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Synthesis and biological activities of 7-aza rebeccamycin analogues bearing the sugar moiety on the nitrogen of the pyridine ring

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Abstract—The synthesis of a new family of 7-aza-rebeccamycin analogues in which the sugar moiety is attached to the nitrogen of the pyridine ring is described. The capacity of the newly synthesized compounds to bind to DNA and to inhibit topoisomerase I has been evaluated. Their cytotoxicities toward four tumor cell lines, one murine leukemia L1210 and three human tumor cell lines, one prostate carcinoma DU145, one colon carcinoma HT29, and one non-small cell lung carcinoma A549, have been determined. Their abilities to inhibit the checkpoint kinase Chk1 have been evaluated.

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1. Introduction

Rebeccamycin and related compounds have triggered considerable interest in the past 15 years because of their ability to inhibit topoisomerase I, a nuclear enzyme essential for DNA replication and transcription.^{1–5} It has been shown that topoisomerase I is the major target for many rebeccamycin derivatives, however, various rebeccamycin analogues may have several other targets such as DNA or kinases.⁵ Recently, with the aim of improving the binding properties to their possible targets, new rebeccamycin analogues, in which one or both indole moieties have been replaced by a 7-azaindole unit, have been synthesized.^{6,7} In mono 7-azaindole compounds, the carbohydrate moiety has been linked either to the indole or to the azaindole (Fig. 1). The *in vitro* antiproliferative activities against a panel of nine tumor cell lines of these 7-aza-rebeccamycin have been determined. Compared to the parent compounds without 7-azaindole unit, the aza-rebeccamycins were much more selective toward the tumor cell lines tested. The most sensitive cells were SK-N-MC neuroblastoma,

NCI-H69 small cell lung carcinoma, and A431 epidermoid carcinoma cells with IC₅₀ values in the nanomolar range. Interestingly, in spite of major differences in DNA binding and topoisomerase I inhibitory capacities between the compounds bearing the sugar moiety either on the indole or on the azaindole, in both series strong cytotoxicities were observed, suggesting other targets than DNA and topoisomerase I for compounds in which the sugar is attached to the azaindole. More recently, 7-aza-rebeccamycins bearing substituents on the aromatic framework, in various positions of the sugar part and on the imide nitrogen, have been synthesized.⁸

In the course of our syntheses of 7-aza-rebeccamycins, in the Mitsunobu coupling reaction carried out with aglycone **A**, we observed the formation of a by-product which could not be purified by chromatography. It was isolated in mixture with triphenylphosphine oxide (Scheme 1). But because of the known tautomerization of 7-azaindole,^{9,10} the formation of compound **10** with the carbohydrate attached to the nitrogen of the pyridine heterocycle was suspected. This compound could be of interest as an intermediate for the synthesis of a new family of 7-aza-rebeccamycin analogues. The position of the sugar moiety may have major effects on DNA affinity and on topoisomerase I poisoning.

Keywords: Rebeccamycin; 7-Azaindole; Antitumor compounds; Chk1 inhibitors; DNA binding agents; Topoisomerase I inhibitors.

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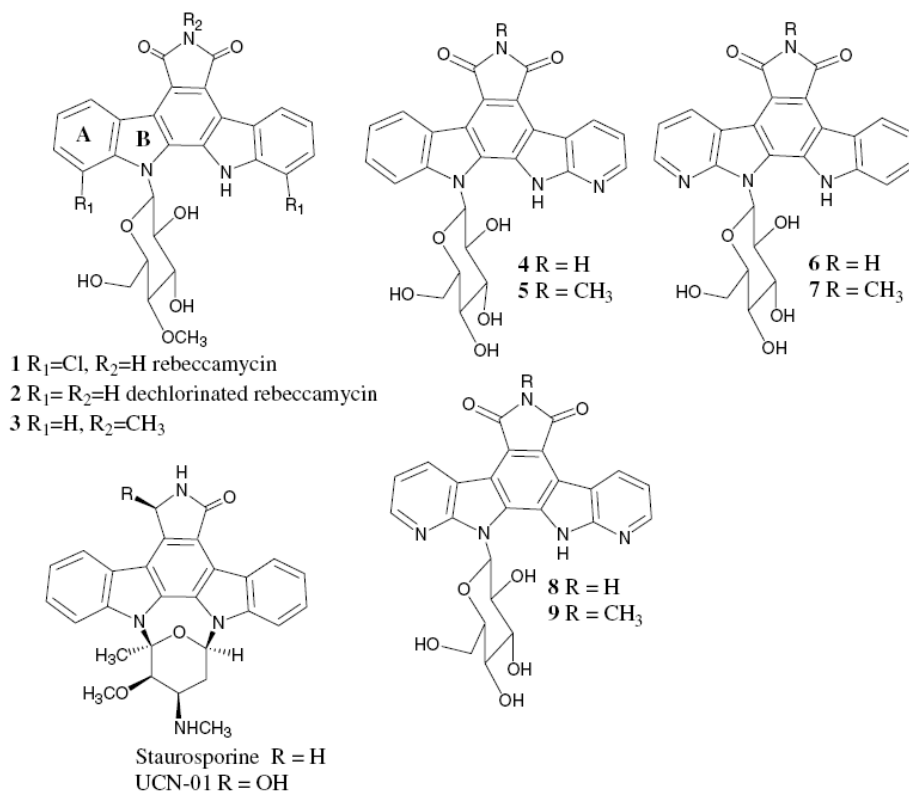


Figure 1. Structures of rebeccamycin and aza analogues previously synthesized.

In this paper, the syntheses of 7-aza-rebeccamycins bearing the sugar moiety on the pyridine ring are described. Their cytotoxicities against four tumor cell lines, their DNA binding properties, and their anti-topoisomerase I activities are reported. Moreover, since structurally related natural products staurosporine and UCN-01 (Fig. 1) are potent Checkpoint 1 kinase (Chk1) inhibitors,¹¹ the capacities of the newly synthesized compounds to inhibit Chk1 have been investigated.

2. Results and discussion

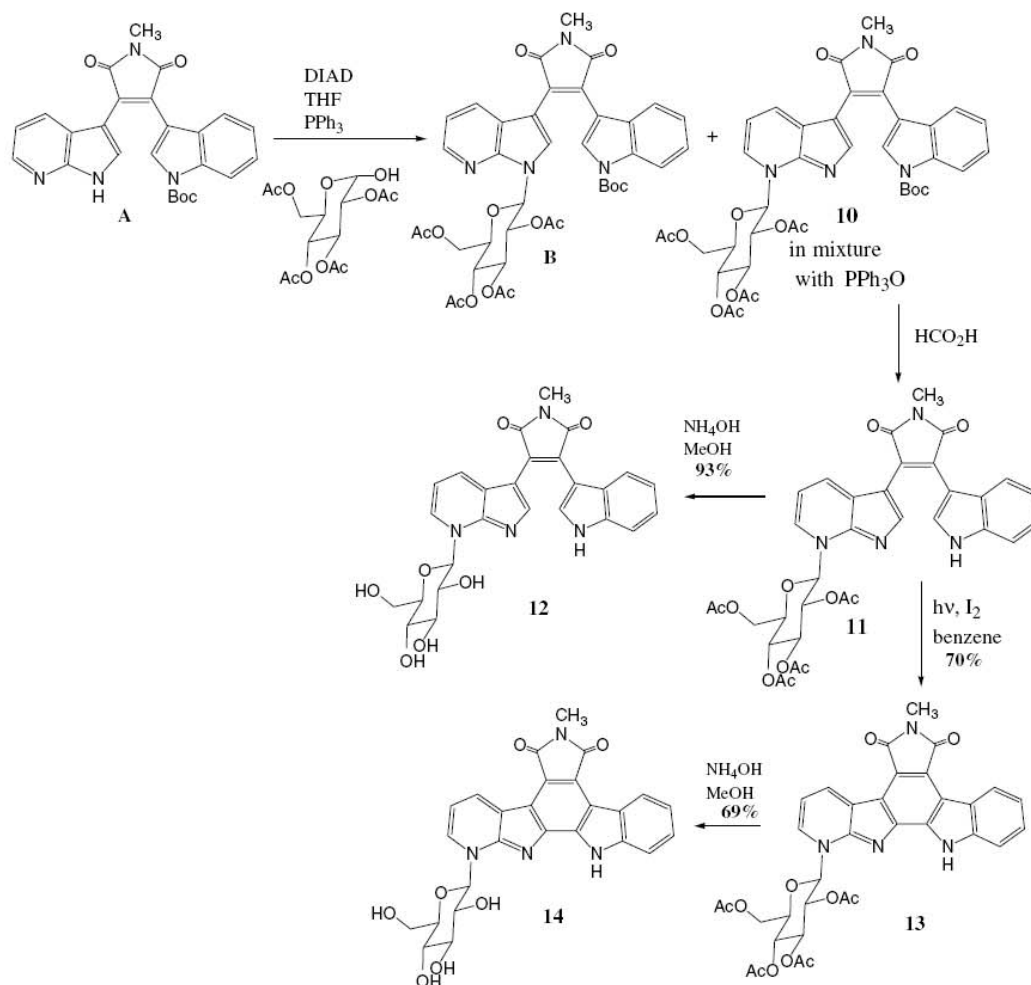
2.1. Chemistry

The Mitsunobu reaction performed with aglycone **A**¹² protected on the indole nitrogen with a Boc group and α -D-2,3,4,6-tetra-*O*-acetyl-glucopyranose led to compound **B** as the major product (Scheme 1), whereas compound **10**, the minor product, could only be isolated in mixture with triphenylphosphine oxide. Compound **10** was partly purified by flash chromatography. Removal of the Boc protective group was performed by reaction of compound **10** (in mixture with triphenylphosphine oxide) with formic acid giving compound **11**. The position of the sugar moiety was assigned from NMR experiments (¹H-¹H COSY and ¹H-¹³C correlations, INEPT-DN) (Scheme 2). The anomeric proton $H_{1'}$ at 7.01 ppm is coupled with the two carbons at 125.7 ppm (CH) and at 150.0 ppm (C quat) of the

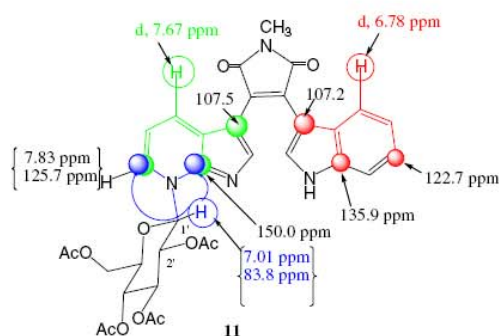
7-azaindole. An axial-axial coupling constant of 9 Hz between $H_{1'}$ and $H_{2'}$ is consistent with a β -coupling. Deprotection of the hydroxyl group of the sugar unit by aminolysis in methanol afforded compound **12**. Oxidative photocyclization of **11**, followed by the removal of the acetyl groups, led to **14**, the first aza-rebeccamycin analogue of this series.

With the aim of introducing various substituents on the imide nitrogen,¹³ *N*-methylated compound **12** was treated with aqueous NaOH followed by a work-up in an acidic medium affording **15** with concomitant hydrolysis of the acetates of the sugar moiety (Scheme 3). Like in *N*-methylated series, the Mitsunobu reaction carried out in *N*-benzyloxymethyl (*N*-BOM) series with aglycone **C**⁶ to obtain analogues without substituents on the imide nitrogen, either with 3,4,6-tri-*O*-benzyl-2-tosyl- α -D-glucopyranose **D**¹⁴ or with 2,3,4,6-tetra-*O*-benzyl- α -D-glucopyranose **E**, led to compounds **F**¹⁴ and **G**, respectively, as the major products. The minor products of the reactions were **16** and **17** isolated in 17% and 25% yields, respectively (Scheme 4). Deprotection of the indole nitrogen with tetrabutylammonium fluoride¹⁵ gave compounds **18** and **19** which were further photocyclized leading to compounds **20** and **21**. Removal of the BOM and benzyl protective groups, using boron tribromide then aqueous NH₄OH, yielded compounds **22** and **23**.

Via a Mitsunobu coupling reaction, compound **11** was isolated with only 8% yield. To enhance the coupling



Scheme 1.



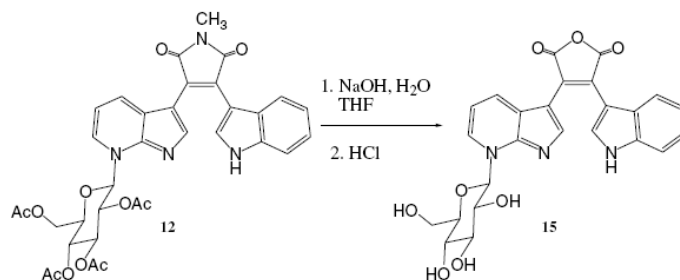
Scheme 2.

on the pyridine nitrogen, the reaction was carried out with aglycone **A** and a 1,2-anhydro sugar according to the method reported by Danishefsky et al.¹⁶ in a total synthesis of rebeccamycin (Scheme 5). The 3,4,6-tri-*O*-benzyl-1,2-anhydro sugar was prepared from 3,4,6-tri-*O*-acetyl-glucal in three steps according to the literature procedure.^{17,18} After coupling of the carbohydrate then

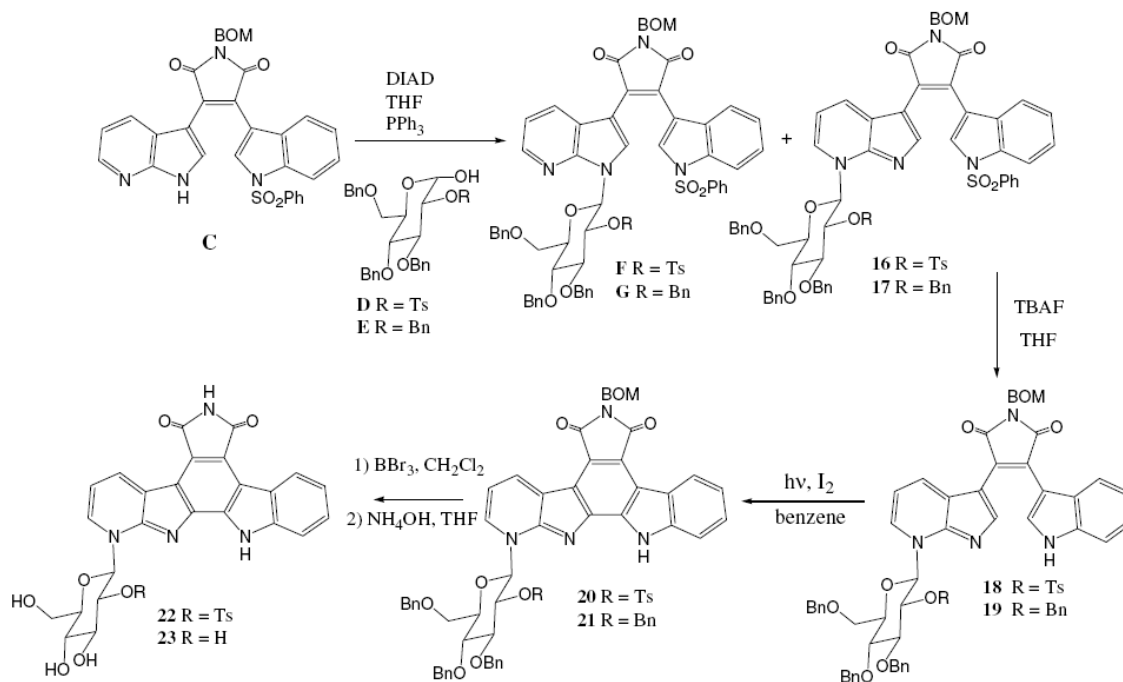
deprotection of the indole nitrogen using formic acid, compound **24** was obtained as the major product in 63% yield, whereas compound **F** was the minor product in only 20% yield. Photocyclization of **24** led to **25** in 69% yield.

2.2. DNA binding

As part of the determination of structure–activity relationships, compounds **14** and **22** were first tested for their ability to interact with DNA. Either calf thymus (CT-DNA) or poly(dAdT)₂ was used in melting temperature studies in the presence of increasing drug/base ratios of the derivatives **14** and **22**. From comparison of the melting temperature (T_m) obtained in the presence or absence (control) of the tested drugs, Figure 2A clearly evidences compound **14** as a potent DNA helix stabilizing molecule, whereas compound **22** clearly does not. The deduced ΔT_m values obtained using increasing drug/DNA ratios of compound **14** in the presence of poly(dAdT)₂ are much higher (up to a ΔT_m value of 10 °C for $R = 1$) than those obtained in the presence



Scheme 3.



Scheme 4.

of CT-DNA (ΔT_m value of 4 °C for $R = 1$). By contrast, the presence of a tosyl group on the sugar ring totally abolished this DNA binding propensity.

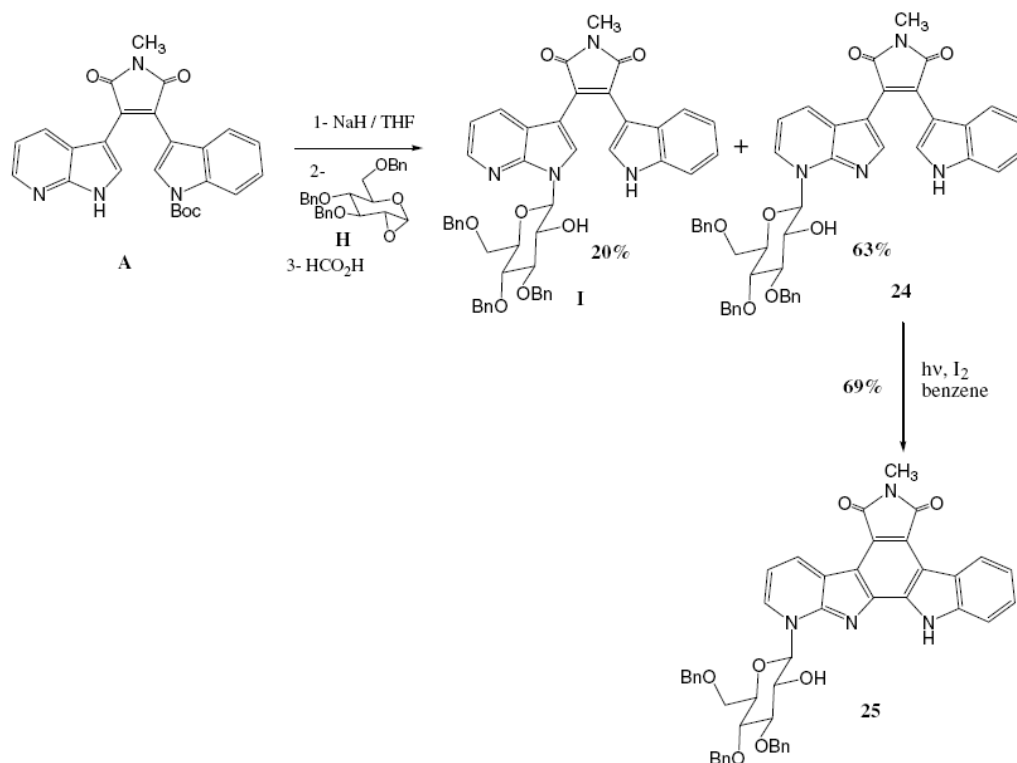
Interestingly, the presence of the sugar ring on the first ring (A-ring) of the rebeccamycin structure enhances the DNA binding propensity since the same structure but bearing the sugar ring on the classical B-ring (compound 7) failed to interact with DNA as previously shown.⁶

In order to get an insight into the nature of the DNA binding, we performed topoisomerase I induced gel relaxation experiments (Fig. 2B). Incubation of increasing concentrations of compound 14 correlates with an increase in the relaxation of the circular plasmid to a fully relaxed form and using a larger amount of compound to a positively supercoiled form typical of the effect of an intercalating compound. By contrast, compound 22 only

weakly changes the DNA relaxation profile induced by topoisomerase I in correlation with its weak DNA binding efficiency.

2.3. Topoisomerase I poisoning

These selected rebeccamycin derivatives were further studied for their ability to poison topoisomerase I enzyme in order to address their mechanism of action. By comparison with the reference molecule camptothecin, increasing concentrations of compounds 14 or 22 were incubated with supercoiled DNA plasmid as described previously for Figure 2B but the samples were separated on an ethidium bromide-containing agarose gel (Fig. 3A). An efficient DNA cleavage (Nck form) is only observed using camptothecin (CPT lane) suggesting that either 14 and 22 are not efficient topoisomerase poisons. An efficient gel shift of the supercoiled DNA form appears with increasing concentrations of 14 in



Scheme 5.

correlation with its efficient DNA binding effect. Migration on a denaturing polyacrylamide gel of a radiolabeled DNA fragment incubated in the presence of increasing concentrations of **14** or **22** reveals that either compounds failed to poison topoisomerase I, only a weak cleavage site being observed using 50 μ M of compounds. By contrast, the control compound camptothecin and the topoisomerase I poisoning rebeccamycin derivative NB-506 used as controls efficiently induced cleavage sites (Fig. 3B).

2.4. In vitro antiproliferative activities

The in vitro antiproliferative activities toward four tumor cell lines: one murine leukemia L1210, and three human tumors: prostate carcinoma DU145, colon carcinoma HT29, and non-small cell lung carcinoma A549 were evaluated. The IC₅₀ values in μ M are reported in Table 1. Compared with the parent compounds **6** and **7** in which the sugar part is attached to the nitrogen of the B-ring, compounds **23** and **14** with the glucosyl moiety attached to the nitrogen of the A-ring were less cytotoxic against L1210 cells. Compound **14** is also less cytotoxic than compound **7** toward all the other tumor cell lines tested. At first sight, it seems that the shifting of the sugar unit from the B-ring to the A-ring decreases the cytotoxicity. Bis-indolyl compound **12** is less cytotoxic than the fully aromatized analogue **7**. The presence of a tosyl group in the 2' position of the sugar increases the cytotoxicity (compare **22** and **23**), this effect could be due to a better penetration into the cells.

2.5. Chk1 inhibitory properties

The transitions in the cell division cycle are regulated predominantly by the activities of cyclin-dependent kinases (CDKs). In the case of DNA damage, however, checkpoints are engaged and will block the cell cycle by eventually inhibiting CDKs to allow time for DNA repair. The activity of the G1/S phase checkpoint requires the p53 protein, which is either absent or mutated in more than 50% of human tumors. In the absence of a G1/S phase checkpoint, tumors become more dependent upon the G2/M phase checkpoint, which is regulated, in part, by Checkpoint 1 kinase (Chk1). An inhibitor of Chk1 should, therefore, selectively force cancer cells to bypass the G2 checkpoint and enter a premature and lethal mitosis. Staurosporine and UCN-01, isolated from cultures of *Streptomyces*, inhibit Chk1 with IC₅₀ values of 8 and 7 nM, respectively.¹¹ Clinical trials are currently undergoing combining UCN-01 with a DNA damaging agent such as cisplatin and topotecan.¹⁹ Compounds **14**, **22**, and **23** are carbazoles structurally related to the potent Chk1 inhibitors staurosporine and UCN-01. Therefore, the inhibitory properties of compounds **14**, **22**, and **23** were determined and compared to that of rebeccamycin **1**. The percentages of Chk1 inhibition at a drug concentration of 10 μ M were evaluated, and for the most efficient inhibitor **23**, the IC₅₀ value was determined (Table 2). Rebeccamycin **1** and compounds **14** and **22** are weak Chk1 inhibitors, whereas compound **23** is a strong Chk1 inhibitor with an IC₅₀ value toward this enzyme of 61 nM. Interestingly, Compound **23** with

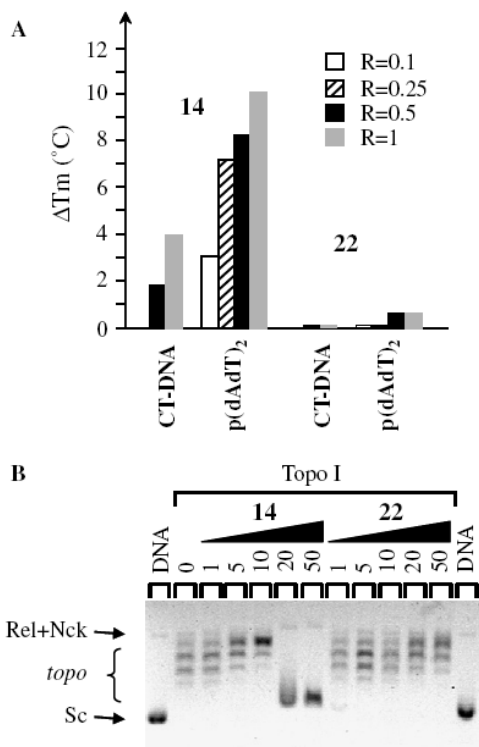


Figure 2. DNA binding efficiencies and mode of binding to the DNA helix of compounds **14** and **22**. (A) Melting temperature studies were performed using either calf thymus DNA or a synthetic poly(dAdT)₂ double stranded oligonucleotide and increasing concentrations of **14** or **22** to a drug/DNA ratio *R* of 0.1 (white boxes), 0.25 (dashed boxes), 0.5 (black boxes) or 1 (gray boxes). The various *T_m* values were deduced from the first derivative plots and were subtracted from the value obtained using either DNA alone to obtain the various ΔT_m values presented in the diagrams. (B) Topoisomerase I DNA relaxation studies were obtained by incubating supercoiled plasmid DNA with increasing concentrations of compounds **14** or **22** prior to the addition of topoisomerase I enzyme as described in the experimental section. The DNA samples were then separated on an agarose gel and stained post-electrophoresis using ethidium bromide. Sc, Rel, Nck, and topo refer to supercoiled, relaxed, nicked, and topoisomer DNA plasmid forms, respectively. Lanes DNA refer to the native plasmid DNA alone.

the glucosyl moiety attached to the nitrogen of the pyridine ring is a considerably stronger Chk1 inhibitor than the parent compound **6** in which the sugar is linked to the nitrogen of the pyrrole. Concerning compound **22**, it may be possible that the bulky tosyl group of compound **22** prevents the binding to the ATP binding site of the enzyme. The weak inhibitory effect of compound **14** is not surprising since the imide nitrogen is not free. In the crystal structures of Chk1 in complex with staurosporine, UCN-01, and isogranulatimide, a natural product with a carbazole structure containing an imide upper heterocycle, two hydrogen bonds are conserved: one between a carbonyl of the upper heterocycle of the drugs and the Cys⁸⁷ residue, the other between the lactam or imide NH and the Glu⁸⁵ residue.^{11,20} The *N*-methylated imide prevents the stabilization of compound **14** inside the ATP binding site of Chk1.

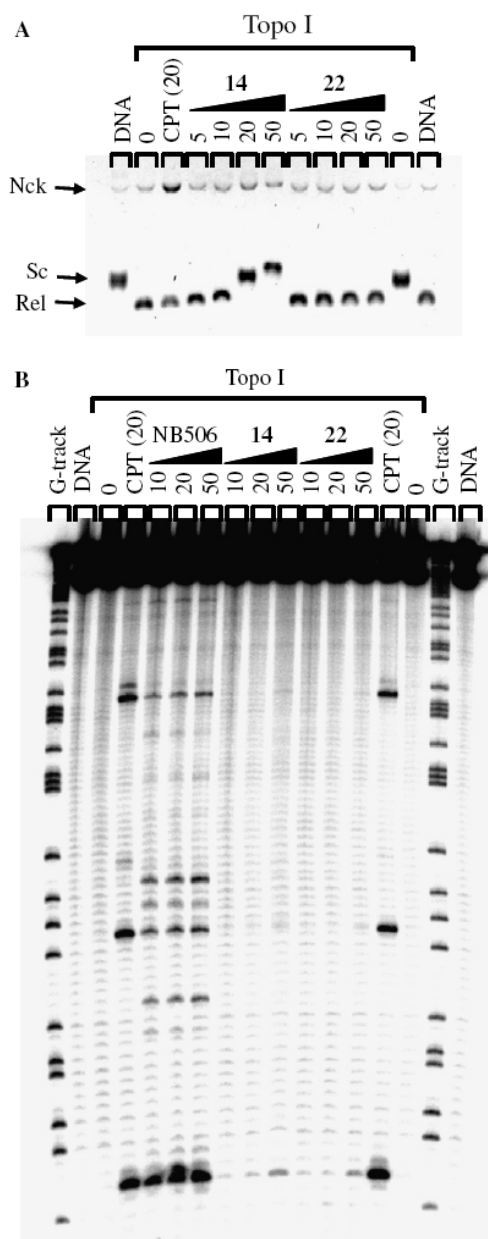


Figure 3. Topoisomerase I DNA poisoning. Increasing concentrations of **14**, **22**, NB506 or 20 μ M of CPT were incubated with supercoiled pUC19 plasmid DNA (A) or the 117 bp radiolabeled DNA fragment (B) prior to further addition of 4 U of human recombinant topoisomerase I enzyme. The DNA samples were then separated on a 1% agarose ethidium bromide-containing gel (A) or a 8% denaturing polyacrylamide gel (B). Sc, Rel, and Nck refer to supercoiled, relaxed, and nicked DNA plasmid forms, respectively. G-track lanes reveal the guanines position on the radiolabeled 117 bp DNA fragment. Lanes DNA correspond to the relative DNA alone.

3. Conclusion

In conclusion, we have prepared a novel series of aza rebeccamycin derivatives in which the glucosyl moiety is attached to the nitrogen of the pyridine ring. It appeared that the shifting of the sugar from the nitrogen

Table 1. In vitro antiproliferative activities (IC₅₀ μM) toward several tumor cell lines: one murine leukemia L1210, and four human tumors prostate carcinoma DU145, colon carcinoma HT29, non-small cell lung carcinoma A549, and colon carcinoma HCT116

| Compound | L1210 | DU145 | HT29 | A549 | HCT116 |
|-----------|-------|-------|------|------|--------|
| 1 | 0.14 | ne | 0.3 | 0.3 | nd |
| 2 | 0.11 | ne | 2.5 | 2 | nd |
| 3 | 0.58 | 0.42 | 0.43 | 0.46 | nd |
| 4 | 0.06 | 0.59 | 4.8 | 5.3 | nd |
| 5 | 1.3 | 6 | 17.8 | 47.2 | nd |
| 6 | 0.13 | 0.36 | >100 | >100 | nd |
| 7 | 0.34 | 0.20 | 67.2 | 59.9 | nd |
| 12 | 85 | 82.6 | 76.2 | >100 | nd |
| 14 | 17.6 | 83 | >100 | >100 | nd |
| 22 | 15.4 | nd | 57.9 | nd | 13.4 |
| 23 | 48 | nd | 100 | nd | 16 |

Table 2. Percentages of Chk1 inhibition at a drug concentration of 10 μM

| Compound | % of Chk1 inhibition at 10 μM | IC ₅₀ in μM |
|-----------|-------------------------------|------------------------|
| 1 | 62 | nd |
| 6 | 15 | nd |
| 14 | 31.8 | nd |
| 22 | 45.5 | nd |
| 23 | 85 | 0.061 |

of the B-ring to the nitrogen of the A-ring enhances binding to the DNA. However, as observed with other rebeccamycin derivatives, the DNA binding affinity is not correlated with topoisomerase I inhibitory properties. The shifting of the sugar from the nitrogen of the B-ring to the nitrogen of the A-ring considerably increases the Chk1 inhibitory effect. Indeed, compound **23** is a potent Chk1 inhibitor. The work reported here provides a new avenue to the design of Chk1 inhibitors.

4. Experimental

4.1. Chemistry

IR spectra were recorded on a Perkin-Elmer 881 spectrometer. NMR spectra were recorded on a Bruker AVANCE 400 (¹H: 400 MHz, ¹³C: 100 MHz) chemical shifts δ in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), pseudo-triplet (pt), doubled triplet (dt), multiplet (m), br s (broad signal), tertiary carbons (C tert), and quaternary carbons (C quat). The signals were assigned from ¹H-¹H COSY and ¹³C-¹H correlations. Low resolution mass spectra (ESI+ and APCI+) were determined on a MS Hewlett Packard engine. HRMS spectra (FAB+) were determined on a high resolution Fisons Autospec-Q spectrometer at CESAMO (Talence, France). Chromatographic purifications were performed by flash silicagel Geduran SI 60 (Merck) 0.040–0.063 mm or Kieselgel 60 (Merck) 0.063–0.200 mm column chromatography. For purity tests, TLC were performed on fluorescent silica gel plates (60 F₂₅₄ from Merck).

4.1.1. 3-(1*H*-Indol-3-yl)-1-methyl-4-[7-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranos-1-yl)-7*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-pyrrole-2,5-dione (11**).** To a solution of aglycone **A** (384 mg, 0.863 mmol) in THF (27 mL) were added 2,3,4,6-*O*-acetyl-α-*D*-glucopyranose (672 mg, 1.93 mmol) and triphenylphosphine (505 mg, 1.93 mmol). The mixture was cooled to −78 °C then diethyl azodicarboxylate (DEAD) (304 μL, 1.93 mmol) was added dropwise. The mixture was allowed to reach room temperature, then was stirred at room temperature for 18 h. Water (50 mL) was added. After extraction with EtOAc, the organic phase was dried over MgSO₄, and the solvent was removed. The residue was purified by flash chromatography (eluent: cyclohexane/AcOEt 7:3 containing NEt₃ 20%) to give a mixture of *N*-Boc **10** and triphenylphosphine oxide (600 mg).

This mixture was dissolved in formic acid (250 mL) and stirred at room temperature for 24 h. After evaporation, the residue was purified by flash chromatography (eluent: cyclohexane/AcOEt from 7:3 to 5:5) to give **11** (48 mg, 0.071 mmol, 8% yield) as a red solid.

Mp: 155 °C. IR (KBr) ν_{C=O} 1700, 1752 cm^{−1}, ν_{NH} 3300–3500 cm^{−1}. HRMS (ESI+) [M + H]⁺ calcd for C₃₄H₃₃N₄O₁₁ 673.2146, found 673.2118. ¹H NMR (400 MHz, CDCl₃): 1.62 (3H, s, CH₃CO), 2.01 (3H, s, CH₃CO), 2.08 (6H, s, CH₃CO), 3.19 (3H, s, N-CH₃), 4.11–4.20 (2H, m, H_{5'}, H_{6'}), 4.34 (1H, dd, *J*₁ = 13.0 Hz, *J*₂ = 5.0 Hz, H_{6'}), 5.27 (1H, t, *J* = 10.0 Hz, H_{4'}), 5.37 (1H, t, *J* = 10.0 Hz, H_{2'}), 5.58 (1H, t, *J* = 9.5 Hz, H_{3'}), 6.64–6.72 (2H, m), 6.78 (1H, d, *J* = 8.0 Hz), 7.01 (1H, d, *J* = 9.0 Hz, H_{1'}), 7.05 (1H, t, *J* = 7.5 Hz), 7.34 (1H, d, *J* = 8.0 Hz), 7.67 (1H, d, *J* = 8.0 Hz), 7.79–7.84 (2H, m), 8.38 (1H, s), 8.82 (1H, s, NH_{ind}). ¹³C NMR (100 MHz, CDCl₃): 20.0, 20.5, 20.6, 20.7 (CH₃CO), 24.2 (N-CH₃), 61.6 (C_{6'}), 67.9, 71.6, 72.5, 75.5 (C_{2'}, C_{3'}, C_{4'}, C_{5'}), 83.8 (C_{1'}), 111.0, 111.4, 120.4, 121.7, 122.7, 125.7, 127.9, 134.7, 149.9 (C tert arom), 107.2, 107.5, 124.9, 125.1, 127.9, 128.5, 136.0, 150.0 (C quat arom), 169.0, 169.6 (2C), 170.5, 172.6 (2C) (C=O).

4.1.2. 3-(1*H*-Indol-3-yl)-1-methyl-4-[(7-β-*D*-glucopyranos-1-yl)-7*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-pyrrole-2,5-dione (12**).** To a solution of **11** (20 mg, 0.0297 mmol) in methanol (10 mL) was added 28% aqueous NH₄OH (16 mL). The mixture was stirred at 65 °C for 22 h. The solvent was removed, then water and EtOAc were added to the residue. After extraction with EtOAc, the organic phase was dried over MgSO₄, and the solvent was removed to give **12** (14 mg, 0.0277 mmol, 93% yield) as a red solid.

Mp: 182–185 °C. IR (KBr) ν_{C=O} 1695, 1750 cm^{−1}, ν_{NH,OH} 3100–3600 cm^{−1}. HRMS (FAB+) [M + H]⁺ calcd for C₂₆H₂₄N₄O₇ 505.1723, found 505.1719. ¹H NMR (400 MHz, DMSO-*d*₆): 3.07 (3H, s, CH₃), 3.48–3.56 (4H, m), 3.72–3.85 (2H, m), 4.68 (1H, t, *J* = 6.0 Hz, OH), 5.25 (1H, d, *J* = 4.5 Hz, OH), 5.39 (2H, d, *J* = 5.5 Hz, OH), 6.53 (1H, d, *J* = 8.5 Hz), 6.71 (1H, d, *J* = 7.0 Hz), 6.77 (1H, d, *J* = 8.0 Hz), 6.87 (1H, t, *J* = 7.0 Hz), 7.05 (1H, t, *J* = 7.0 Hz), 7.45 (1H, d, *J* = 8.5 Hz), 7.66 (1H, d, *J* = 8.0 Hz), 7.79 (1H, d, *J* = 1.5 Hz), 8.08 (1H, s), 8.26 (1H, d, *J* = 6.5 Hz),

11.72 (1H, s, NH_{ind}). ¹³C NMR (100 MHz, DMSO-*d*₆): 23.9 (N-CH₃), 60.8 (C_{6'}), 69.5, 72.5, 76.9, 80.7, 86.6 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 110.5, 111.8, 119.4, 120.9, 121.6, 128.3, 128.6, 133.5, 148.7 (C tert arom), 105.5, 106.2, 123.8, 124.8, 126.7, 128.2, 136.0, 150.2 (C quat arom), 171.9, 172.0 (C=O).

4.1.3. 6-Methyl-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranos-1-yl)-1*H*-pyrido[3',2':4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(12*H*)-dione (13). A mixture of **11** (70 mg, 0.104 mmol) and iodine (290 mg, 1.14 mmol) in benzene (150 mL) was irradiated for 4 h with a medium-pressure mercury lamp (400 W). The solvent was removed, and the residue was dissolved in EtOAc and washed with saturated aqueous sodium thiosulfate (100 mL), then with brine (100 mL). The organic phase was dried over MgSO₄. After removal of the solvent, the residue was purified by flash chromatography (eluent: EtOAc/cyclohexane 5:5) to give **13** (49 mg, 0.073 mmol, 70% yield) as an orange solid.

Mp: 128–130 °C. IR (KBr) ν_{C=O} 1700, 1750 cm⁻¹, ν_{NH} 3200–3600 cm⁻¹. HRMS (ESI+) [M + H]⁺ calcd for C₃₄H₃₁N₄O₁₁ 671.1989, found 671.1982. ¹H NMR (400 MHz, CDCl₃): 1.33 (3H, s, CH₃CO), 1.97 (3H, s, CH₃CO), 2.10 (3H, s, CH₃CO), 2.11 (3H, s, CH₃CO), 3.15 (3H, s, N-CH₃), 4.34–4.43 (3H, m), 5.29 (1H, t, *J* = 9.5 Hz), 5.38 (1H, t, *J* = 9.5 Hz), 5.65 (1H, t, *J* = 9.5 Hz), 6.72 (1H, t, *J* = 7.0 Hz), 6.79 (1H, d, *J* = 9.0 Hz, H_{1'}), 7.27 (1H, dt, *J*₁ = 8.0 Hz, *J*₂ = 1.5 Hz), 7.42 (2H, t, *J* = 6.5 Hz), 7.79 (1H, d, *J* = 7.0 Hz), 8.89 (1H, d, *J* = 8.0 Hz), 8.93 (1H, d, *J* = 7.0 Hz), 9.66 (1H, s, NH_{ind}). ¹³C NMR (100 MHz, CDCl₃): 19.8, 20.5, 20.7, 20.9 (CH₃CO), 23.6 (N-CH₃), 62.2 (C_{6'}), 67.8, 72.4, 73.6, 75.5, 83.7 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 109.9, 111.0, 120.6, 125.3, 127.0, 128.5, 136.3 (C tert arom), 115.0, 117.7, 119.3, 120.1, 122.3, 124.9, 132.6, 140.1, 141.1, 152.9 (C quat arom), 169.0, 169.6, 169.9, 170.1, 170.3, 170.8 (C=O).

4.1.4. 1-(β-D-Glucopyranos-1-yl)-6-methyl-1*H*-pyrido[3',2':4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(12*H*)-dione (14). To a solution of **13** (45 mg, 0.067 mmol) in methanol (20 mL) was added 28 % aqueous NH₄OH (31 mL). The mixture was stirred at 65 °C for 22 h. After removal of the solvent, water and EtOAc were added and the mixture was filtered off. The solid residue was washed with EtOAc to give **14** (23 mg, 0.046 mmol, 69 % yield) as a red solid.

Mp: 290–295 °C (decomposition). IR (KBr) ν_{C=O} 1690, 1740 cm⁻¹, ν_{NH,OH} 3200–3600 cm⁻¹. HRMS (FAB+) [M+H]⁺ calcd for C₂₆H₂₃N₄O₇ 503.1566, found: 503.1569. ¹H NMR (400 MHz, DMSO-*d*₆): 3.18 (3H, s, CH₃), 3.48–3.60 (4H, m), 3.81 (1H, d, *J* = 4.0 Hz), 3.96 (1H, m), 4.71 (1H, br s, OH), 5.33 (1H, br s, OH), 5.48 (1H, br s, OH), 5.57 (1H, d, *J* = 4.5 Hz, OH), 6.78 (1H, d, *J* = 9.0 Hz, H_{1'}), 7.33 (1H, t, *J* = 7.5 Hz), 7.39 (1H, t, *J* = 7.0 Hz), 7.52 (1H, t, *J* = 7.5 Hz), 7.68 (1H, d, *J* = 8.0 Hz), 8.67 (1H, d, *J* = 6.5 Hz), 8.97 (1H, d, *J* = 7.5 Hz), 9.40 (1H, d, *J* = 7.0 Hz), 12.55 (1H, s, NH_{ind}). ¹³C NMR (100 MHz, DMSO-*d*₆): 23.5 (N-CH₃), 60.9 (C_{6'}), 69.5,

72.6, 77.1, 81.1, 86.5 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 109.8, 111.9, 119.8, 124.0, 126.5, 128.3, 135.6 (C tert arom), 114.9, 116.1, 118.1, 119.6, 121.6, 124.1, 132.6, 140.8, 142.7, 154.1 (C quat arom), 170.3 (2 C=O).

4.1.5. 3-[7-(β-D-Glucopyranos-1-yl)-7*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-4-(1*H*-indol-3-yl)-furan-2,5-dione (15). To a suspension of **12** (50 mg, 0.074 mmol) in water (40 mL) were added NaOH (44.5 mg, 1.1 mmol) and THF (5 mL). The mixture was stirred at room temperature for 2 h, then was acidified to pH 1 with 2 N HCl. The mixture was stirred overnight at room temperature. After evaporation, the residue was purified by flash chromatography (eluent: THF/methanol 95:5) to yield **15** (21.8 mg, 0.044 mmol, 60% yield) as a red solid.

Mp: 168–170 °C. IR (KBr) ν_{C=O} 1750, 1820 cm⁻¹, ν_{NH,OH} 3000–3600 cm⁻¹. HRMS (ESI+) [M + H]⁺ calcd for C₂₅H₂₂N₃O₈ 492.1407, found 492.1403. ¹H NMR (400 MHz, DMSO-*d*₆): 3.45–3.55 (3H, m), 3.72–3.85 (2H, m), 4.10 (1H, m), 4.64 (1H, t, *J* = 5.5 Hz, OH), 5.22 (1H, d, *J* = 5.5 Hz, OH), 5.30–5.40 (2H, m, 2OH), 6.51 (1H, d, *J* = 9.5 Hz, H_{1'}), 6.75 (1H, d, *J* = 7.5 Hz), 6.80 (1H, d, *J* = 8.0 Hz), 6.96 (1H, t, *J* = 7.0 Hz), 7.07 (1H, t, *J* = 8.0 Hz), 7.46 (1H, d, *J* = 8.0 Hz), 7.73 (1H, d, *J* = 7.5 Hz), 7.88 (1H, d, *J* = 3.0 Hz), 8.10 (1H, s), 8.33 (1H, d, *J* = 6.5 Hz), 12.10 (1H, s, NH_{ind}). ¹³C NMR (100 MHz, DMSO-*d*₆): 60.7 (C_{6'}), 69.5, 72.5, 76.9, 80.7, 86.7 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 111.4, 112.2, 119.8, 121.1, 122.0, 129.0, 129.7, 134.1, 149.8 (C tert arom), 104.7, 105.4, 124.3, 124.4, 126.3, 129.1, 136.1, 150.7 (C quat arom), 166.6, 166.7 (2C, C=O).

4.1.6. 1-Benzyloxymethyl-3-[1-phenylsulfonyl-indol-3-yl]-4-[7-(2-*O*-tosyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranos-1-yl)-7*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-pyrrole-2,5-dione (16). To a solution of aglycone **C** (200 mg, 0.341 mmol) in THF (18 mL) were added **D** (459 mg, 0.76 mmol) and triphenylphosphine (199 mg, 0.76 mmol). The mixture was cooled to -78 °C, then diisopropyl azodicarboxylate (DIAD) (147 μL, 0.76 mmol) was added dropwise. The mixture was allowed to reach room temperature, then was stirred at room temperature for 18 h. Water (50 mL) was added. After extraction with EtOAc, the organic phase was dried over MgSO₄, and the solvent was removed. The residue was purified by flash chromatography (eluent: CH₂Cl₂/EtOAc 9:1) to give **16** (69 mg, 0.058 mmol, 17% yield) as a red solid.

Mp: 63–65 °C. IR (KBr) ν_{C=O} 1706 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): 2.11 (3H, s, CH₃), 3.51–3.60 (1H, m), 3.63 (1H, dd, *J*₁ = 11.0 Hz, *J*₂ = 3.5 Hz), 3.70 (1H, d, *J* = 10.0 Hz), 3.82 (1H, t, *J* = 9.0 Hz), 3.90 (1H, t, *J* = 9.0 Hz), 4.34 (1H, d, *J* = 12.0 Hz), 4.42 (1H, d, *J* = 12.0 Hz), 4.43 (1H, d, *J* = 10.5 Hz), 4.56 (1H, d, *J* = 10.5 Hz), 4.62 (2H, s), 4.68 (1H, d, *J* = 11.0 Hz), 4.69 (1H, d, *J* = 10.5 Hz), 4.84 (1H, t, *J* = 9.0 Hz, H_{2'}), 5.14 (2H, AB system, *J* = 10.5 Hz, Δ*v* = 7.5 Hz), 6.37 (1H, t, *J* = 7.0 Hz), 6.64–6.73 (2H, m), 6.76 (1H, d, *J* = 9.0 Hz, H_{1'}), 6.82 (2H, d, *J* = 8.5 Hz), 6.92 (2H, d, *J* = 8.0 Hz), 6.96–7.02 (3H, m), 7.05 (1H, dt, *J*₁ = 8.5 Hz, *J*₂ = 1.5 Hz), 7.09–7.23 (14H_{arom}), 7.24 (1H, d, *J* = 7.5 Hz), 7.28 (2H, d, *J* = 7.5 Hz), 7.38 (2H,

t, $J = 7.5$ Hz), 7.48 (1H, t, $J = 7.0$ Hz), 7.64 (1H, m), 7.68 (1H, d, $J = 6.5$ Hz), 7.86 (3H, d, $J = 7.5$ Hz), 7.95 (1H, s), 8.27 (1H, s). ^{13}C NMR (100 MHz, CDCl_3): 16.3 (CH_3), 62.2, 62.6, 64.8, 66.5, 70.1, 70.6 (C_6' + 5CH_2), 71.8, 73.1, 74.6, 77.4, 78.6 (C_1' , C_2' , C_3' , C_4' , C_5'), 106.3, 108.2, 117.0, 118.5, 119.8, 121.3–127.5, 128.9, 146.5 (C tert arom), 101.5, 107.6, 114.8, 127.3, 127.5, 128.9, 129.3, 132.1, 132.2, 132.3, 132.5, 132.6, 139.6, 145.7 (C quat arom), 165.6, 165.9 (C=O).

4.1.7. 1-Benzyloxymethyl-3-(1H-indol-3-yl)-4-[7-(2-O-tosyl-3,4,6-tri-O-benzyl- β -D-glucopyranos-1-yl)-7H-pyrrolo[2,3-b]pyridin-3-yl]-pyrrole-2,5-dione (18). To a solution of **16** (69 mg, 0.059 mmol) in THF (3 mL) was added 1.1 M *n*-Bu₄NF in THF (177 mL). The mixture was stirred at room temperature for 2.5 h. Water (45 mL) was added. After extraction with EtOAc, the organic phase was dried over MgSO₄. The solvent was removed and the residue was purified by flash chromatography (eluent: CH₂Cl₂ 100% to CH₂Cl₂/EtOAc 8:2) to give **18** (36 mg, 0.035 mmol, 59% yield) as a red solid.

Mp: 93–95 °C. IR (KBr) $\nu_{\text{C=O}}$ 1706, 1755 cm^{-1} , ν_{NH} 3500 cm^{-1} . Mass (ESI+) $[\text{M}+\text{H}]^+$ 1035. ^1H NMR (400 MHz, CDCl_3): 2.14 (3H, s, CH₃), 3.61 (1H, d, $J = 10.5$ Hz), 3.67 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 3.0$ Hz), 3.75 (1H, m), 3.82–3.92 (2H, m), 4.38 (1H, d, $J = 12.0$ Hz), 4.45 (1H, d, $J = 12.0$ Hz), 4.47 (1H, d, $J = 11.0$ Hz), 4.57 (1H, d, $J = 10.5$ Hz), 4.66 (2H, s), 4.71 (1H, d, $J = 10.5$ Hz), 4.73 (1H, d, $J = 9.5$ Hz), 4.91 (1H, t, $J = 8.5$ Hz, H_{2'}), 5.16 (2H, AB system, $J = 11.0$ Hz, $\Delta\nu = 8.0$ Hz), 6.55 (1H, t, $J = 7.0$ Hz), 6.66 (1H, t, $J = 7.5$ Hz), 6.80–6.90 (3H + H_{1'}, m), 6.90–7.00 (3H, m), 7.00–7.08 (3H, m), 7.10–7.30 (17H_{arom}), 7.33 (2H, d, $J = 7.5$ Hz), 7.37 (1H, d, $J = 7.5$ Hz), 7.65 (1H, d, $J = 2.5$ Hz), 7.70 (1H, d, $J = 6.5$ Hz), 8.32 (1H, s), 8.90 (1H, s). ^{13}C NMR (100 MHz, CDCl_3): 21.5 (CH_3), 67.2, 67.9, 71.6, 73.4, 75.4, 75.9 (C_6' + 5CH_2), 77.2, 78.4, 80.0, 82.8, 83.8 (C_1' , C_2' , C_3' , C_4' , C_5'), 111.0, 111.4, 120.7, 122.1, 122.7, 126.1, 126.8, 126.9–128.6, 129.5 (2C), 134.3, 150.5 (C tert arom), 107.0, 107.2, 124.5, 125.3, 128.9, 132.6, 135.9, 137.5, 137.6, 137.7, 137.8, 144.8, 150.5 (C quat arom), 171.9, 172.0 (C=O).

4.1.8. 6-Benzyloxymethyl-1-(2-O-tosyl-3,4,6-tri-O-benzyl- β -D-glucopyranos-1-yl)-1H-pyrido[3',2':4,5]pyrrolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(12H)-dione (20). A mixture of **18** (180 mg, 0.174 mmol) and iodine (66 mg, 0.259 mmol) in benzene (300 mL) was irradiated according to the procedure described for the synthesis of compound **13**. After identical work-up, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 7:3) to give **20** (135 mg, 0.130 mmol, 75% yield) as a yellow solid.

Mp: 53–55 °C. IR (KBr) $\nu_{\text{C=O}}$ 1707, 1753 cm^{-1} , ν_{NH} 2926 cm^{-1} . Mass (APCI+) $[\text{M}-\text{OTs}]^+$ 861. ^1H NMR (400 MHz, CDCl_3): 1.54 (3H, s, CH₃), 3.70–3.81 (2H, m), 3.88–4.10 (3H, m), 4.48 (1H, d, $J = 12.5$ Hz), 4.53 (1H, d, $J = 11.0$ Hz), 4.54 (1H, d, $J = 11.5$ Hz), 4.57 (1H, d, $J = 9.5$ Hz), 4.71 (2H, s), 4.79 (1H, d, $J = 11.0$ Hz), 4.82 (1H, d, $J = 10.0$ Hz), 4.99 (1H, br s),

5.25 (2H, AB system, $J = 11.0$ Hz, $\Delta\nu = 10.0$ Hz), 6.07 (2H, d, $J = 8.5$ Hz), 6.70 (2H, d, $J = 8.0$ Hz), 6.74 (1H, t, $J = 8.0$ Hz), 6.80 (1H, m), 7.05–7.50 (23H_{arom}), 7.77 (1H, d, $J = 6.5$ Hz), 8.94–8.99 (2H, m), 10.27 (1H, s, NH). ^{13}C NMR (100 MHz, CDCl_3): 21.9 (CH_3), 66.9, 68.0, 71.6, 73.5, 75.1, 75.9 (C_6' + 5CH_2), 70.1, 77.1, 78.6, 80.7, 81.9 (C_1' , C_2' , C_3' , C_4' , C_5'), 112.1, 121.2, 125.1, 126.0, 127.8–129.0, 130.0, 130.9 (C tert arom), 118.3, 118.8, 121.3, 130.6, 132.4, 137.6 (several C), 137.7, 141.0, 144.4, 156.7 (C quat arom), 168.9 (2C, C=O).

4.1.9. 1-(2-O-Tosyl- β -D-glucopyranos-1-yl)-1H-pyrido[3',2':4,5]pyrrolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7-(6H,12H)-dione (22). To a suspension of **20** (120 mg, 0.115 mmol) in CH₂Cl₂ (12 mL) at –78 °C was added 1M BBr₃ in CH₂Cl₂ (1.87 mL). The mixture was stirred at –78 °C for 30 min, then water was added. After extraction with EtOAc, the organic phase was dried over MgSO₄, and the solvent was removed. To a solution of the residue (41 mg) in THF (6 mL) was added 28% aqueous NH₄OH (12 mL). The mixture was stirred at room temperature overnight. After evaporation, the residue was purified by flash chromatography (eluent: EtOAc 100% to EtOAc/methanol 95:5) to give **22** (30 mg, 0.047 mmol, 41% yield) as an orange solid.

Mp: 200 °C (degradation). IR (KBr) $\nu_{\text{C=O}}$ 1705, 1741 cm^{-1} , ν_{NH} 3018–3642 cm^{-1} . HRMS (FAB+) $[\text{M}+\text{H}]^+$ calcd for C₃₂H₂₆N₄O₉S 669.0846, found 669.0856. ^1H NMR (400 MHz, DMSO-*d*₆): 1.83 (3H, s, CH₃), 3.50–3.61 (2H, m), 3.64 (1H, m), 3.74 (1H, m, H_{3'}), 3.81 (1H, dd, $J_1 = 11.5$ Hz, $J_2 = 4.0$ Hz, H_{6'}), 4.74 (1H, t, $J = 5.5$ Hz, OH_{6'}), 5.15 (1H, t, $J = 9.0$ Hz, H_{2'}), 5.63 (1H, br s, OH), 5.92 (1H, d, $J = 4.0$ Hz, OH_{3'}), 5.60 (2H, d, $J = 8.0$ Hz), 6.92 (2H, d, $J = 8.5$ Hz), 6.93 (1H, d, $J = 9.0$ Hz, H_{1'}), 7.28 (1H, t, $J = 7.0$ Hz), 7.32 (1H, t, $J = 7.0$ Hz), 7.53 (1H, t, $J = 7.5$ Hz), 7.68 (1H, d, $J = 8.0$ Hz), 8.70 (1H, d, $J = 6.5$ Hz), 8.98 (1H, d, $J = 8.0$ Hz), 8.19 (1H, d, $J = 7.5$ Hz), 10.94 (1H, br s, NH), 12.45 (1H, s, NH). ^{13}C NMR (100 MHz, CDCl_3): 20.3 (CH_3), 60.7 (C_6'), 69.4, 74.6, 81.0, 81.9, 83.1 (C_1' , C_2' , C_3' , C_4' , C_5'), 109.9, 111.8, 119.7, 124.1, 125.6 (2C), 126.5, 128.6 (2C), 131.8, 135.7 (C tert arom), 114.9, 116.1, 119.1, 120.4, 121.6, 124.2, 133.0, 133.5, 140.8, 142.7, 143.5, 153.2 (C quat arom), 171.5, 171.7 (C=O).

4.1.10. 1-Benzyloxymethyl-4-(1-phenylsulfonyl-indol-3-yl)-3-[7-(2,3,4,6-tetra-O-benzyl- β -D-glucopyranos-1-yl)-7H-pyrrolo[2,3-b]pyridin-3-yl]-pyrrole-2,5-dione (17). To a solution of aglycone **C** (500 mg, 0.852 mmol) in THF (5 mL) were added 2,3,4,6-tetra-O-benzyl- α -D-glucopyranose **E** (1.364 g, 2.25 mmol) and triphenylphosphine (786 mg, 2.25 mmol). The mixture was cooled to –78 °C, then DIAD (500 μL , 2.25 mmol) was added dropwise. After identical work-up as described for the synthesis of compound **16**, the residue was purified by flash chromatography (eluent: CH₂Cl₂/EtOAc 95:5) to give **G** (in mixture with starting products) and **17** (234 mg, 0.215 mmol, 25% yield) as red solids. Compound **G** cannot be purified by chromatography.

Mp: 83–85 °C. IR (KBr) $\nu_{\text{C=O}}$ 1707, 1762 cm^{-1} . Mass (ESI+) $[\text{M} + \text{Na}]^+$ 1133, $[\text{M} + \text{K}]^+$ 1149. ^1H NMR (400 MHz, CDCl_3): 3.57–3.61 (2H, m), 3.70 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 3.0$ Hz), 3.73–3.81 (3H, m), 3.94 (1H, t, $J = 8.5$ Hz), 4.19 (1H, d, $J = 11.0$ Hz), 4.38 (1H, d, $J = 12.0$ Hz), 4.45 (1H, d, $J = 12.0$ Hz), 4.53 (1H, d, $J = 11.0$ Hz), 4.65 (2H, s), 4.78 (1H, d, $J = 11.0$ Hz), 4.81 (2H, s), 5.15 (2H, s), 6.29 (1H, t, $J = 7.0$ Hz), 6.39–6.49 (3H, m), 6.64 (1H, d, $J = 8.0$ Hz), 6.90 (2H, t, $J = 7.5$ Hz), 6.97 (1H, t, $J = 8.0$ Hz), 7.02 (1H, t, $J = 8.0$ Hz), 7.09–7.13 (2H, m), 7.16–7.28 (17H_{arom}), 7.31 (2H, d, $J = 7.5$ Hz), 7.37–7.43 (3H, m), 7.48 (1H, d, $J = 7.5$ Hz), 7.52 (1H, d, $J = 7.0$ Hz), 7.86 (1H, s), 7.88 (2H, d, $J = 8.0$ Hz), 8.00 (1H, s), 8.39 (1H, s). ^{13}C NMR (100 MHz, CDCl_3): 67.4, 68.2, 71.7, 73.5, 74.3, 75.2, 75.9 ($\text{CH}_2 + \text{C}_6'$), 70.0, 77.2, 78.0, 81.1, 85.4 (C_1' , C_2' , C_3' , C_4' , C_5'), 111.5, 113.5, 122.1, 123.3, 125.1, 126.9, 127.2–129.0, 129.5, 133.7, 134.1, 152.1 (C tert arom), 106.5, 112.9, 120.0, 133.0, 134.6, 136.7, 137.7, 137.8, 138.0, 151.1, 156.4 (C quat arom), 170.9, 171.2 (C=O).

4.1.11. 1-Benzylloxymethyl-3-(1*H*-indol-3-yl)-4-[7-(2,3,4,6-tetra-*O*-benzyl- β -D-glucopyranos-1-yl)-7*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-pyrrole-2,5-dione (19). To a solution of **17** (223 mg, 0.201 mmol) in THF (18 mL) was added 1.1 M *n*-Bu₄NF in THF (0.9 mL). The mixture was stirred overnight at room temperature. Water (45 mL) was added. After extraction with EtOAc, the organic phase was dried over MgSO₄, then the solvent was removed and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 8:2) to give **19** (109 mg, 0.112 mmol, 56% yield) as a red solid.

Mp: 93–95 °C. IR (KBr) $\nu_{\text{C=O}}$ 1704, 1760 cm^{-1} , ν_{NH} 3406 cm^{-1} . Mass (ESI+) $[\text{M} + \text{H}]^+$ 971. ^1H NMR (400 MHz, CDCl_3): 3.65 (1H, d, $J = 10.0$ Hz), 3.72–3.80 (2H, m), 3.80–3.88 (2H, m), 3.94 (1H, t, $J = 8.5$ Hz), 4.24 (1H, d, $J = 11.0$ Hz), 4.44 (1H, d, $J = 12.0$ Hz), 4.52 (1H, d, $J = 12.0$ Hz), 4.58 (1H, d, $J = 11.0$ Hz), 4.71 (2H, s), 4.80–4.88 (3H, m), 5.21 (2H, s), 6.41 (1H, t, $J = 7.5$ Hz), 6.48 (1H, t, $J = 7.0$ Hz), 6.53 (2H, d, $J = 8.0$ Hz), 6.78 (1H, d, $J = 8.0$ Hz), 6.90 (1H, t, $J = 8.0$ Hz), 6.99 (2H, t, $J = 7.5$ Hz), 7.09 (1H, t, $J = 7.0$ Hz), 7.13–7.18 (2H, m), 7.19–7.33 (20H_{arom}), 7.38 (2H, d, $J = 7.5$ Hz), 7.57 (1H, d, $J = 6.5$ Hz), 7.60 (1H, d, $J = 7.5$ Hz), 7.77 (1H, d, $J = 2.5$ Hz), 9.34 (1H, s, NH). ^{13}C NMR (100 MHz, CDCl_3): 67.2, 68.3, 71.6, 73.5, 74.3, 75.1, 75.8 ($\text{C}_6' + \text{CH}_2$), 77.2, 78.1, 81.2, 85.4 (C_1' , C_2' , C_3' , C_4' , C_5'), 110.9, 111.5, 120.3, 121.8, 122.6, 127.5–128.7, 133.9, 150.7 (C tert arom), 106.9, 107.1, 124.6, 125.1, 128.8, 136.0, 136.5, 137.8, 137.9, 138.0, 138.2, 150.6 (C quat arom), 172.0, 172.1 (C=O).

4.1.12. 6-Benzylloxymethyl-1-(2,3,4,6-tetra-*O*-benzyl- β -D-glucopyranos-1-yl)-1*H*-pyrido[3',2':4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(12*H*)-dione (21). A mixture of **19** (70 mg, 0.072 mmol) and iodine (29 mg, 0.106 mmol) in benzene (150 mL) was irradiated according to the procedure described for the synthesis of compound **13**. After

identical work-up, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 7:3) to give **21** (53 mg, 0.055 mmol, 76% yield) as a yellow solid.

Mp 68–73 °C. IR (KBr) $\nu_{\text{C=O}}$ 1702, 1751 cm^{-1} , ν_{NH} 3396 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): 3.63–3.89 (6H, m), 4.28 (1H, d, $J = 11.5$ Hz), 4.41 (1H, d, $J = 12.0$ Hz), 4.50 (1H, d, $J = 12.0$ Hz), 4.54 (1H, d, $J = 11.0$ Hz), 4.61–4.68 (2H, m), 4.70 (2H, s), 4.77 (1H, d, $J = 10.0$ Hz), 5.25 (2H, s), 6.34 (2H, d, $J = 7.5$ Hz), 6.63 (2H, t, $J = 7.0$ Hz), 7.04–7.32 (23H_{arom}), 7.36 (2H, d, $J = 7.5$ Hz), 7.38–7.42 (2H, m), 7.68 (1H, d, $J = 6.5$ Hz), 9.01 (1H, d, $J = 8.0$ Hz), 9.22 (1H, br s), 10.47 (1H, br s, NH). ^{13}C NMR (100 MHz, CDCl_3): 66.9, 68.3, 71.5, 73.6, 74.5, 75.1, 75.7 ($\text{C}_6' + \text{CH}_2$), 77.2, 78.1, 82.0, 85.7 (C_1' , C_2' , C_3' , C_4' , C_5'), 111.5, 116.2, 120.4, 120.7, 125.3, 127.2, 127.5–128.5, 129.7, 132.4, 136.7 (C tert arom), 115.2, 118.1, 119.4, 119.7, 122.4, 136.3, 137.8, 137.9, 138.1, 140.5 (C quat arom), 169.9, 170.2 (C=O).

4.1.13. 1-(β -D-Glucopyranos-1-yl)-1*H*-pyrido[3',2':4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*,12*H*)-dione (23). To a suspension of **21** (53 mg, 0.055 mmol) in CH_2Cl_2 (5.5 mL) at -78 °C was added 1 M BBr_3 in CH_2Cl_2 (1.1 mL). The reaction was carried out as described for the synthesis of compound **22**.

After removal of the solvent, the solid residue was washed with CH_2Cl_2 then with water to give **23** (9.7 mg, 0.020 mmol, 36% yield) as a red solid.

Mp > 200 °C (decomposition). IR (KBr) $\nu_{\text{C=O}}$ 1703, 1743 cm^{-1} , ν_{NH} 3004–3654 cm^{-1} . HRMS (FAB+) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{20}\text{N}_4\text{O}_7$ 489.1410, found 489.1419. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 3.46 (1H, m, H_6'), 3.52–3.64 (3H, m, H_3' , H_5' , H_4'), 3.84 (1H, dd, $J_1 = 10.5$ Hz, $J_2 = 5.5$ Hz), 3.94 (1H, dt, $J_1 = 9.0$ Hz, $J_2 = 5.0$ Hz, H_2'), 4.74 (1H, t, $J = 6.0$ Hz, OH_6'), 5.33 (1H, d, $J = 6.0$ Hz, OH_4'), 5.46 (1H, d, $J = 4.5$ Hz, OH_3'), 5.57 (1H, d, $J = 5.0$ Hz, OH_2'), 6.82 (1H, d, $J = 9.5$ Hz, H_1'), 7.35 (1H, t, $J = 7.0$ Hz), 7.39 (1H, t, $J = 7.0$ Hz), 7.55 (1H, t, $J = 7.5$ Hz), 7.71 (1H, d, $J = 8.0$ Hz), 8.68 (1H, d, $J = 6.5$ Hz), 9.00 (1H, d, $J = 8.0$ Hz), 9.43 (1H, d, $J = 7.0$ Hz), 10.94 (1H, br s, NH), 12.56 (1H, s, NH). ^{13}C NMR (100 MHz, CDCl_3): 60.9 (C_6'), 69.5, 72.6, 77.2, 81.1, 86.5 (C_1' , C_2' , C_3' , C_4' , C_5'), 109.8, 111.8, 119.7, 124.2, 126.4, 132.5, 135.7 (C tert arom), 114.8, 116.0, 119.1, 120.6, 121.7, 124.1, 133.6, 140.7, 142.9, 154.0 (C quat arom), 171.5, 171.7 (C=O).

4.1.14. 3-(1*H*-Indol-3-yl)-1-methyl-4-[1-(3,4,6-tri-*O*-benzyl- β -D-glucopyranos-1-yl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-pyrrole-2,5-dione (I) and 3-(1*H*-indol-3-yl)-4-[7-(3,4,6-tri-*O*-benzyl- β -D-glucopyranos-1-yl)-7*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1-methyl-pyrrole-2,5-dione (24). To a solution of 3,4,6-tri-*O*-benzyl-glucal (280 mg, 0.680 mmol) in CH_2Cl_2 (4 mL) was added at 0 °C a solution of 0.07–0.09 M dimethyldioxirane in acetone (15 mL).^{17,18} The mixture was stirred at 0 °C for 40 min. After evaporation under reduced pressure at 0 °C, the residue was dried under vacuum for 2 h. To a solution of aglycone

A (100 mg, 0.225 mmol) in THF (5 mL) was added NaH (11.8 mg, 60% dispersion in mineral oil). The mixture was stirred for 30 min at room temperature before dropwise addition of a solution of H in THF (4 mL). After refluxing for 48 h, the solvent was removed and the residue was dried under vacuum for 2 h. The residue was dissolved in formic acid and stirred at room temperature for 24 h. After evaporation, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc from 7:3 to 5:5) to give **1** (34.8 mg, 0.045 mmol, 20% yield) and **24** (111 mg, 0.143 mmol, 63% yield) as red solids.

Compound 1

Mp: 72–75 °C. IR (KBr) $\nu_{\text{C=O}}$ 1700, 1751 cm^{-1} , $\nu_{\text{NH,OH}}$ 3300–3500 cm^{-1} . Mass (FAB+) $[\text{M} + \text{H}]^+$ 775. ^1H NMR (400 MHz, CDCl_3): 3.15 (3H, s, N-CH₃), 3.65–3.84 (5H, m), 3.94 (1H, t, $J = 9.0$ Hz, H₂), 4.44 (1H, d, $J = 12.0$ Hz), 4.53 (1H, d, $J = 12.0$ Hz), 4.60 (1H, d, $J = 11.0$ Hz), 4.87 (1H, d, $J = 11.0$ Hz), 4.89 (1H, d, $J = 11.5$ Hz), 4.94 (1H, d, $J = 11.5$ Hz), 5.96 (1H, d, $J = 9.0$ Hz, H₁), 6.77 (2H, m), 6.95 (1H, d, $J = 8.5$ Hz), 7.03 (1H, t, $J = 7.5$ Hz), 7.17–7.43 (17H_{arom}), 7.47 (1H, d, $J = 3.0$ Hz), 7.80 (1H, s), 8.21 (1H, dd, $J_1 = 4.5$ Hz, $J_2 = 1.5$ Hz), 9.15 (1H, d, $J = 2.5$ Hz, NH_{ind}). ^{13}C NMR (100 MHz, CDCl_3): 24.2 (N-CH₃), 68.6 (C₆'), 73.5, 75.0, 75.5 (CH₂ of OBn), 73.6, 77.4, 77.6, 82.9, 85.5 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 111.8, 117.2, 120.4, 121.9, 122.7, 127.5–128.7, 129.2, 130.9, 142.0, 143.7 (C tert arom), 106.3, 118.9, 124.7, 126.0, 129.1, 136.1, 137.9, 138.1, 138.6, 148.0 (C quat arom), 172.0 (2C, C=O).

Compound 24

Mp: 92–95 °C. IR (KBr) $\nu_{\text{C=O}}$ 1690, 1750 cm^{-1} , $\nu_{\text{NH,OH}}$ 3200–3600 cm^{-1} . HRMS (ESI+) $[\text{M} + \text{H}]^+$ calcd for C₄₇H₄₃N₄O₇ 775.3132, found 775.3115. ^1H NMR (400 MHz, CDCl_3): 3.08 (3H, s, N-CH₃), 3.64–3.88 (6H, m), 4.42 (1H, d, $J = 12.0$ Hz), 4.48 (1H, d, $J = 12.0$ Hz), 4.51 (1H, d, $J = 11.0$ Hz), 4.79 (1H, d, $J = 11.0$ Hz), 4.80 (1H, d, $J = 11.0$ Hz), 4.91 (1H, d, $J = 11.0$ Hz), 6.53 (1H, d, $J = 9.0$ Hz, H₁), 6.62–6.74 (3H, m), 6.97 (1H, dt, $J_1 = 8.0$ Hz, $J_2 = 1.5$ Hz), 7.10–7.14 (2H, m), 7.19–7.30 (14H_{arom}), 7.63 (1H, d, $J = 2.5$ Hz), 7.67 (1H, d, $J = 7.5$ Hz), 7.83 (1H, d, $J = 6.0$ Hz), 8.11 (1H, s), 9.10 (1H, s, NH_{ind}). ^{13}C NMR (100 MHz, CDCl_3): 24.1 (N-CH₃), 68.4 (C₆'), 73.4, 74.9, 75.5 (CH₂ of OBn), 76.3, 76.6, 78.2, 85.7, 86.8 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 111.1, 111.5, 120.3, 121.8, 122.4, 126.0, 127.7–128.4, 134.4, 148.9 (C tert arom), 106.7, 107.2, 124.9, 125.2, 127.5, 128.1, 136.0, 137.7, 137.9, 138.4, 150.2 (C quat arom), 172.6, 172.7 (C=O).

4.1.15. 1-(3,4,6-Tri-*O*-benzyl- β -D-glucopyranos-1-yl)-6-methyl-1*H*-pyrido[3',2':4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(12*H*)-dione (25). A mixture of **24** (100 mg, 0.129 mmol) and iodine (388 mg, 1.52 mmol) in benzene (150 mL) was irradiated as described for the synthesis of compound **13**. After identical work-up, the residue was purified by flash chromatography (eluent: EtOAc/cyclohexane from 5:5 to 8:2) to give **25** (68 mg, 0.088 mmol,

69% yield) as a yellow solid. Mp: 237–240 °C (decomposition). IR (KBr) $\nu_{\text{C=O}}$ 1690, 1745 cm^{-1} , $\nu_{\text{NH,OH}}$ 3200–3600 cm^{-1} . HRMS (FAB+) $[\text{M} + \text{H}]^+$ calcd for C₄₇H₄₁N₄O₇ 773.2975, found 773.2956. ^1H NMR (400 MHz, CDCl_3): 3.18 (3H, s, N-CH₃), 3.72–3.86 (5H, m), 3.90 (1H, m), 4.47 (1H, d, $J = 12.5$ Hz), 4.54 (1H, d, $J = 12.0$ Hz), 4.63 (1H, d, $J = 10.5$ Hz), 4.85 (1H, d, $J = 11.0$ Hz), 4.87 (1H, d, $J = 11.0$ Hz), 5.02 (1H, d, $J = 11.5$ Hz), 5.93 (1H, d, $J = 6.0$ Hz, OH), 6.81 (1H, d, $J = 9.5$ Hz, H₁), 7.20–7.38 (15H_{arom}), 7.41 (2H, t, $J = 7.0$ Hz), 7.52 (1H, dt, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz), 7.69 (1H, d, $J = 8.0$ Hz), 8.74 (1H, d, $J = 6.0$ Hz), 8.98 (1H, d, $J = 8.0$ Hz), 9.43 (1H, dd, $J_1 = 7.5$ Hz, $J_2 = 1.0$ Hz), 12.50 (1H, s, NH_{ind}). ^{13}C NMR (100 MHz, CDCl_3): 23.5 (N-CH₃), 68.5 (C₆'), 72.3, 74.3, 74.4 (CH₂ of OBn), 72.7, 76.7, 77.7, 85.2, 86.8 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 109.9, 111.9, 119.8, 124.0, 126.5, 127.3–128.3, 132.3, 135.8 (C tert arom), 115.0, 116.2, 118.2, 119.6, 121.6, 124.3, 133.5, 137.9, 138.0, 138.8, 140.9, 142.7, 154.1 (C quat arom), 170.2, 170.3 (C=O).

4.2. Melting temperature studies

The concentrations of CT-DNA and double stranded poly(dAdT) oligonucleotide (Sigma–Aldrich) were determined from their molar extinction coefficients of 6600 $\text{M}^{-1}\text{cm}^{-1}$. Compounds **14** and **22**, NB-506, and camptothecin (Sigma) were prepared in DMSO (10 mM) and more diluted solutions were freshly prepared in the appropriate experimental buffers.

CT-DNA or poly(dAdT)₂ (20 μM) was incubated with increasing concentrations of compounds **14** or **22** in 1 mL of BPE buffer for a final drug/base pair ratio of 0.1, 0.25, 0.5 or 1. The absorbency at 260 nm was measured using an UVikon 943 spectrophotometer thermostated with a Neslab RTE111 cryostat with a point measured every min over a range of 20–100 °C with an increment of 1 °C per min. The T_m values were deduced from the midpoint of the hyperchromic transition and the variation of melting temperature (ΔT_m) calculated from the formula: $\Delta T_m = T_{m[\text{drug}+\text{DNA}]} - T_{m[\text{DNA alone}]}$.

4.3. Topoisomerase I-mediated DNA cleavage

Graded concentrations of compounds **14**, **22**, NB506 or 20 μM of CPT were incubated with supercoiled pUC19 plasmid DNA (120 ng) or 117 base pairs 3'-end-labeled DNA fragments in 20 μL of relaxation buffer for 15 min at 37 °C to ensure binding equilibrium prior to adding human recombinant topoisomerase I enzyme (4 U) for a further 30 min incubation at 37 °C. The cleavage reactions were then stopped through digestion of the topoisomerase I protein by adding SDS (0.25%) and proteinase K (250 $\mu\text{g}/\text{mL}$) for 30 min at 50 °C. The DNA samples were then differently separated depending on the size of the various DNA substrates. On the one hand, the plasmid DNA samples were loaded onto a 1% agarose ethidium bromide-containing gel after addition of 2 μL of the loading buffer. After a 2 h electrophoresis in TBE buffer at 120 V at room temperature, the

agarose gel was photographed under UV light. On the other hand, the radiolabeled DNA samples were precipitated to be then diluted in 5 μ L of denaturing loading buffer. The samples were heated at 90 °C for 3 min and subsequently cooled on ice to then be separated on a 8% denaturing polyacrylamide gel for 90 min at 65 W in TBE buffer. The cleaved bands were identified using the Molecular Dynamics 445SI PhosphorImager. The precise localization of the bases on the DNA fragment was established from comparison with the position of guanines in the G-track lane obtained from Maxam and Gilbert classical procedure.

4.4. Growth inhibition assays

Tumor cells were provided by American Type Culture Collection (Frederik, MD, USA). They were cultivated in RPMI 1640 medium (Life Science technologies, Cergy-Pontoise, France) supplemented with 10 % fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 10 mM Hepes buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.²¹ Cells were continuously exposed to graded concentrations of the compounds for four doubling times, then 15 μ L of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well and the plates were incubated for 4 h at 37 °C. The medium was then aspirated and the formazan solubilized by 100 μ L DMSO. Results are expressed as IC₅₀, concentration which reduced by 50% the optical density of treated cells with respect to untreated controls.

4.5. Chk1 inhibitory assays

Human Chk1 full-length enzyme with an N-terminal GST sequence was purchased from Upstate Biochemicals (No. 14-346). Assays for compound testing were based upon the method described by Davies et al.²² except that the final ATP concentration was 15 μ M. Compounds (10 μ M final concentration) were tested in duplicate and the average inhibition was calculated as a % relative to samples without compound. The reproducibility of assays was monitored by testing a control compound in every experiment.

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References and notes

1. Prudhomme, M. *Curr. Med. Chem.* **2000**, *7*, 1189–1212.
2. Long, B. H.; Rose, W. C.; Vyas, D. M.; Matson, J. A.; Forenza, S. *Curr. Med. Chem. AntiCancer Agents* **2002**, *2*, 255–266.
3. Prudhomme, M. *Eur. J. Med. Chem.* **2003**, *38*, 123–140.
4. Balasubramanian, B. N.; St. Laurent, D. R.; Saulnier, M. G.; Long, B. H.; Bachand, C.; Beaulieu, F.; Clarke, W.; Deshpande, M.; Eumme, J.; Fairchild, C. R.; Frennesson, D. B.; Kramer, R.; Lee, F. Y.; Mahler, M.; Martel, A.; Narasimhulu Naidu, B.; Rose, W. C.; Russell, J.; Ruediger, E.; Solomon, C.; Stoffan, K. M.; Wong, H.; Zimmermann, K.; Vyas, D. M. *J. Med. Chem.* **2004**, *47*, 1609–1612.
5. Prudhomme, M. *Curr. Med. Chem. AntiCancer Agents* **2004**, *4*, 509–521.
6. Marminon, C.; Pierré, A.; Pfeiffer, B.; Pérez, V.; Léonce, S.; Joubert, A.; Bailly, C.; Renard, P.; Hickman, J.; Prudhomme, M. *J. Med. Chem.* **2003**, *46*, 609–622.
7. Marminon, C.; Pierré, A.; Pfeiffer, B.; Pérez, V.; Léonce, S.; Renard, P.; Prudhomme, M. *Bioorg. Med. Chem.* **2003**, *11*, 679–687.
8. Messaoudi, S.; Anizon, F.; Léonce, S.; Pierré, A.; Pfeiffer, B.; Prudhomme, M. *Eur. J. Med. Chem.* **2005**, *40*, 961–971.
9. Mente, S.; Maroncelli, M. *J. Phys. Chem. A* **1998**, *102*, 3860–3876.
10. Mérour, J. Y.; Joseph, B. *Curr. Org. Chem.* **2001**, *5*, 471–506.
11. Zhao, B.; Bower, M. J.; McDevitt, P. J.; Zhao, H.; Davis, S. T.; Johanson, K. O.; Green, S. M.; Concha, N. O.; Zhou, B. B. S. *J. Biol. Chem.* **2002**, *277*, 46609–46615.
12. Routier, S.; Coudert, G.; Mérour, J. Y.; Caignard, D. H. *Tetrahedron Lett.* **2002**, *43*, 2561–2564.
13. Rodrigues Pereira, E.; Belin, L.; Sancelme, M.; Prudhomme, M.; Ollier, M.; Rapp, M.; Severe, D.; Riou, J.-F.; Fabbro, D.; Meyer, T. *J. Med. Chem.* **1996**, *39*, 4471–4477.
14. Messaoudi, S.; Anizon, F.; Pfeiffer, B.; Prudhomme, M. *Tetrahedron* **2005**, *61*, 7304–7316.
15. Ward, D. E.; Gai, Y.; Kaller, B. F. *J. Org. Chem.* **1995**, *60*, 7830–7836.
16. Gallant, M.; Link, J. T.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 343–349.
17. Murray, R. W.; Singh, M. *Org. Synth.* **1997**, *74*, 91–97.
18. Bovin, N. V.; Zurabyan, S. E.; Khorlin, A. Y. *Carbohydr. Res.* **1981**, *98*, 25–35.
19. Prudhomme, M. *Curr. Med. Chem. AntiCancer Agents* **2004**, *4*, 435–438.
20. Jiang, X.; Zhao, B.; Britton, R.; Lim, L. Y.; Leong, D.; Sanghera, J. S.; Zhou, B. B. S.; Piers, E.; Andersen, R. J.; Roberge, M. *Mol. Cancer Ther.* **2004**, *3*, 1221–1227.
21. Léonce, S.; Pérez, V.; Casabianca-Pignède, M. R.; Anstett, M.; Bisagni, E.; Atassi, G. *Invest. New Drugs* **1996**, *14*, 169–180.
22. Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.* **2000**, *351*, 95–105.