 2.2, 11.0 Hz, H-1), 5.01 (d, 1H, J = 2.6 Hz, –OH), 4.96 (d, 1H, J = 5.1 Hz, –OH), 4.08–4.12 (m, 2H, H-3, H-4), 3.92–3.99 (m, 2H, H-5ab), 2.16–2.19 (m, 1H, H-2a), 1.97–2.02 (m, 1H, H-2b); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4, 169.7, 142.0, 140.8, 132.2, 130.6, 128.3, 128.3, 128.2, 128.1, 127.2, 127.0, 127.2, 127.0, 125.6, 125.2, 123.1, 122.2, 121.9, 121.3, 116.9, 87.8, 69.4, 67.8, 66.6, 59.8, 32.4, 20.8, 14.1; ESI–MS (m/z): 453.2 [M+H]$^+$, (Calcd 453.14).

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