Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 3709–3722

BIOORGANIC & MEDICINAL CHEMISTRY

Rebeccamycin Analogues Bearing Amine Substituents or Other Groups on the Sugar Moiety

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Received 28 February 2003; accepted 16 May 2003

Abstract—In the course of structure–activity relationship studies on rebeccamycin analogues, a series of compounds bearing an amino function on the sugar moiety were synthesized with the aim of improving the solubility and interaction with the macromolecular target(s). The syntheses of amino derivatives and the corresponding chloro, iodo and azido intermediates are described. Their interaction with DNA and effects on human DNA topoisomerases I and II were investigated. Their antimicrobial activities against two Gram-positive bacteria, *Bacillus cereus* and *Streptomyces chartreusis*, a Gram-negative bacterium *Escherichia coli* and a yeast *Candida albicans* were also determined. 6′-Amino compound **7** and 6′-*N*-methylamino **14** very efficiently inhibit the growth of *E. coli*. The introduction of an amino group at the 6′-position strongly enhances the capacity of the drugs to interact with DNA but almost abolishes their poisoning effect on topoisomerase I. Unlike the vast majority of rebeccamycin analogues previously studied, the newly designed compounds do not stimulate DNA cleavage by topoisomerase I. The enhanced capacity of the 6′-amino glycosyl rebeccamycin derivatives to bind to DNA likely account for the improved biological profiles. DNA and topoisomerase I represent two independent targets which can both be used for the development of antitumor rebeccamycin derivatives.

Introduction

Indolocarbazoles represent a very promising series of antitumor agents.[1,2] A few synthetic compounds structurally related to the antibiotics staurosporine and rebeccamycin are currently undergoing clinical trials as anticancer agents.[3,4] For the staurosporine analogues, such as the 7-hydroxy derivative UCN-01 (Chart 1), the main targets are kinases.[5–7] In contrast, rebeccamycin analogues show no significant effect on PKC but generally bind to DNA and inhibit topoisomerase I.[8–12] In this series, the synthetic drug NB-506 (Chart 1) was initially tested in the clinic[13] but it has now been replaced with a more potent analogue, named J-107088.[14] This compound is more potent than NB-506 in terms of cytotoxicity but it is also more soluble in aqueous media and its plasma stability is also significantly reinforced.[15] The limited water solubility of indolocarbazoles needs to be taken into account for the development of analogues which could be more easily used in the clinic. Therefore, several attemps have been undertaken to design water soluble indolocarbazole drugs. To date, at least three different approaches have been followed to achieve this goal. The first is to introduce a hydrophilic group on the imide nitrogen, such as the *N*-bis(hydroxymethyl)methylamino group of J-107088 in place of the *N*-formylamino group of NB-506. A similar side chain at the N6 position also exists in the rebeccamycin analogue NSC655649 which appears promising for the treatment of solid tumors, especially in children[16,17] (Chart 1). The synthesis of various 6-*N*-amino analogues of NB-506 has been recently reported.[18] The second possibility consisted to elongate the carbohydrate side chain. A disaccharide analogue of NB-506 has been prepared and several indolocarbazole diglycosides related to the antitumor antibiotic AT2433-B1 have been synthesized and studied.[19–21] In most cases, the water solubility was effectively increased but the replacement of the glucosyl moiety of NB-506 by a maltosyl chain or a more complex disaccharide (meth-

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doi:10.1016/S0968-0896(03)00343-2

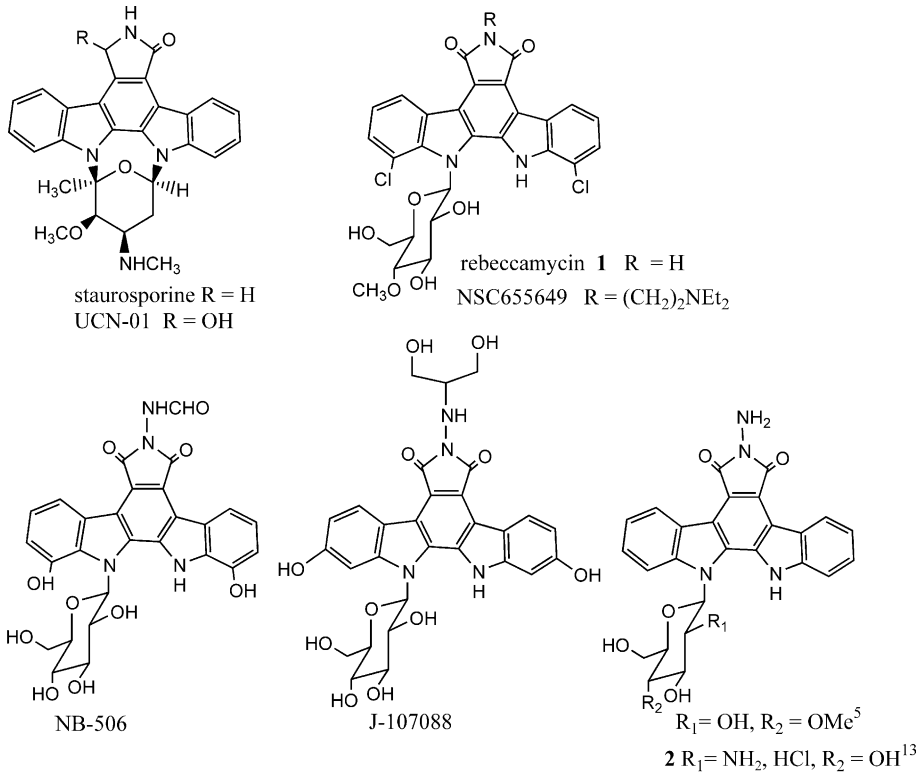


Chart 1.

oxyglucose-2,4-dideoxy-4-methylamino-L-xylose) was found to abolish the capacity of the drug to stimulate DNA cleavage by topoisomerase I whereas the cytotoxic potential was preserved. The third potential approach is to substitute the uncharged sugar residue of rebeccamycin for a positively charged amino-carbohydrate. This approach has been recently tested and it is extended here.[22]

We have previously reported the design of a rebeccamycin analogue bearing a 2′-amino group on the carbohydrate domain (Chart 1, compound **2**).[22] We found that the incorporation of the 2′-amino function reinforces the interaction of the drug with DNA without affecting the capacity of the compound to stabilize topoisomerase I–DNA covalent complexes and without significant modification of the cytotoxicity profiles. To extend this approach, we decided to construct other rebeccamycin derivatives containing an amino function on the sugar moiety, at either the 3′-position or the 6′-position. In this paper, we describe the syntheses and biological properties of rebeccamycin analogues possessing a primary, secondary or tertiary amine on the sugar residue as well as those of the chloro, azido, and iodo intermediates.

Results

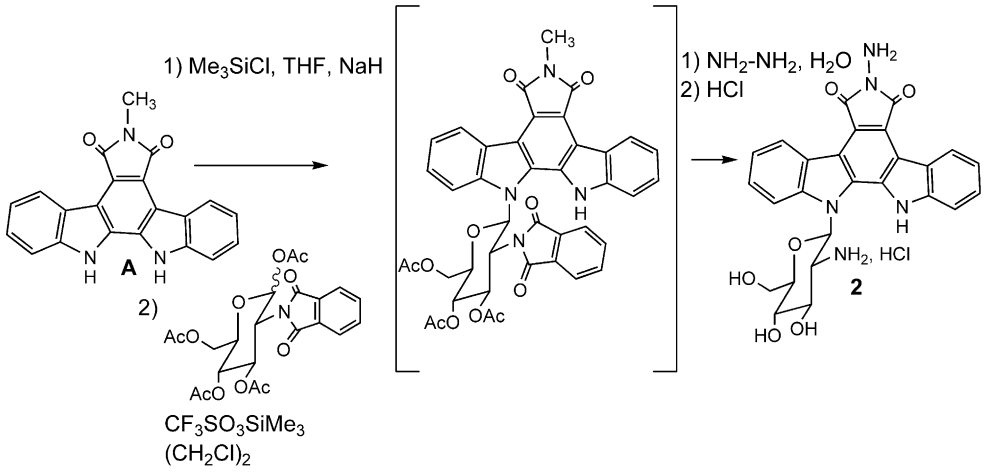
Chemistry

To introduce an amino group on the sugar moiety, several routes were investigated. Firstly, by total synthesis via the coupling of a sugar containing a protected amine function to an indolocarbazole aglycone. The coupling was performed with *N*-methylmaleimide indolocarbazole trimethylsilylated at one of the indole nitrogens in the presence of trimethylsilyltriflate, then hydrazinolysis of the intermediate led to diamine **2** (Scheme 1).[22]

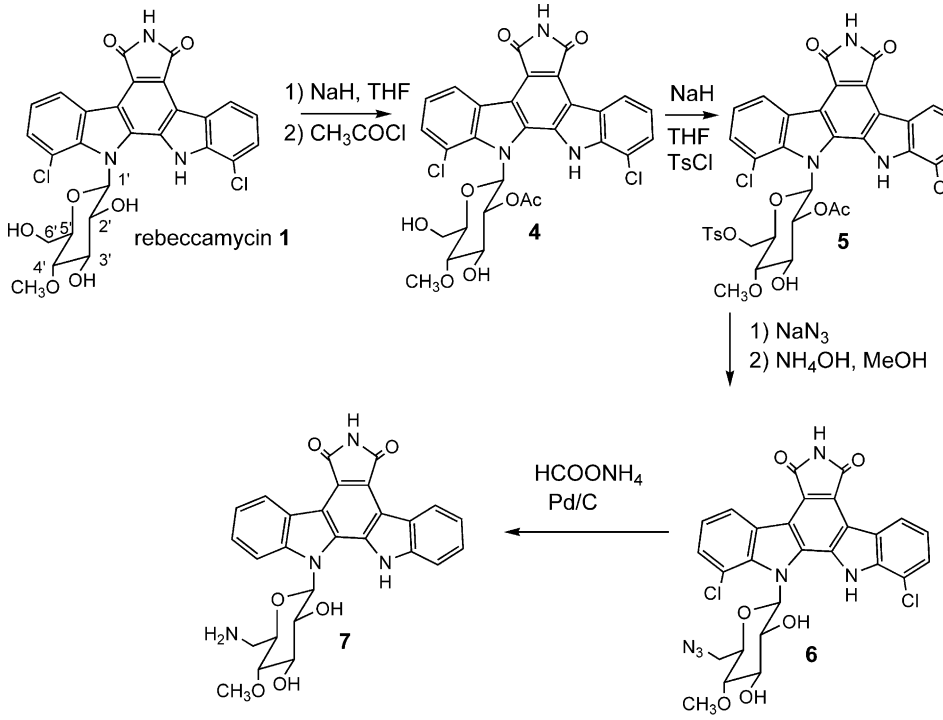
Semi-synthesis from the bacterial metabolite rebeccamycin allowed the introduction of an amine function on the sugar moiety at the 6′-position or 3′-position. The initial reactions sequence performed to obtain the amine at the 6′-position is shown in Scheme 2: protection of the hydroxy group of rebeccamycin at the 2′-position was performed yielding **4**,[23] tosylation at the 6′-position, nucleophilic substitution with sodium azide, deprotection of the hydroxy group at the 2′-position, and finally reduction of the azide **6** to dechlorinated amine **7** using ammonium formate in the presence of Pd/C.[24]

However, this long procedure was not satisfactory. It needed five steps, major difficulties were encountered for the purification of compound **4**, a low and variable yield was obtained for compound **5**. For these reasons, another chemical strategy was investigated (Scheme 3).

A one-step replacement of the hydroxy group at the 6′-position by a chlorine atom was performed using triphenylphosphine and CCl_4 in pyridine[25] leading to a mixture of **8** and **9** (Scheme 3a). The same reaction performed with dechlorinated rebeccamycin **10** led to **11** in an excellent yield (Scheme 3b). Compound **11** was converted to the iodo derivative **12** by halogen exchange. Compound **12** treated either with dimethylamine, methylamine and azabicyclooctylamine led to amines **13**, **14** and **15**, respectively, which were converted to the



Scheme 1.

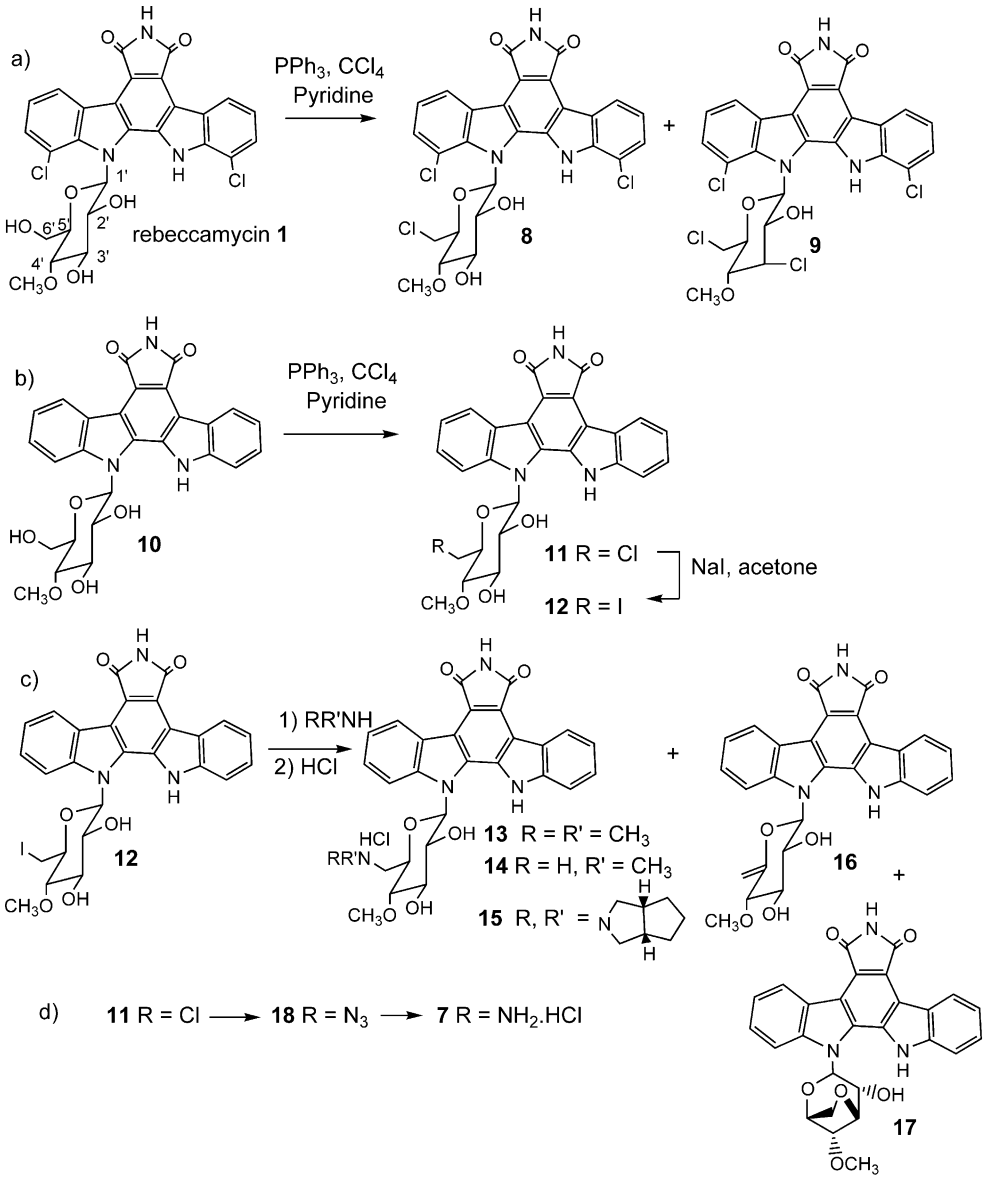


Scheme 2.

corresponding hydrochlorides (Scheme 3c). In addition to amines **13**, **14** and **15**, compound **16**, possessing an exocyclic methylene on the carbohydrate moiety and 3′,6′-anhydro **17**, resulting from elimination of hydroiodic acid were obtained as minor products of the reactions. A nucleophilic substitution on the chloro derivative **11** with sodium azide yielded the azido compound **18** which was further converted to amine **7** using hydrogen in the presence of catalytic amounts of Pd/C (Scheme 3d). The introduction of an amine function at the 3′-axial position was achieved according to the sequence of reactions shown in Scheme 4.

Rebeccamycin was monotosylated at the 2′-position. Substitution using sodium azide gave compounds **19** and **20**[26] by either nucleophilic attack of the azide ion at the 3′-position of the transient epoxide or by nucleophilic substitution by the indole nitrogen. The stereochemistry of compound **20** has been assigned from X-ray diffraction studies (unpublished results). Azide **19** was then converted to the corresponding dechlorinated amine hydrochloride **21** by reduction with ammonium formate and Pd/C followed by an acidic treatment.

For most of the reactions, the products were obtained as conformers due to possible hydrogen bonds between the functional groups of the sugar moiety and the indole N–H. In the case of compounds bearing the chlorine atoms on the indolocarbazole unit, hydrogen bonds between the indole N–H and the neighbouring halogen atom could modify the orientation of the sugar moiety.[23] For each compound in which conformers were hypothesized from ^{1}H NMR spectra, the spectra have been recorded at various temperatures and in different



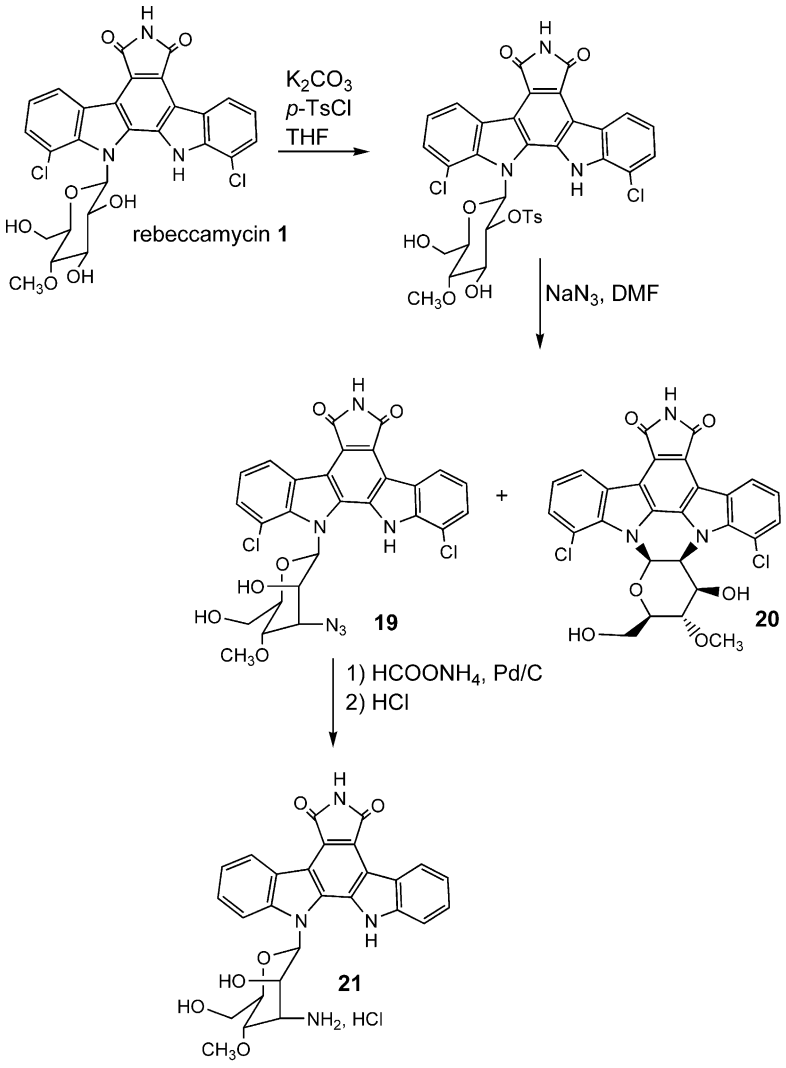
Scheme 3.

solvents to confirm this hypothesis and the ratio of the conformers at room temperature is given in the experimental section. Gilbert et al.[27] have shown that in the absence of chlorine atoms on the indolocarbazole framework, hydrogen bonds can be formed between the indole NH and the oxygen of the pyranose ring and the oxygen at C6′. These possible hydrogen bonds could stabilize a 'closed' conformation. In an open conformation, the indolocarbazole NH is hydrogen bonded to the solvent (Scheme 5). The same conformations can be postulated for compounds having at C6′ an atom with non-bonding electrons.

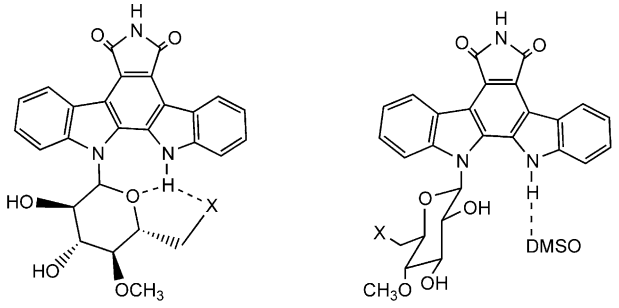
DNA interaction

We examined the ability of the indolocarbazoles to affect the thermal denaturation profile of the polynucleotide poly(dA-dT)·poly(dA-dT) (Fig. 1).

The very weak stabilizing effect of rebeccamycin (**1**) has been previously attributed to the chlorine atoms at position 1,11.[9] Dechlorinated rebeccamycin (**10**) binds to DNA much more tightly than the parent antibiotic. It is therefore unsurprising that compounds **8** and **9** which possess chloro groups on both the chomophore and the sugar do not stabilize the polynucleotide against heat denaturation. Compounds **7**, **10**–**18** only differ by the nature of the 6′ substituent on the carbohydrate. Substitution with a chloro group (**11**) only slightly reduces the extent of DNA binding compared to dechlorinated rebeccamycin (**10**) whereas substitution with a bulky iodo group (**12**) apparently prevents the drug from interacting with DNA. The incorporation of a 6′-amino group reinforces the capacity of the drugs to interact with DNA. The measured ΔT_m values reach about 40 °C (at a drug/DNA-nucleotide ratio of 0.5) with the 6′-NH_2 (**7**), 6′-$NHCH_3$ (**14**) and 6′-$N(CH_3)_2$ (**13**) derivatives. The presence of a hydrogen bond donor group is therefore not necessary for promoting the interaction with the nucleic acid. The azabi-



Scheme 4.



Scheme 5.

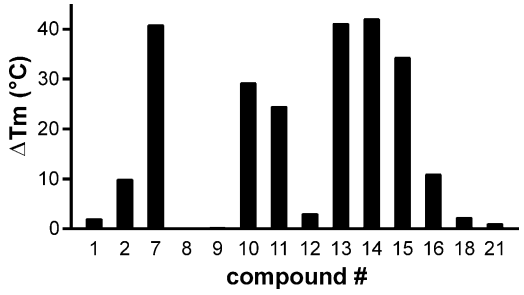


Figure 1. Variation in melting temperature (ΔT_m) of poly(dA-dT)·(dA-dT) induced by the binding of the test drugs. T_m measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM $Na_2H_2PO_4$, 1 mM EDTA) using 20 μM polynucleotide and 10 μM drug, in 1-mL quartz cuvettes at 260 nm with a heating rate of 1 °C/min.

cyclooctylamine derivative **15** protects poly(dA-dT)·poly(dA-dT) against thermal denaturation much more strongly than compound **16** bearing an exocyclic methylene group and the azido compound **18**.

The ΔT_m values vary considerably depending on the position of the newly added NH_2 group at position 2′ (**2**), 3′ (**21**) or 6′ (**7**) on the carbohydrate residue. There is no doubt that the incorporation of the NH_2 group at position 6′ is by far the most favorable position. The ΔT_m value is more than 3 times higher with **7** compared to **2**. At first sight, the 3′-amino substitution is apparently not favorable but the carbohydrate's stereochemistry of **21** is different from that of **2** and **7** and, for this reason, no strict comparison can be made. The increased capacity of the 6′-amino derivative (**7**) to bind

to DNA compared to its 2′-amino analogue (**2**) is also clearly evident from the DNA relaxation experiment (see below). The same T_m experiments were performed with DNA from calf thymus (42% GC) and similar results were observed but the differences between the compounds were much less pronounced due to the higher stability of this natural DNA ($T_m = 60 \pm 1$ °C in BPE buffer, 16 mM Na) compared to the synthetic polynucleotide ($T_m = 42 \pm 1$ °C). It was not possible to study the variations of thermal stability with poly(dG-dC)·poly(dG-dC) due to the very high stability of this polynucleotide, under the ionic conditions used in these experiments ($T_m > 90$ °C in BPE buffer) but we used the polynucleotide poly(dI-dC)·poly(dI-dC) which contains inosine residues in place of guanosines. Here again, we observed that the 6′-amino derivative **7** was far more potent than its 2′ analogue **2** stabilizing the double helical structure ($\Delta T_m = 33°$ and 9 °C for **7** and **2**, respectively).

Topoisomerases inhibition

All indolocarbazole compounds we have synthesized so far have no effect on topoisomerase II but it has been reported that the afore-mentioned rebeccamycin analogue NSC655649 is an inhibitor of topoisomerase II. These considerations prompted us to investigate the effects of the test drugs on the relaxation of supercoiled DNA by human topoisomerase II (p170 isoform from TopoGen Inc.). None of the compounds proved to be a poison for topoisomerase II. Only with the 6′-amino compound (**7**) we detected a very weak effect.

As shown in Figure 2, this compound interferes with the catalytic activity of topoisomerase II in two ways. The drug-induced inhibition of the relaxation of supercoiled DNA can be attributed to its high affinity for DNA. In addition, this compound weakly promotes DNA cleavage by the enzyme, as revealed by the production of a faint band corresponding to linear DNA. This band due to double-stranded DNA cleavage is considerably weaker than that observed under identical conditions with the reference topoisomerase II poison etoposide but the band is however detectable (see the contrast image below the main gel in Fig. 2). No such band was detected with the 2′-amino derivative **2**. In addition to the linear DNA band, we observed with both the 2′ and 6′-amino compounds a DNA band that migrates between the bands corresponding to nicked and linear DNA. This band, as yet unidentified, suggests that the drugs exert a peculiar effect on DNA and/or DNA-topoisomerase complexes. The effect of compound **7** on the catalytic activity of topoisomerase II is real but very weak and for this reason, we think it is unlikely that topoisomerase II is a target for the compound into cells.

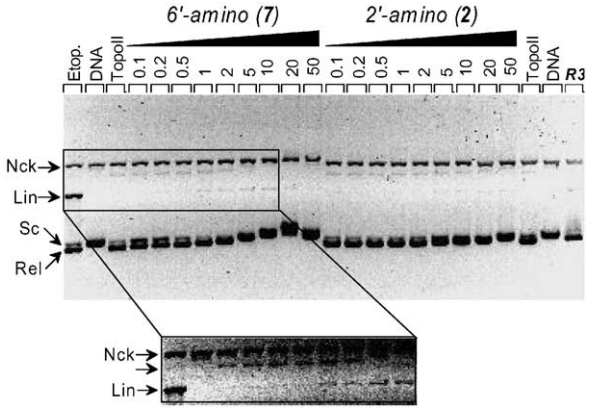


Figure 2. Effect of increasing concentrations of **2** and **7** on the relaxation of plasmid DNA by human topoisomerase II. Supercoiled DNA (lane DNA) was incubated with topoisomerase II (lane TopoII) in the absence and presence of the test drug at the indicated concentration (μM). DNA samples were separated by electrophoresis on an agarose gel containing ethidium bromide. The gel was photographed under UV light. Nck, nicked; Rel, relaxed; Sc, supercoiled; Lin, linear DNA. Etoposide (Etop.) and the indolocarbazole drug R3 were used at 50 μM. A high contrast image of a portion of the gel is presented to better show the bands corresponding to nicked and linear DNA, and the intermediate band.

Similar relaxation experiments were performed with the corresponding type I enzyme. Closed circular DNA was treated with human topoisomerase I in the absence and presence of the test drugs at 50 μM.

As shown in Figure 3, supercoiled DNA is fully relaxed by the enzyme in the absence of drug (compare lanes DNA and Topo I). The relaxed DNA migrates faster than the supercoiled plasmid on an agarose gel containing ethidium bromide. Some compounds, such as compounds **8**, **9**, **18** and **21** have almost no effect on topoisomerase I-mediated relaxation of DNA. With other compounds, in particular with **13**, **14** and **7**, the electrophoretic mobility of the supercoiled DNA is strongly reduced. This reflects the tight binding to DNA of the 6′-NH_2 (**7**), 6′-$NHCH_3$ (**14**) and 6′-$N(CH_3)_2$ (**13**) derivatives, in perfect agreement with the T_m data reported above. We can conclude at this stage that the increased DNA binding capacity provided by the 6′-amino substitution is at the expense of topoisomerase I inhibition. In some cases the intensity of the band corresponding to the nicked form of DNA has increased significantly. This effect, observed with camptothecin (lane Cpt), reflects the stabilization of topoisomerase I–DNA cleavable complexes. This is the case also with

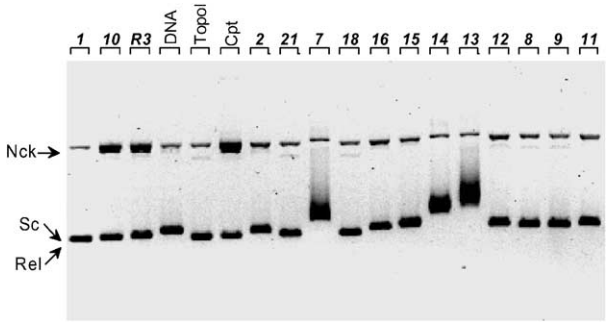


Figure 3. Inhibition of topoisomerase I-mediated relaxation of DNA by rebeccamycin analogues. Native supercoiled pKMp27 DNA (0.5 μg) (lane DNA) was incubated for 30 min at 37 °C with 4 units human topoisomerase I in the absence (lane Topo I) or presence of the drug at 25 μM. Reactions were stopped with sodium dodecylsulphate and treatment with proteinase K. The DNA samples were run on an agarose gel containing ethidium bromide (1 mg/mL). Nck, nicked; Rel, relaxed; Sc, supercoiled. The gel was photographed under UV light.

dechlorinated rebeccamycin (**10**) and its *N6*-OH analogue named **R3** which we have extensively studied.[9,27–30] A weaker but substantial increase of the nicked DNA form was observed with different compounds. In this assay, the compounds rank in the order **R3**, **10**≫**16**, **12**>**11**>**2**, **8**>**9**, **15**, **18**, **21**>**7**, **13**, **14**.

We compared further the effects of the 6′-amino (**7**) and 2′-amino (**2**) derivatives using the same relaxation assay.

The concentration-dependent profiles presented in Figure 4 clearly show that the 6′-amino compound has only a marginal topoisomerase I poisoning capacity compared to the corresponding 2′-amino derivative. But this amino compound is itself much less efficient than camptothecin or the indolocarbazole derivative **R3** at stimulating DNA cleavage by topoisomerase I. This gel also indicates that the 6′-amino compound binds tightly to DNA, as judged from its unwinding effect on supercoiled DNA. Linear dichroism experiments revealed, as expected, that this compound typically behaves as a strong DNA intercalating agent (data not shown). We have previously demonstrated that the incorporation of an amino group at the 2′ position does not hinder the capacity of the compound to intercalate into DNA.[13]

In order to better compare the compounds, their topoisomerase I inhibitory properties were further examined using the 117-mer *Eco*RI-*Pvu*II fragment of the pBS plasmid as a substrate. The labeled DNA fragment was incubated with topoisomerase I in the presence and absence of the indolocarbazoles (25 μM each) and the resulting DNA cleavage products were analyzed by polyacrylamide gel electrophoresis under denaturing conditions. A typical example of a gel is shown in Figure 5.

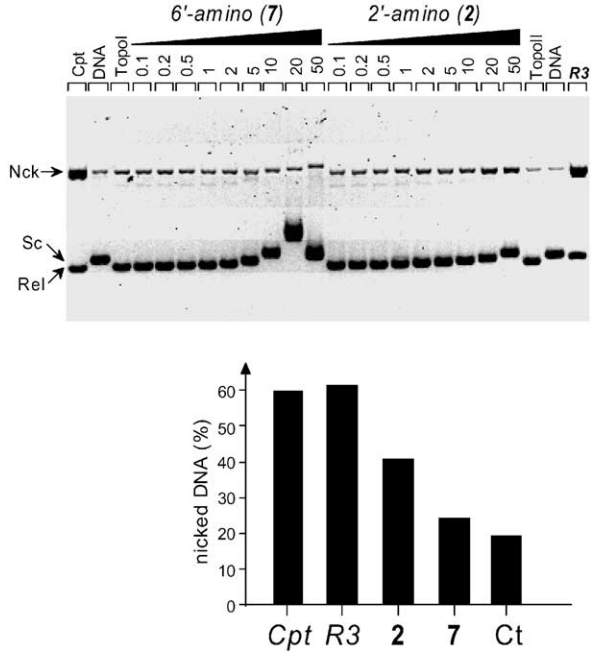


Figure 4. Effect of increasing concentrations of **2** and **7** on the relaxation of plasmid DNA by human topoisomerase I. Supercoiled DNA (lane DNA) was incubated with topoisomerase I (lane TopoI) in the absence and presence of the test drug at the indicated concentration (μM). DNA samples were separated by electrophoresis on an agarose gel containing ethidium bromide. The gel was photographed under UV light. Nck, nicked; Rel, relaxed; Sc, supercoiled. Camptothecin (Cpt) and the indolocarbazole drug **R3** were used at 50 μM. The histogram indicates the extent of single strand DNA cleavage (% nicked DNA, form II) obtained in the presence of topoisomerase I alone (Ct) or with camptothecin (Cpt) or the indolocarbazole drugs **R3**, **2** and **7** (50 μM each).

Important differences can be seen between the compounds. All compounds stimulate DNA cleavage by topoisomerase I at identical sites but the extent of cleavage varies considerably from one indolocarbazole to another. Prominent cleavage was observed with the 2′-amino compound (**2**) whereas the 6′-amino (**7**) and 3′-amino (**21**) derivatives showed no effect. The *N*-methyl and *N*-dimethyl analogues (**13**, **14**) were also inactive whereas the chloro (**11**), iodo (**12**) and methylene (**16**) derivatives efficiently stimulated cleavage of DNA by topoisomerase I. The results obtained from these sequencing experiments are in good agreement with the results obtained from the relaxation assays and thus, reinforce the conclusion that tight binding to DNA is detrimental to topoisomerase I inhibition. In terms of sequence analysis, the results are consistent with previous studies. The cleavage sites stimulated by indolocarbazoles such as **R3** or compound **2** correspond essentially to TG and CG sites whereas the cleavage is essentially restricted to TG steps with camptothecin.[6,26]

Antimicrobial properties

Since the discovery of *Escherichia coli* topoisomerase I, researchers have isolated Type I topoisomerases from both prokaryotes and eukaryotes.[31,32] Consequently, we investigated the antimicrobial activities of compounds **1**, **2**, **4**, **7**, **8**, **11**–**16**, **18**, **19**, **21** against two Gram-positive bacteria (*Bacillus cereus* and *Streptomyces chartreusis*), a Gram-negative bacterium (*E. coli*) and a yeast (*Candida albicans*) (Table 1). Except amines **7** and **14** which exhibited a strong antimicrobial activity against *E. coli*, the other compounds tested in this series were inactive against this Gram-negative bacterium and against *C. albicans*. The most efficient compounds against the two Gram-positive bacteria tested were the amines **7**, **13** and **14**. The antimicrobial activity of **16** was found in the same range than that of rebeccamycin. The efficiency of amines **7** and **14** against *E. coli* are exceptional for indolocarbazole compounds. The MICs values against *B. cereus* were determined. Compounds **12** and **14** were found to be the most active with MICs values of about 1 μM, while the MIC value for the primary amine **7** was about 5 times higher. The discrepancy observed between the MIC values and the diameters of growth inhibition of compounds **7**, **13** and **12** could be explained by a limited diffusion of some compounds in the agar medium.

Conclusion

Compounds **7**, **13** and **14** exhibit promising antimicrobial activities which can be directly associated

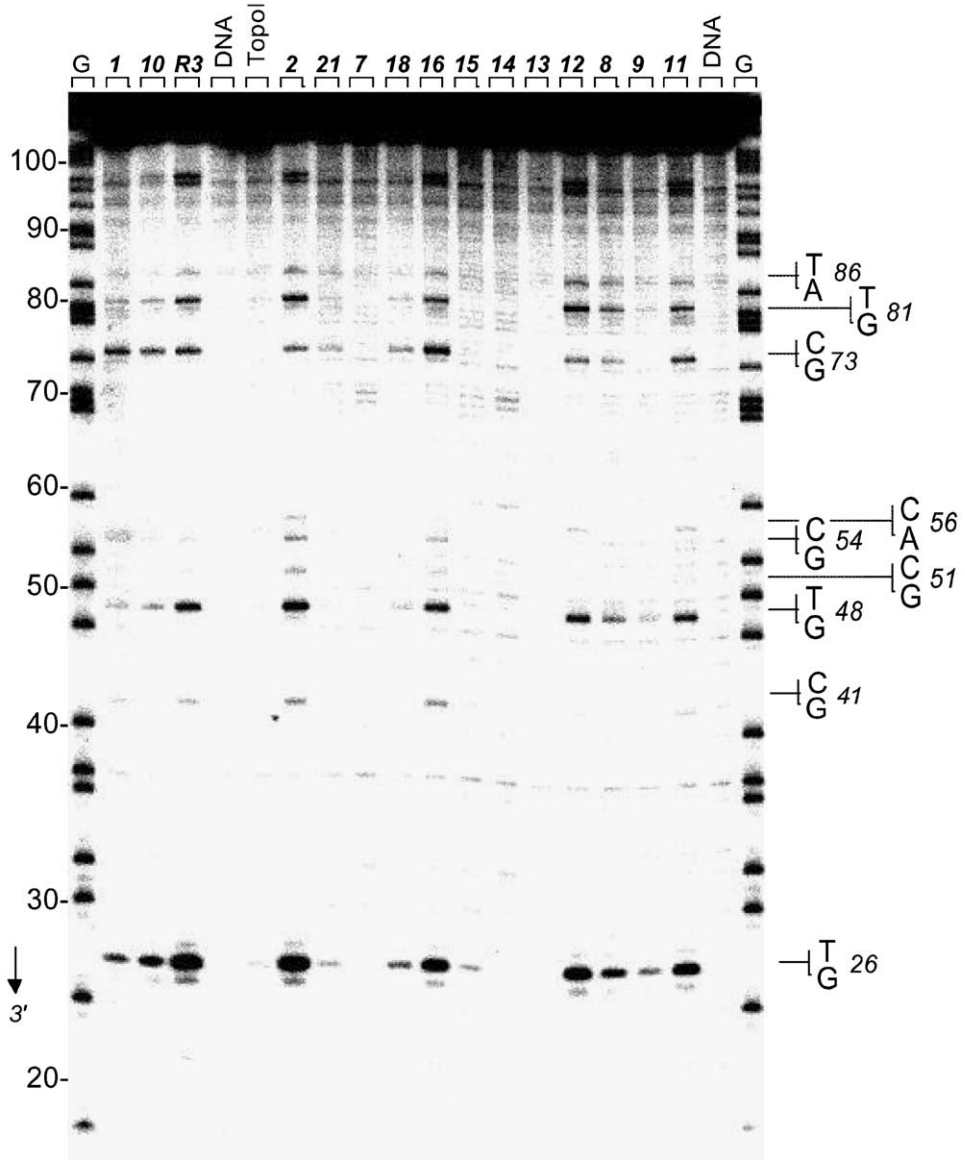


Figure 5. Sequencing of topoisomerase I-mediated DNA cleavage sites observed in the presence of the drugs. The 117-mer *Eco*RI-*Pvu*II fragment from plasmid pBS was 3′-end labeled at the *Eco*RI site with [α-^{32}P]dATP in the presence of AMV reverse transcriptase and then subjected to cleavage by topoisomerase I. The cleavage products were resolved on an 8% polyacrylamide gel containing 7 M urea. Guanine-specific sequence markers obtained by treatment of the DNA with dimethylsulfate followed by piperidine were run in lanes marked G. The control track (DNA) contained no drug and no enzyme. The lane Topo I refers to the radiolabeled DNA substrate incubated with the enzyme but with no drug. Each indolocarbazole drug was used at 50 μM. Numbers on the left side of the gel refer to the standard numbering scheme for the nucleotide sequence of the DNA fragment. The positions and nucleotide sequences of the main topoisomerase I cleavage sites are indicated.

with their enhanced capacity to interact with DNA. These three compounds do not stabilize topoisomerase I–DNA covalent complexes, in contrast to indolocarbazole compounds like **2** and **R3**. As such, this observation is consistent with the recent discovery that topoisomerase I may be dispensable for the rebeccamycin-type compounds to exert their cytotoxic activity. Indeed, we found that a maltosyl–indolocarbazole compound which has totally lost the capacity to stimulate DNA cleavage by topoisomerase I remained as potent as NB-506 to inhibit the growth of various human and murine tumour cell lines.[19] At least in this case, topoisomerase I appears not to contribute to the cytotoxic action of the indolocarbazole compounds. But the situation is not so simple because there are examples where the cytotoxicity was directly proportional to the effect of the compound on topoisomerase I. We have previously reported that a highly cytotoxic regio-isomeric form of the antitumor drug NB-506 was most effective against topoisomerase I but did not bind to DNA.[33] Therefore, it seems now clear that interaction with DNA and topoisomerase I inhibition correspond in fact to two separate mechanisms. Both DNA and topoisomerase I are valid targets for the development of antitumor rebeccamycin derivatives. Studies are in progress to determine the in vitro antiproliferative activities of the new rebeccamycin analogues against a panel of tumor cell lines.

Table 1. Antimicrobial activities against two Gram-positive bacteria (*B. cereus* and *S. chartreusis*), one Gram-negative bacterium (*E. coli*) and one yeast (*C. albicans*)[a,b]

Compd	*B. cereus* ATCC 14579	*S. chartreusis* NRRL 11407	*E. coli ATCC 11303*	*C. albicans IP 444*	*B.cereus MIC* (μM)
1	+ +	+ +	–	–	10.9
2	–	–	–	–	nd
4	–	–	–	–	>81
7	+ + +	+ + + +	+ + +	–	6.24
8	–	–	–	–	85
11	±	±	–	–	3.0
12	+	±	–	–	1.27
13	+ + + +	+ + + +	–	–	5.90
14	+ + + +	+ + + +	+ + +	–	1.42
15	±	±	–	–	4.96
16	+ +	+ + +	–	–	3.23
18	–	–	–	–	>95
19	–	–	–	–	>84
21	–	–	–	–	>100

[a]Minimal inhibitory concentrations for *B. cereus* (MIC in μM).
[b]The size of zones of growth inhibition was 13–16 mm (+ + + +), 10–12 mm (+ + +), 8–9 mm (+ +), 7–8 mm (+), and 6–7 mm (±).

Experimental

Chemistry

IR spectra were recorded on a Perkin-Elmer 881 spectrometer (ν in cm^{-1}). NMR spectra were performed on a Bruker AC 400 (^1H: 400 MHz, ^{13}C: 100 MHz) (chemical shifts δ in ppm, the following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), triplet (t), pseudo-triplet (pt), multiplet (m), tertiary carbons (C tert), quaternary carbons (C quat). The signals were assigned from ^1H–^1H COSY and ^{13}C–^1H correlations. Mass spectra (FAB+) were determined at CESAMO (Talence, France) on a high resolution Fisons Autospec-Q spectrometer. 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 F_{254} from Merck). Rebeccamycin was from our laboratory stock sample.

1,11-Dichloro-12-(6-chloro-6-deoxy-4-*O*-methyl-β-D-glucopyrannosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7-dione (8) and 1,11-dichloro-12-(3,6-deoxy-3,6-dichloro-4-*O*-methyl-β-D-allopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole-5,7-dione (9). Rebeccamycin (50 mg, 0.088 mmol) was dissolved in pyridine (0.5 mL). PPh_3 (92 mg, 0.351 mmol) then CCl_4 (0.175 mmol, 17 μL) were added. The mixture was stirred at room temperature for 3.5 h, then poured into water. EtOAc was added and the organic phase was washed successively with 1 N HCl, water, saturated aqueous $NaHCO_3$ then dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography (eluent, EtOAc–cyclohexane, 20:80 for the elution of compound **9**, then 40:60 for **8**) to give **8** (19.4 mg, 0.033 mmol, 38% yield) and **9** (13.1 mg, 0.022 mmol, 25% yield) as yellow solids.

8 (Conformers ratio at room temperature in DMSO-d_6: 12/1). Mp >245 °C (decomposition). IR (KBr) ν_{CO} 1710, 1750 cm^{-1}, $\nu_{NH,OH}$ 3100–3600 cm^{-1}. HRMS (FAB+) (M^+) calcd for $C_{27}H_{20}N_3O_6Cl_3$ 587.0418, found 587.0422. ^1H NMR (400 MHz, DMSO-d_6) of the major conformer δ 3.65 (3H, s, OCH_3), 3.55–3.80 (3H, m), 4.23 (2H, m), 4.34 (1H, d, J=10.3 Hz), 5.11 (1H, d, J=6.3 Hz, OH), 5.59 (1H, d, J=5.5 Hz, OH), 6.93 (1H, d, J=8.7 Hz, $H_{1'}$), 7.41 (1H, t, J=8.0 Hz), 7.45 (1H, d, J=8.0 Hz), 7.67 (1H, d, J=8.0 Hz), 7.70 (1H, d, J=8.0 Hz), 8.99 (1H, d, J=8.0 Hz), 9.21 (1H, d, J=8.0 Hz), 10.20 (1H, s, NH), 11.35 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) of the major conformer δ 44.4 ($C_{6'}$), 60.4 (OCH_3), 72.0, 77.2, 78.6, 80.5, 84.6 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 115.7, 116.4, 117.5, 119.7, 120.7, 122.7, 123.1, 125.2, 129.4, 129.6, 136.8, 138.1 (C quat arom), 122.1, 122.9, 123.6, 124.2, 126.9, 129.8 (C tert arom), 170.1, 170.4 (C=O).

9 (Conformers ratio at room temperature in DMSO-d_6: 4.2/1). Mp >265 °C (decomposition). IR (KBr) ν_{CO} 1710, 1760 cm^{-1}, $\nu_{NH,OH}$ 3200–3600 cm^{-1}. HRMS (FAB+) (M^+) calcd for $C_{27}H_{19}N_3O_5Cl_4$ 605.0079 found 605.0080. ^1H NMR (400 MHz, DMSO-d_6) of the major conformer δ 3.45 (3H, s, OCH_3), 4.15 (2H, m, $H_{4'}$ and $H_{6'}$), 4.33 (2H, m, $H_{2'}$ and $H_{6'}$), 4.56 (1H, m, $H_{5'}$), 5.08 (1H, t, J=3.0 Hz, $H_{3'}$), 5.36 (1H, *d*, J=5.4 Hz, $OH_{2'}$), 7.34 (1H, *d*, J=9.3 Hz, $H_{1'}$), 7.39 (1H, t, J=7.9 Hz), 7.46 (1H, t, J=8.4 Hz), 7.68 (1H, d, J=7.4 Hz), 7.69 (1H, d, J=7.9 Hz), 8.99 (1H, d, J=7.9 Hz), 9.21 (1H, d, J=7.9 Hz), 10.15, 11.36 (2H, 2 s, N_{imide}–H and N_{indole}–H). ^{13}C NMR (100 MHz, DMSO-d_6) of both conformers δ 44.2, 44.3 ($C_{6'}$), 55.8, 56.1 (OCH_3), 63.5, 64.3, 66.6, 67.5, 73.6, 74.1, 75.5, 76.5, 82.1, 85.8 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 116.0, 116.6, 117.7, 118.0, 119.0, 119.9, 120.3, 120.6, 122.6, 122.9, 123.2, 125.4, 127.2, 129.6, 129.9, 134.0, 136.9, 137.6, 137.8, 138.2 (C quat arom), 121.8, 122.2, 123.0, 123.5, 124.1, 126.7, 126.9, 129.4, 130.0 (C tert arom), 170.1, 170.3, 170.4, 170.5 (C=O).

12-(6-Chloro-6-deoxy-4-*O*-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]-pyrrolo[3,4-*c*]-carbazole-5,7-dione (11). Dechlorinated rebeccamycin **10** (299 mg, 0.597 mmol) was dissolved in pyridine (3 mL). PPh_3 (627 mg, 2.39 mmol) then CCl_4 (1.194 mmol, 115

μL) were added. The mixture was stirred at room temperature for 1.5 h. After identical work up as described above, the residue was purified by flash chromatography (eluent, EtOAc–cyclohexane, 50:50) to give **11** (256 mg, 0.493 mmol, 82% yield) as a yellow solid. The conformers ratio observed at room temperature in DMSO-d_6 was: 1.3/1. Mp >185 °C (decomposition). IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, $\nu_{NH,OH}$ 3150–3700 cm^{-1}. HRMS (FAB+) (M^+) calcd for $C_{27}H_{22}N_3O_6Cl$ 519.1197, found 519.1208. ^1H NMR (400 MHz, DMSO-d_6), [a]major conformer, [b]minor conformer δ 3.57 ($1H^b$, t, $J=9.4$ Hz), 3.71 ($3H^b$, s, OCH_3), 3.75 ($3H^a$, s, OCH_3), 3.84, 3.96, 4.12, 4.34, 4.58 ($6H^a+5H^b$, 4m), 5.22 ($1H^a$, d, $J=5.1$ Hz, OH), 5.45 ($1H^b$, d, $J=5.1$ Hz, OH), 5.53 ($1H^a+1H^b$, m, OH), 6.45 ($1H^a$, d, $J=8.0$ Hz, $H_{1'}$), 6.47 ($1H^b$, d, $J=7.0$ Hz, $H_{1'}$), 7.45 ($2H^a+2H^b$, m), 7.64 ($2H^a+2H^b$, m), 7.74 ($1H^a$, d, $J=8.1$ Hz), 7.83 ($1H^b$, d, $J=8.2$ Hz), 8.06 ($1H^a$, d, $J=8.6$ Hz), 8.09 ($1H^b$, d, $J=8.6$ Hz), 9.16 ($1H^a$, d, $J=8.0$ Hz), 9.19 ($1H^b$, d, $J=7.8$ Hz), 9.21 ($1H^a$, d, $J=7.8$ Hz), 9.29 ($1H^b$, d, $J=8.0$ Hz), 10.68 ($1H^a$, s, NH), 11.17 ($1H^b$, s, NH), 11.25 ($1H^a$, s, NH), 11.82 ($1H^b$, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 45.3, 45.8 ($C_{6'}$), 60.4 (OCH_3), 71.0, 73.1, 75.5, 76.0, 76.2, 76.7, 78.7, 79.5, 84.7, 86.5 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.7 (several C), 114.6, 120.4, 120.6, 121.0, 124.5, 124.7, 125.0, 126.6, 127.1, 127.3, 127.5 (C tert arom), 116.7, 116.9, 117.8, 118.6, 119.7, 120.0, 120.5, 120.9, 121.2, 121.7, 123.0, 128.0, 128.2, 129.2, 130.1, 139.1, 140.1, 140.8, 142.4 (C quat arom), 170.9, 171.0 (C=O).

12-(6-Deoxy-6-iodo-4-*O*-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]-pyrrolo[3,4-*c*]-carbazole-5,7-dione (12). To a solution of compound **11** (807 mg, 1.55 mmol) in acetone (80 mL) was added NaI (4.7 g, 31 mmol) and the mixture was refluxed for a week. The solvent was removed, EtOAc was added and the mixture washed with brine. The organic phase was washed with saturated aqueous sodium thiosulfate then brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography (eluent, EtOAc–cyclohexane, 40:60) to give **12** (845 mg, 1.38 mmol, 89% yield). The conformers ratio observed at room temperature in DMSO-d_6 was: 2/1) as an orange solid. Mp >160 °C (decomposition). IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, $\nu_{OH,NH}$ 3200–3600 cm^{-1}. HRMS (FAB+) (M^+) calcd for $C_{27}H_{22}IN_3O_6$ 611.0553 found 611.0555. ^1H NMR (400 MHz, DMSO-d_6) [a]major conformer, [b]minor conformer δ 3.40–4.12 ($6H^a+6H^b$, m), 3.74 ($3H^a$, s, OCH_3), 3.79 ($3H^b$, s, OCH_3), 5.19 ($1H^b$, d, $J=5.1$ Hz, OH), 5.42 ($1H^a$, d, $J=5.1$ Hz, OH), 5.50 ($1H^a$, d, $J=6.4$ Hz, OH), 5.52 ($1H^b$, d, $J=6.4$ Hz, OH), 6.42 ($1H^b$, d, $J=9.0$ Hz, $H_{1'}$), 6.47 ($1H^a$, d, $J=8.4$ Hz, $H_{1'}$), 7.44 ($2H^a+2H^b$, m), 7.63 ($2H^a+2H^b$, m), 7.82 ($1H^a$, d, $J=8.3$ Hz), 8.02 ($1H^b$, d, $J=7.7$ Hz), 8.08 ($1H^b$, d, $J=8.4$ Hz), 8.18 ($1H^a$, d, $J=8.4$ Hz), 9.17 ($1H^a+2H^b$, m), 9.28 ($1H^a$, d, $J=7.7$ Hz), 10.44 ($1H^b$, s, NH), 11.14 ($1H^a$, s, NH), 11.24 ($1H^b$, s, NH), 11.78 ($1H^a$, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 10.0, 10.6 ($C_{6'}$), 60.5 (OCH_3), 71.0, 73.0, 74.2, 75.6, 75.9, 76.8, 82.7, 84.8, 86.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.6, 111.7, 112.6, 115.0, 120.4, 120.9, 121.1, 121.2, 124.4, 124.6, 124.8, 126.5, 127.1, 127.2, 127.4 (C tert arom), 116.6, 117.0, 117.7, 118.5, 119.6, 119.9, 120.4, 120.8, 121.1, 121.6, 122.9, 127.9, 128.1, 129.0, 130.0, 139.0, 140.0, 140.7, 142.4 (C quat arom), 170.8, 170.9, 171.0 (C=O).

12-(6-Deoxy-6-dimethylamino-4-*O*-methyl-β-D-glucopyranosyl) - 6,7,12,13 - tetrahydro(5*H*) - indolo[2,3 - *a*] - pyrrolo[3,4-*c*]-carbazole-5,7-dione, hydrochloride (13) and 12 - (5,6 - anhydro - 4 - *O* - methyl - β - D - glucopyranosyl) - 6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]-pyrrolo[3,4-*c*]-carbazole-5,7-dione (16). To a solution of **12** (94 mg, 0.154 mmol) in DMF (3 mL) was added a 2 M solution of dimethylamine in DMF (2 mL). The mixture was stirred at 65 °C for 16 h in a sealed tube. EtOAc was added and the organic phase was washed with water then dried over $MgSO_4$. After removal of the solvent, methanol (200 μL) was added to the residue then 1 N HCl (170 μL). Addition of CH_2Cl_2 yielded the precipitation of the hydrochloride **13** as a yellow solid which was filtered off (68 mg, 0.120 mmol, 78% yield). The filtrate was evaporated and the residue dissolved in EtOAc, the organic phase was washed with water and dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography (eluent, EtOAc–CH_2Cl_2, 30:70) to give **16** (7.3 mg, 0.015 mmol, 10% yield) as a yellow solid.

13. The conformers ratio at room temperature in DMSO-d_6 was 9/1. Mp >245 °C (decomposition). IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, $\nu_{OH,NH}$ 3100–3700 cm^{-1}. HRMS (FAB+) ($M+H^+$) calcd for $C_{29}H_{29}N_4O_6$ 529.2087, found 529.2102. ^1H NMR (400 MHz, DMSO-d_6) major conformer δ 2.84 (3H, d, $J=4.3$ Hz), 2.95 (3H, d, $J=4.3$ Hz), 3.67 (3H, s, OCH_3), 3.45-3.64 (3H, m), 4.04 (1H, t, $J=8.9$ Hz), 4.15 (1H, t, $J=8.9$ Hz), 5.05 (1H, m), 5.38 (2H, br s, OH), 6.83 (1H, d, $J=8.7$ Hz, $H_{1'}$), 7.40 (1H, t, $J=7.4$ Hz), 7.47 (1H, t, $J=7.5$ Hz), 7.59 (1H, t, $J=7.1$ Hz), 7.62 (1H, t, $J=7.2$ Hz), 8.05 (1H, d, $J=8.2$ Hz), 8.09 (1H, d, $J=8.3$ Hz), 9.15 (1H, d, $J=7.9$ Hz), 9.27 (1H, d, $J=7.8$ Hz), 9.65 (1H, s, NH), 11.14 (1H, s, NH), 12.78 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) major conformer δ 42.1, 44.3 (CH_3), 57.9 (CH_2N), 59.8 (OCH_3), 70.6, 70.7, 75.9, 80.5, 85.8 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.5, 114.8, 120.2, 121.1, 124.2, 124.7, 126.5, 126.9 (C tert arom), 116.5, 117.6, 119.3, 120.5, 120.6, 123.1, 128.1, 130.3, 139.2, 141.2 (C quat arom), 171.0, 171.1 (C=O).

16. Mp >220 °C (decomposition). IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, $\nu_{NH,OH}$ 3100–3600 cm^{-1}. HRMS (FAB+) (M^+) calcd for $C_{27}H_{21}N_3O_6$ 483.1430, found 483.1416. ^1H NMR (400 MHz, DMSO-d_6 at 60 °C only one conformer was observed) δ 3.68 (3H, s, OCH_3), 4.04 (1H, m), 4.14 (1H, m), 4.35 (1H, t, $J=8.3$ Hz), 4.84 (1H, s), 4.90 (1H, s), 5.53 (2H, br s), 6.54 (1H, d, $J=8.8$ Hz, $H_{1'}$), 7.42 (1H, t, $J=7.4$ Hz), 7.47 (1H, t, $J=7.5$ Hz), 7.63 (2H, t, $J=7.5$ Hz), 7.80 (1H, d, $J=8.1$ Hz), 7.93 (1H, d, $J=8.0$ Hz), 9.18 (1H, d, $J=7.9$ Hz), 9.30 (1H, d, $J=7.9$ Hz), 10.99 (1H, br s, NH), 11.35 (1H, br s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 59.6 (OCH_3), 70.9, 74.0, 80.8, 87.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$), 95.0 ($C_{6'}$), 111.9, 114.3, 120.5, 121.2, 124.5, 124.9, 126.7, 127.3 (C tert

arom), 116.6, 117.8, 119.6, 120.8, 122.9, 128.1, 129.9, 138.7, 140.8 (C quat arom), 156.0 ($C_{5'}$), 171.0, 171.1 (C=O).

12-(3,6-Anhydro-4-*O*-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]-pyrrolo[3,4-*c*]-carbazole-5,7-dione (17). A mixture of **12** (94 mg, 0.154 mmol), DMF (5 mL) and a 40% w/w aqueous solution of methylamine (220 μL, 2.56 mmol) was stirred in a sealed tube at room temperature for 19 h. The mixture was poured into water, extracted with EtOAc. The organic phase was dried over $MgSO_4$ and the solvent was removed. To a solution of the residue in methanol (200 μL) was slowly added 1 N HCl (170 μL). The hydrochloride was precipitated by addition of CH_2Cl_2, filtered off and washed with CH_2Cl_2 to give a mixture of **13** and **14** (51 mg) as a yellow solid. From the filtrate, the solvent was removed and the residue was purified by flash chromatography (eluent, EtOAc–CH_2Cl_2, 30:70) to give **17** (7.7 mg, 0.016 mmol, 10% yield) and **16** (11.0 mg, 0.023 mmol, 15% yield) as yellow solids.

17. Mp >270 °C (decomposition). IR (KBr): ν_{CO} 1700, 1750 cm^{-1}, $\nu_{OH,NH}$ 3100–3600 cm^{-1}. HRMS (FAB+) $(M+H)^+$: calcd for $C_{27}H_{22}N_3O_6$, 484.1509, found, 484.1509. ^1H NMR (400 MHz, DMSO-d_6) δ 3.72 (3H, s, OCH_3), 4.08 (1H, d, $J=9.5$ Hz), 4.24 (1H, d, $J=9.9$ Hz), 4.44 (2H, m), 4.57 (1H, d, $J=7.8$ Hz), 4.84 (1H, s), 6.14 (1H, br s, OH), 7.00 (1H, d, $J=7.8$ Hz, $H_{1'}$), 7.44 (1H, t, $J=7.7$ Hz), 7.48 (1H, t, $J=7.3$ Hz), 7.64 (1H, dt, $J_1=8.3$ Hz, $J_2=1.2$ Hz), 7.67 (1H, dt, $J_1=7.0$ Hz, $J_2=1.0$ Hz), 7.76 (1H, d, $J=8.3$ Hz), 7.80 (1H, d, $J=8.4$ Hz), 9.13 (1H, d, $J=7.9$ Hz), 9.23 (1H, d, $J=7.7$ Hz), 10.97 (1H, br s), 11.20 (1H, br s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 58.1 (OCH_3), 71.2 ($C_{6'}$), 73.1, 74.7, 74.9, 80.0, 82.0 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.0, 112.2, 120.8, 121.2, 124.5, 124.9, 127.0, 127.4 (C tert arom), 117.1, 117.8, 120.0, 120.9, 121.3, 122.4, 129.0, 129.8, 140.1, 140.7 (C quat arom), 170.9, 171.0 (C=O).

12-(6-Deoxy-4-*O*-methyl-β-D-6-methylamino-glucopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]-pyrrolo[3,4-*c*]-carbazole-5,7-dione, hydrochloride (14). To a solution of **12** (94 mg, 0.154 mmol) in THF (5 mL) was added a 40% w/w aqueous solution of $MeNH_2$ (180 μL). The mixture was stirred at room temperature for 11 days in a sealed tube. EtOAc was added and the organic phase was washed with water then dried over $MgSO_4$. After removal of the solvent, methanol (200 μL) was added to the residue then 1 N HCl (170 μL). Addition of CH_2Cl_2 yielded the precipitation of the hydrochloride **14** (56 mg, 0.102 mmol, 67% yield) as a yellow solid. The conformers ratio at room temperature in DMSO was 6.9/1. Mp >260 °C (decomposition). IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, $\nu_{OH,NH}$ 3100–3650 cm^{-1}. HRMS (ESI) (M^+) calcd for $C_{24}H_{26}N_4O_6$ 515.1931, found 515.1923. ^1H NMR (400 MHz, DMSO-d_6) major conformer δ 2.60 (3H, s, CH_3), 3.35–3.54 (3H, m), 3.67 (3H, s, OCH_3), 4.08 (1H, m), 4.20 (1H, m), 4.58 (1H, m), 5.45 (1H, d, $J=5.3$ Hz, OH), 5.60 (1H, d, $J=5.5$ Hz, OH), 6.58 (1H, d, $J=8.7$ Hz, $H_{1'}$), 7.41 (1H, t, $J=7.4$ Hz), 7.47 (1H, t, $J=7.5$ Hz), 7.60 (1H, t, $J=7.4$ Hz), 7.63 (1H, t, $J=7.5$ Hz), 7.97 (1H, d, $J=7.8$ Hz), 8.08 (1H, d, $J=8.3$ Hz), 8.74 (1H, br s), 8.89 (1H, br s), 9.16 (1H, d, $J=7.9$ Hz), 9.27 (1H, d, $J=7.9$ Hz), 11.15 (1H, s, NH), 12.32 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ major conformer 33.2 (NCH_3), 49.4 ($C_{6'}$), 60.0 (OCH_3), 70.8, 72.2, 76.0, 80.6, 86.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.2, 114.8, 120.3, 121.1, 124.3, 124.7, 126.5, 127.0 (C tert arom), 116.5, 117.7, 119.4, 120.5, 120.7, 123.0, 128.1, 130.2, 139.1, 141.0 (C quat arom), 170.9, 171.0 (C=O).

12-(6-(3-Azabicyclo[3.3.0]-octyl)-4-*O*-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione hydrochloride (15). To a solution of bicyclic amine (348 mg, 3.14 mmol) in THF (5 mL) was added **12** (94 mg, 0.154 mmol). The mixture was stirred at room temperature for 48 h. Water was added and the mixture was extracted with EtOAc. The organic phase was dried over $MgSO_4$ and the solvent was removed. To the residue dissolved in methanol (300 μL) was added 1 N HCl (170 μL) and the mixture was stirred, the hydrochloride which precipitated by addition of CH_2Cl_2 was filtered off and washed with CH_2Cl_2 to give **15** (74 mg, 0.117 mmol, 76% yield) as a yellow solid. The 3 conformers ratio observed at room temperature in DMSO was 75/17/8. Mp >240 °C. IR (KBr) ν_{CO} 1690, 1750 cm^{-1}, $\nu_{OH,NH}$ 3100–3700 cm^{-1}. HRMS (FAB+) $(M+H)^+$ calcd for $C_{34}H_{35}N_4O_6$ 595.2556, found 595.2553. ^1H NMR (400 MHz, DMSO-d_6) major conformer δ 1.30–1.85 (6H, m), 2.60–2.85 (4H, m), 3.30-3.75 (4H, m), 3.66 (3H, s, OCH_3), 4.02 (1H, m), 4.14 (2H, m), 4.91 (1H, m), 5.37 (1H, d, $J=5.0$ Hz, OH), 5.57 (1H, d, $J=5.4$ Hz, OH), 6.77 (1H, d, $J=8.6$ Hz, $H_{1'}$), 7.40 (1H, t, $J=7.5$ Hz), 7.47 (1H, t, $J=7.6$ Hz), 7.60 (1H, t, $J=7.6$ Hz), 7.62 (1H, t, $J=7.6$ Hz), 8.03 (1H, d, $J=8.1$ Hz), 8.09 (1H, d, $J=8.3$ Hz), 9.15 (1H, d, $J=8.0$ Hz), 9.27 (1H, d, $J=7.9$ Hz), 9.85 (1H, br s), 11.15 (1H, s, NH), 12.71 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) major conformer δ 23.8, 30.2, 30.3 (CH_2), 40.6, 40.7 (CH), 54.3, 58.6, 60.0 (2 $CH_2N+C_{6'}$), 59.9 (OCH_3), 70.8, 71.9, 75.9, 80.5, 86.0 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.5, 114.9, 120.3, 121.1, 124.2, 124.8, 126.5, 126.9 (C tert arom), 116.5, 117.6, 119.4, 120.5, 120.7, 123.2, 128.2, 130.3, 139.3, 141.2 (C quat arom), 171.0, 171.1 (C=O).

12-(6-Azido-6-deoxy-4-*O*-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]-pyrrolo[3,4-*c*]-carbazole-5,7-dione (18). A mixture of compound **11** (172 mg, 0.331 mmol) in DMF (9 mL) and NaN_3 (215 mg, 3.31 mmol) was maintained at 90 °C for 45 h. After addition of EtOAc, the mixture was washed with brine. The organic phase was dried over $MgSO_4$. The solvent was removed and purification of the residue by flash chromatography (eluent, EtOAc–cyclohexane, 50:50) afforded **18** (145 mg, 0.276 mmol, 83% yield) as an orange solid. The conformers ratio at room temperature in DMSO was 1.2/1. Mp >180 °C (decomposition). IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, $\nu_{N=N}$ 2100 cm^{-1}, $\nu_{NH,OH}$ 3200–3600 cm^{-1}. HRMS (FAB+) (M^+) calcd for $C_{27}H_{22}N_6O_6$ 526.1601 found 526.1617. ^1H NMR (400 MHz, DMSO-d_6) [a]major conformer, [b]minor conformer δ 3.45–4.32 ($6H^a+6H^b$, m), 3.66 ($3H^b$, s,

OCH_3), 3.73 ($3H^a$, s, OCH_3), 5.20 ($1H^a$, d, $J=5.4$ Hz, OH), 5.44 ($1H^b$, d, $J=4.9$ Hz, OH), 5.47 ($1H^a$, d, $J=6.4$ Hz, OH), 5.50 ($1H^b$, d, $J=5.9$ Hz, OH), 6.39 ($1H^a$, d, $J=8.9$ Hz, $H_{1'}$), 6.43 ($1H^b$, d, $J=8.9$ Hz, $H_{1'}$), 7.45 ($2H^a+2H^b$, m), 7.65 ($2H^a+2H^b$, m), 7.77 ($1H^a$, d, $J=8.4$ Hz), 7.84 ($1H^b$, d, $J=8.4$ Hz), 8.02 ($1H^a$, d, $J=8.4$ Hz), 8.07 ($1H^b$, d, $J=8.4$ Hz), 9.15 ($1H^a$, d, $J=7.9$ Hz), 9.18 ($1H^b$, d, $J=7.9$ Hz), 9.21 ($1H^a$, d, $J=7.4$ Hz), 9.28 ($1H^b$, d, $J=8.4$ Hz), 10.83 ($1H^a$, s, NH), 11.16 ($1H^b$, s, NH). 11.22 ($1H^a$, s, NH), 11.82 ($1H^b$, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 51.0, 51.5 ($C_{6'}$), 60.3 (OCH_3), 71.0, 73.2, 75.3, 75.8, 76.5, 76.6, 78.8, 79.8, 84.5, 86.7 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.4, 111.7, 111.8, 114.5, 120.9, 121.0, 124.6, 124.7, 124.8, 126.7, 127.1, 127.4, 127.7 (C tert arom), 116.7, 117.0, 117.9, 118.6, 119.7, 119.9, 121.2, 121.3, 121.7, 123.0, 128.1, 128.2, 129.3, 130.1, 139.1, 140.3, 140.8, 142.2 (C quat arom), 171.0, 171.1 (C=O).

12-(6-Amino-6-deoxy-4-*O*-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]-pyrrolo[3,4-*c*]-carbazole-5,7-dione, hydrochloride (7). To a suspension of compound **18** (105 mg, 0.199 mmol) in methanol (10 mL) was added 10% Pd/C (10 mg). The mixture was hydrogenated at 1 bar for 25 h. The mixture was filtered off over Celite and the residue washed with methanol. The solvent was removed. To a suspension of the residue in methanol (300 μL) was added 1 N HCl (230 μL). The mixture was stirred. Addition of CH_2Cl_2 yielded the precipitation of the hydrochloride **7** which was filtered off and washed with CH_2Cl_2. Compound **7** (88 mg, 0.164 mmol, 82% yield) was obtained as a yellow solid. The conformers ratio at room temperature in DMSO was 8/1. Mp >300 °C (decomposition). IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, $\nu_{OH,NH}$ 3100–3600 cm^{-1}. HRMS (FAB+) (M^+) calcd for $C_{27}H_{24}N_4O_6$ 500.1696 found 500.1707. ^1H NMR (400 MHz, DMSO-d_6) major conformer δ 3.19–3.54 (3H, m), 3.66 (3H, s, OCH_3), 4.09 (1H, pt, $J=8.9$ Hz), 4.20 (1H, pt, $J=8.9$ Hz), 4.46 (1H, pt, $J=8.9$ Hz), 5.44 (1H, br s, OH), 5.59 (1H, br s, OH), 6.54 (1H, d, $J=8.9$ Hz, $H_{1'}$), 7.40 (1H, t, $J=7.9$ Hz), 7.45 (1H, t, $J=7.9$ Hz), 7.60 (1H, t, $J=7.9$ Hz), 7.62 (1H, t, $J=7.9$ Hz), 7.97 (1H, d, $J=8.4$ Hz), 8.09 (1H, d, $J=8.4$ Hz), 8.22 (3H, br s), 9.16 (1H, d, $J=7.9$ Hz), 9.27 (1H, d, $J=7.9$ Hz), 11.15 (1H, s, NH), 12.35 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) major conformer δ $C_{6'}$ is under the signal of DMSO, 60.0 (OCH_3), 71.0, 73.4, 76.1, 80.4, 86.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.2, 114.9, 120.4, 121.1, 124.3, 124.7, 126.5, 127.1 (C tert arom), 116.5, 117.7, 119.5, 120.5, 120.8, 123.0, 128.2, 130.2, 139.1, 141.1 (C quat arom), 170.9, 171.0 (C=O).

12(3-Amino-3-deoxy-4-*O*-methyl-β-D-altropyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]-pyrrolo[3,4-*c*]-carbazole-5,7-dione (21). A mixture of azide **19** (89 mg, 0.15 mmol), methanol (20 mL), ammonium formate (267 mg) and 10% Pd/C (267 mg) was stirred at room temperature with light protection for a week. After filtration over Celite, the solid residue was washed with THF and the filtrate was evaporated. The residue was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, the organic phase was dried over $MgSO_4$ and evaporated. To form the corresponding hydrochloride, the amine was dissolved in CH_2Cl_2 and 1.1 N HCl (1.1 equiv) was added. The hydrochloride **21** was obtained as an orange solid (49 mg, 0.098 mmol, 65% yield).

Mp >280 °C. IR (KBr) ν_{CO} 1700, 1740 cm^{-1}, $\nu_{NH,OH}$ 3000–3680 cm^{-1}. HRMS (FAB+) ($M+H)^+$ calcd for $C_{27}H_{25}N_4O_6$, 501.1774 found 501.1805. ^1H NMR (400 MHz, DMSO-d_6) δ 3.57 (3H, s, OCH_3), 4.05 (4H, m), 4.40 (1H, d, $J=7.9$ Hz), 4.65 (1H, d, $J=9.3$ Hz), 5.81 (1H, s, OH), 6.27 (1H, s, OH), 7.35 (2H, m), 7.48 (1H, t, $J=6.9$ Hz), 7.57 (1H, t, $J=7.9$ Hz), 7.66 (1H, br s), 7.73 (1H, d, $J=7.9$ Hz), 8.12 (1H, br s), 8.66 (2H, s, NH_2), 9.12 (1H, d, $J=7.9$ Hz), 9.32 (1H, d, $J=7.9$ Hz), 11.10 (1H, s, NH), 11.85 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 50.4, 57.3, 67.6, 69.0, 75.1, 81.6 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 58.7 ($C_{6'}$), 111.0, 112.0, 119.7, 121.1, 124.3, 124.8, 126.6, 126.9 (C tert arom), 116.3, 117.3, 118.6, 120.8, 120.9, 121.7, 129.7, 130.4, 139.3, 139.9 (C quat arom), 171.1, 171.3 (C=O).

Biochemicals

Restriction endonucleases *Pvu*II and *Eco*RI, and avian myeloblastosis virus (AMV) reverse transcriptase were purchased from Boehringer (Mannheim, Germany) and used according to the supplier's recommended protocol in the activity buffer provided. Experiments were performed with either human topoisomerase I from TopoGen Inc. (Columbus, OH) or calf thymus topoisomerase I from Life Science technologies (Cergy-Pontoise, France). The double-stranded polymers poly(dA-dT)·poly(dA-dT) was from Pharmacia (Uppsala, Sweden). The nucleoside triphosphate labeled with [^{32}P] (α-dATP) was obtained from Amersham.

Melting temperature studies

Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. For each series of measurements, 12 samples were placed in a thermostatically controlled cell-holder, and the quartz cuvettes (10 mm pathlength) were heated by circulating water. The measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette was measured with a platinum probe; it was increased over the range 20–100 °C with a heating rate of 1 °C/min. The 'melting' temperature T_m was taken as the midpoint of the hyperchromic transition determined from first derivatives plots. The reproducibility of the T_m measurements is ±1 °C.

Purification and radiolabeling of the DNA substrates

The 117-mer fragment was rendered radioactive by 3′-[^{32}P]-end labeling of the *Eco*RI-*Pvu*II double digest of the plasmid pBS (Stratagene, La Jolla, CA, USA) using [α-^{32}P]-dATP (6000 Ci/mmol) and avian myeloblastosis virus reverse transcriptase. The labeled digestion products were separated on a 6% polyacrylamide gel under non-denaturing conditions in TBE buffer (89 mM Tris–borate pH 8.3, 1 mM EDTA). After autoradiography, the requisite band of DNA was excised, crushed and

soaked in elution buffer (500 mM ammonium acetate, 10 mM magnesium acetate) overnight at 37 °C. This suspension was filtered through a Millipore 0.22-μ filter and the DNA was precipitated with ethanol. Following washing with 70% ethanol and vacuum drying of the precipitate, the labeled DNA was resuspended in 10 mM Tris adjusted to pH 7.0 containing 10 mM NaCl.

DNA relaxation experiments

Supercoiled pKMp27 DNA (0.5 μg) was incubated with 4 units topoisomerase I or topoisomerase II (TopoGen Inc., Columbus, OH) at 37 °C for 1 h in relaxation buffer (50 mM Tris pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA) in the presence of varying concentrations of the drug under study. Reactions were terminated by adding SDS to 0.25% and proteinase K to 250 μg/mL. DNA samples were then added to the electrophoresis dye mixture (3 μL) and electrophoresed in a 1% agarose gel containing ethidium bromide (1 mg/mL), at room temperature for 3 h. Gels were washed and photographed under UV light.

Sequencing of topoisomerase I-mediated DNA cleavage sites

Each reaction mixture contained 2 μL of 3′-end [^{32}P] labeled DNA (~1μM), 5 μL of water, 2 μL of 10× topoisomerase I buffer, 10 μL of drug solution at the desired concentration, usually 10–50 μM. After at least 30 min incubation to ensure equilibration, the reaction was initiated by addition of 20 units calf thymus topoisomerase I (Life Science Technologies, Cergy-Pontoise, France). Samples were incubated for 40 min at 37 °C prior to adding SDS to 0.25% and proteinase K to 250 μg/mL to dissociate the drug–DNA–topoisomerase I cleavable complexes. The DNA was precipitated with ethanol and then resuspended in 5 μL of formamide–TBE loading buffer, denatured at 90 °C for 4 min then chilled in ice for 4 min prior to loading on to the sequencing gel. A Molecular Dynamics 445SI PhosphorImager was used to collect all data which were analyzed using the ImageQuant version 4.1 software. Each resolved band on the autoradiograph was assigned to a particular bond within the DNA fragment by comparison of its position relative to sequencing standards.

Antibiogram tests and MIC determination

Four strains were tested, two Gram-positive bacteria (*B. cereus* ATCC 14579, *S. chartreusis* NRRL 11407), a Gram-negative bacterium (*E. coli* ATCC 11303) and a yeast (*C. albicans* 444 from the Pasteur Institute, Paris, France). Antimicrobial activity was determined by the conventional paper disk (Durieux No. 268; 6 mm in diameter) diffusion method using the following nutrient media: Mueller-Hinton (Difco) for *B. cereus* and *E. coli*, Sabouraud agar (Difco) for *C. albicans* and Emerson agar (0.4% beef extract, 0.4% peptone, 1% dextrose, 0.25% NaCl, 2% agar, pH 7.0) for the *Streptomyces* strains. Paper disks impregnated with solutions of **1**–**21** in DMSO (300 μg of drug per disk) were placed on Petri dishes. Growth inhibition was examined after 24 h incubation at 27 °C. MICs were determined on *B. cereus* ATCC 14579 in Mueller–Hilton broth, pH 7.4 (Difco), after 24 h incubation at 27 °C. The compounds diluted in DMSO were added to 12 tubes; the concentration range was from 100 to 0.05 μg/mL.

Acknowledgements

This work was done under the support of research grants (to C.B.) from the Ligue Nationale Française Contre le Cancer (Equipe labellisée LA LIGUE). The authors are grateful to Nicole Grangemare and Aline Voldoire for assistance in the isolation of rebeccamycin.

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