Syntheses and Biological Activities (Topoisomerase Inhibition and Antitumor and Antimicrobial Properties) of Rebeccamycin Analogues Bearing Modified Sugar Moieties and Substituted on the Imide Nitrogen with a Methyl Group

Fabrice Anizon,[†] Laure Belin,[†] Pascale Moreau,[†] Martine Sancelme,[†] Aline Voldoire,[†] Michelle Prudhomme,*[,†] Monique Ollier,[‡] Danièle Sevère,[§] Jean-François Riou,[§] Christian Bailly,[▽] Doriano Fabbro,[||] and Thomas Meyer[||]

Synthèse, Electrosynthèse et Etude de Systèmes à Intérêt Biologique, Université Blaise Pascal, UMR 6504, 63177 Aubière, France, INSERM U71, Rue Montalembert, 63005 Clermont-Ferrand, France, Rhône-Poulenc Rorer, 13, Quai Jules Guesde, 93403 Vitry sur Seine, France, Centre Oscar Lambret et INSERM U124, Place de Verdun, 59045 Lille, France, and Département d'Oncologie, Novartis, K-125-409, CH-4002 Bâle, Switzerland

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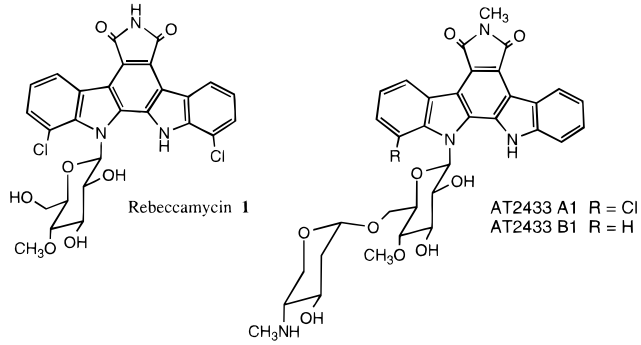
As a part of studies on structure−activity relationships, several potential topoisomerase I inhibitors were prepared. Different analogues of the antitumor antibiotic rebeccamycin substituted on the imide nitrogen with a methyl group were synthesized. These compounds bore either the sugar residue of rebeccamycin, with or without the chlorine atoms on the indole moieties, or modified sugar residues (galactopyranosyl, glucopyranosyl, or fucopyranosyl) linked to the aglycone *via* a *β*- or α-*N*-glycosidic bond. Their inhibitory properties toward protein kinase C, topoisomerase I, and topoisomerase II were examined, and their DNA-binding properties were investigated. Their *in vitro* antitumor activities against murine B16 melanoma and P388 leukemia cells were determined. Their antimicrobial activities were tested against Gram-positive bacteria *Bacillus cereus* and *Streptomyces chartreusis*, Gram-negative bacterium *Escherichia coli,* and yeast *Candida albicans*. These compounds are inactive toward topoisomerase II but inhibit topoisomerase I. A substitution with a methyl group on the imide nitrogen led to a loss of proteine kinase C inhibition in the maleimide indolocarbazole series but did not prevent topoisomerase I inhibition. Compounds possessing a *β*-*N*-glycosidic bond, which fully intercalated into DNA, were more efficient at inhibiting topoisomerase I than their analogues with an α-*N*-glycosidic bond; however, both were equally toxic toward P388 leukemia cells. Dechlorinated rebeccamycin possessing a methyl group on the imide nitrogen was about 10 times more efficient in terms of cytotoxicity and inhibition of topoisomerase I than the natural metabolite.

Introduction

Topoisomerase I participates in the control of the topological state of DNA, and as such this enzyme is essential for DNA transcription and replication as well as other vital processes including chromosome condensation/opening and mitosis.[1−3] Topoisomerase I represents a privileged target for different classes of anticancer drugs, in particular for camptothecin and its derivatives[4,5] but also for various benzophenanthridine alkaloids (fagaronine, berberine, coralyne)[6−8] and indolocarbazole derivatives.

Indolocarbazoles related to the antibiotics K-252a and BE-13793C interfere with topoisomerase I and display a useful spectrum of antitumor activity.[9] In recent years, it has been shown that the synthetic indolocarbazole derivatives NB-506 and ED-110 are potent topoisomerase I inhibitors endowed with remarkable antitumor effects in transplanted tumors in mice.[10−12] The antibiotic rebeccamycin (Chart 1) which belongs to another series of glycosyl-substituted indolocarbazoles was found to display potential antitumor activity.[13,14] In order to develop indolocarbazoles endowed with better antitumor activities and to identify the structural features of the drugs responsible for topoisomerase I inhibition, we have investigated the structure−activity relationships of rebeccamycin analogues. In a recent paper, we have shown that the rebeccamycin analogues are much more active than the corresponding aglycones lacking the sugar moiety.[15] The sugar residue on the indolocarbazole ring system is a key element for both DNA binding and topoisomerase I inhibition.[16] In the present study, we have extended our investigation to a series of rebeccamycin analogues for which a methyl group is introduced on the imide nitrogen on the indolocarbazole chromophore. This methyl substituent was added to reduce the activity of the drugs on protein kinase C (PKC). PKC is a family of different subspecies involved in signal transduction pathways leading to a variety of cellular responses such as gene expression and proliferation as well as muscle contraction, secretions, and exocytosis.[17] As shown in our laboratory and by

Chart 1



[†] Université Blaise Pascal.
[‡] INSERM U71.
[§] Rhône-Poulenc Rorer.
[▽] Centre Oscar Lambret et INSERM U124.
[||] Novartis.
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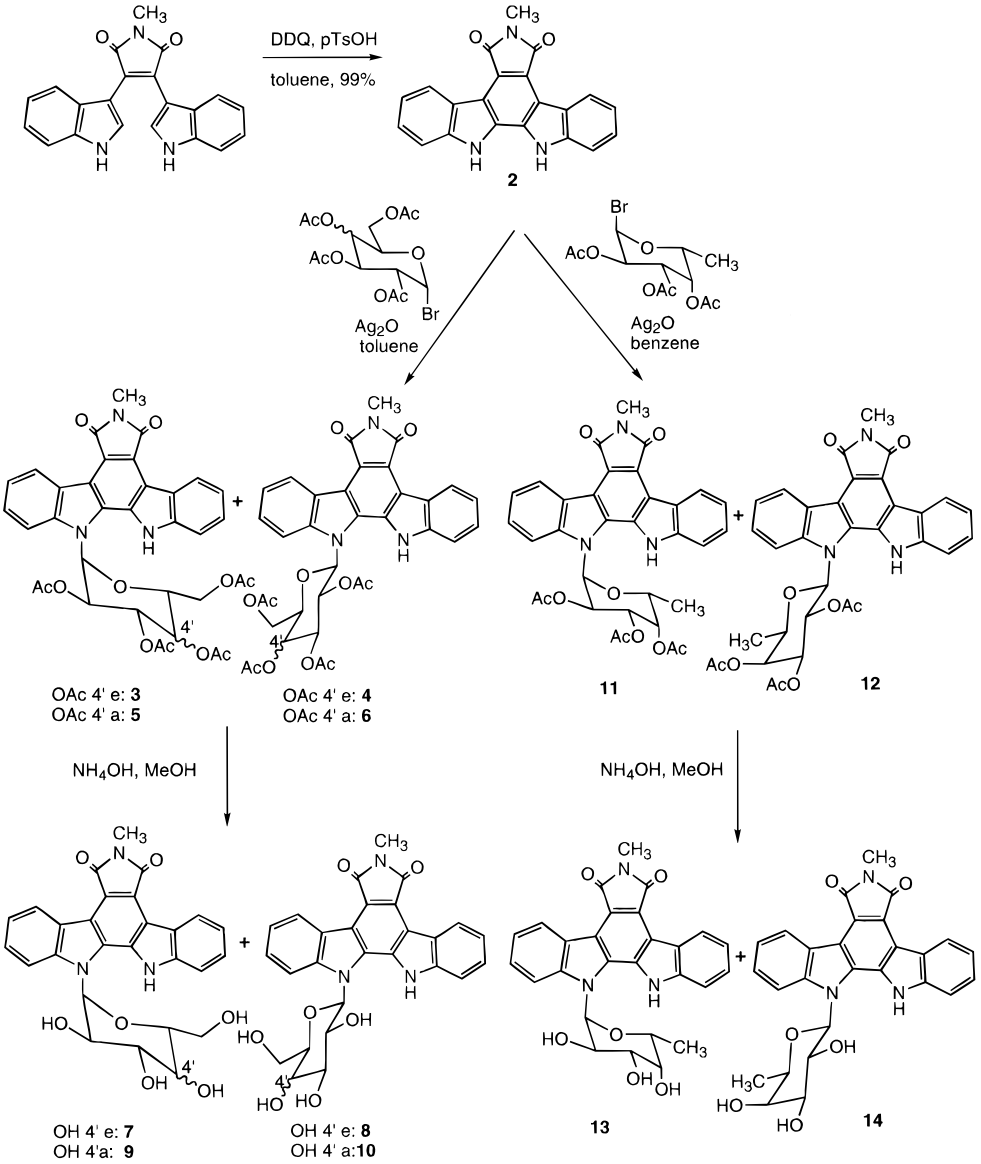
Scheme 1



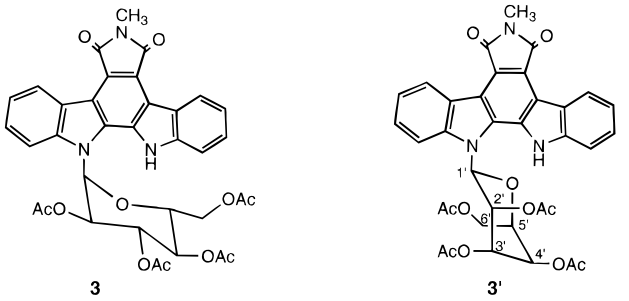
another group,[18] in the aglycone series, a substitution with a methyl group on the imide nitrogen results in a loss of PKC inhibition (IC_{50} > 100 μM) whereas a substitution with a hydroxy or an amino group led to marked PKC inhibition.[15] Moreover, there is good reason to believe that designing methyl-containing indolocarbazoles will provide a profitable route for the development of antitumor drugs since two antibiotics of the rebeccamycin chemotype, AT2433-A1 and AT2433-B1 (Chart 1) having a methyl on the imide nitrogen, exhibit significant antitumor properties, most likely *via* an inhibition of topoisomerase I.[19]

To evaluate the role of the sugar residue on DNA binding and inhibition of topoisomerase I as well as on the antibacterial and antitumor activities, we have synthesized a series of *N*-methyl(indolocarbazolyl)maleimides with one of the indolic nitrogens attached to different sugars, either a glucose, galactose, or fucose, by the intermediate of an α- or a *β*-*N*-glycosidic bond. We have also prepared *N*-methylated derivatives of rebeccamycin and dechlorinated rebeccamycin with the aim to assess the influence of the methyl substituent on the imide nitrogen found in the bacterial metabolites AT2433-A1 and AT2433-B1.

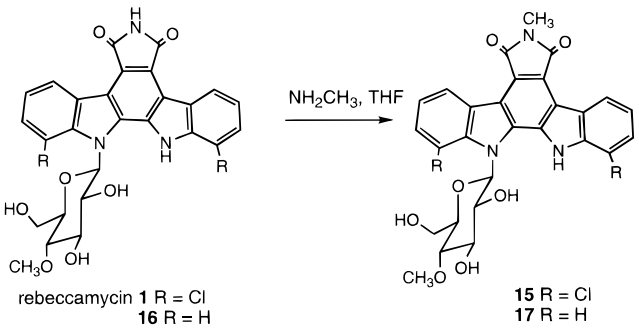
Chemistry

N-Methylmaleimide indolocarbazole (**2**)[20] was prepared by oxidation of *N*-methylmaleimide bis(indole)[21] using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in toluene in the presence of *p*-toluenesulfonic acid. Coupling of the sugar moiety with aglycone **2** (Scheme 1) was realized in refluxing toluene or benzene with silver oxide according to the method described by Tanaka *et al.*[22] for the synthesis of the antitumor drug ED-110 and by Kaneko *et al.*[23] for the first total synthesis of rebeccamycin. Two coupling products resulting from α- and *β*-*N*-glycosidic bonds were obtained from both 2,3,4,6-tetra-*O*-acetyl-α-D-bromoglucopyranosyl and galactopyranosyl bromides and from 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide as observed by Danishefsky[24] in a total synthesis of rebeccamycin using a 1,2-anhydro sugar. The yields for the α- and *β*-compounds were respectively 85% and 8% for **3** and **4**, 28% and 7% for **5** and **6**, and 39% and 52% for **11** and **12**. For the introduction of a fucose moiety, 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide was prepared from commercial L-fucose according to the method described by Flowers *et al.*[25]

Chart 2



Scheme 2



The α- and *β*-structures were identified from the ^{1}H NMR coupling constants between $H_{1'}$ and $H_{2'}$ of the sugar moiety. In the compounds with an α-*N*-glycosidic bond, this coupling a−e was found to be about 5 Hz, while in the compounds with a *β*-*N*-glycosidic bond, the coupling a−a was found to be about 9 Hz. Moreover, ^{1}H−^{13}C correlations allowed unambiguously the assignment of $C_{1'}$ which was shifted at >90 ppm values for compounds having an α-*N*-glycosidic bond, in agreement with that observed for α-glucopyranose ($C_{1'}$ shifted at 92.8 ppm).[26]

Molecular modeling experiments for conformational searches using the SYBYL software package (Tripos Associates Inc.) were carried out on compound **3** (simulated annealing process, using Tripos force field with a dielectric constant of water $\epsilon = 78$) and yielded conformation **3′** shown in Chart 2 with a global energy of 57.46 kcal. Conformation **3′** is derivated from **3** by chair−chair inversion and is stabilized by one hydrogen bond, between the hydrogen of the indolic NH and the oxygen of the carbonyl on $C_{4'}$, and electrostatic interactions between the hydrogen of the indolic NH and the oxygens of the sugar ring and of the carbonyl on $C_{2'}$.

N-Methylrebeccamycin (**15**) and its dechlorinated analogue **17** (Scheme 2) were prepared respectively by reaction of a THF solution of *N*-methylamine on either the bacterial metabolite rebeccamycin (**1**) or its dechlorinated analogue **16** obtained from rebeccamycin by hydrogenolysis using Raney nickel in aqueous sodium hydroxide.[27]

Results and Discussion

DNA Binding. We investigated the DNA-binding properties of pairs of compounds with different sugar moieties: compounds **7** and **8** with an α- or a *β*-glucose, compounds **9** and **10** with an α- or a *β*-galactose, and compounds **13** and **14** with an α- or a *β*-fucose residue, respectively. In addition, we studied compounds **15** and **17** both bearing a *β*-glucose residue in the presence or absence of chlorine atoms on the indolocarbazole chromophore. The binding process was examined by gel electrophoresis-based techniques using plasmid DNA or P^{32}-labeled restriction fragments. The results can be summarized as follows.

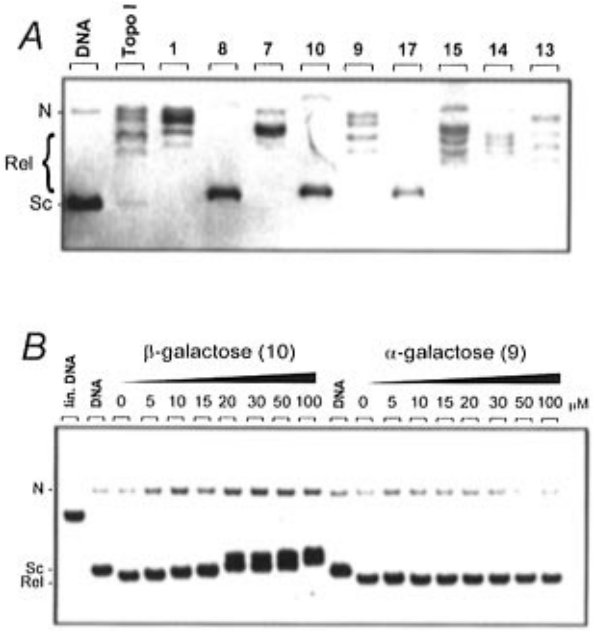


Figure 1. (A) Inhibition of DNA relaxation by rebeccamycin analogues. Native supercoiled pAT DNA (0.5 *μ*g) (lane DNA) was incubated for 30 min at 37 °C with 6 units of topoisomerase I in the absence (lane Topo I) or presence of drug at 30 *μ*M. Reactions were stopped with sodium dodecyl sulfate and treatment with proteinase K. The DNA was analyzed by native agarose gel electrophoresis. N, nicked; Rel, relaxed; Sc, supercoiled. The gel was stained with ethidium bromide and photographed under UV light. (B) DNA samples treated with increasing concentrations of compounds **9** and **10** run on an agarose gel containing ethidium bromide.

DNA Unwinding. Closed circular DNA was treated with topoisomerase I in the absence and presence of the drug at 30 *μ*M. This topoisomerization assay provides a direct means to determine whether the drugs affect the unwinding of closed circular duplex DNA.

As shown in Figure 1A, in the absence of drug, supercoiled DNA is relaxed by topoisomerase I (lane Topo I). In the presence of compounds **8** and **10** containing a sugar residue in the *β*-conformation, the relaxation is totally inhibited, whereas the reaction is much less affected with the corresponding α-anomers **7** and **9**. The effect is much less marked with the fucose derivatives, but it can be seen that the *β*-anomer **14** has a slight effect on the relaxation whereas the α-anomer **13** has no effect. The presence of chlorine atoms is detrimental to the unwinding activity. Indeed, rebeccamycin and compound **15** which both have chloro groups on the indolocarbazole have little effect on the topoisomerase I-mediated DNA relaxation. In constrast, the analogue **17** lacking the bulky chlorine atoms completely inhibits the relaxation of supercoiled DNA. The *β*-sugar-containing derivatives markedly affect the unwinding of circular DNA so as to shift its topoisomer distribution.

Figure 1B compares the results obtained with increasing concentrations of the galactose derivatives **9** and **10**. In this case, the DNA samples were run on an agarose gel containing 0.5 *μ*g/mL ethidium bromide. In these conditions, the relaxed DNA migrates slightly

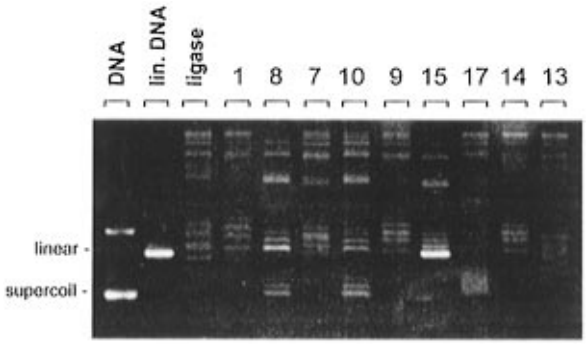


Figure 2. Drug effects on the ligation of DNA with T4 ligase. The pAT plasmid (lane DNA) was linearized with *Eco*RI (lane lin. DNA) and treated with 10 units of ligase in the absence (lane ligase) and presence of the drug at 30 μM. Other details are as for Figure 1.

faster than the supercoiled plasmid (compare lanes DNA and Topo I). The difference between the two drugs is most obvious. Compound **9** with an α-galactose residue has no effect on the activity of topoisomerase I, the mobility of plasmid DNA being similar in the absence or presence of the drug, even at a concentration as high as 100 μM. In contrast, compound **10** with a β-galactose residue induces a strong shift of the DNA band in the gel reflecting a marked alteration in DNA conformation. Similar results were obtained when comparing compounds **7** and **8** or **13** and **14** (not shown). From these data, we conclude that only the drugs possessing a sugar moiety in the β-conformation behave as typical intercalating agents.

Effect on DNA Structure and Flexibility. We ressort to the linear DNA ligation assay using T4 DNA ligase. This simple test has previously been used to characterize the effect of intercalating agents including the antitumor drugs adriamycin and amsacrine as well as indolocarbazole derivatives which are potent topoisomerase I inhibitors.[9] The assay is based on the circularization of the linear pAT DNA (cut with *Eco*RI) in the presence of DNA ligase and consists in determining to which extent the various indolocarbazoles interfere with the rate of formation of circular DNA molecules and multimers.

As shown in Figure 2, when the linear DNA fragment was incubated with the ligase for 30 min at room temperature, a DNA species that comigrates with the supercoiled DNA (native plasmid) is obtained with the β-anomers **8** and **10** but not with the corresponding α-anomers **7** and **9**. The β-fucose-containing derivative **14** is considerably less effective in promoting DNA religation than its glucose and galactose counterparts. This is consistent with the unwinding data presented above indicating that the fucose derivatives do not intercalate efficiently into DNA. This result reflects directly the influence of the hydroxyl group at position 5 on the sugar moiety which must play a decisive role in the interaction with DNA. The unwinding and ligation data concur that the effect of the 4-*O*-methyl-β-glucose derivative **17** is significantly less pronounced than that of the β-glucose derivative **8** indicating that intercalation of the methoxy derivative may not be as complete as it is with the hydroxy derivative. In conclusion, in terms of DNA intercalation, the test molecules rank in the order **10** > **8** > **17** > **14**. So far as the data go, the DNA-binding properties of this series

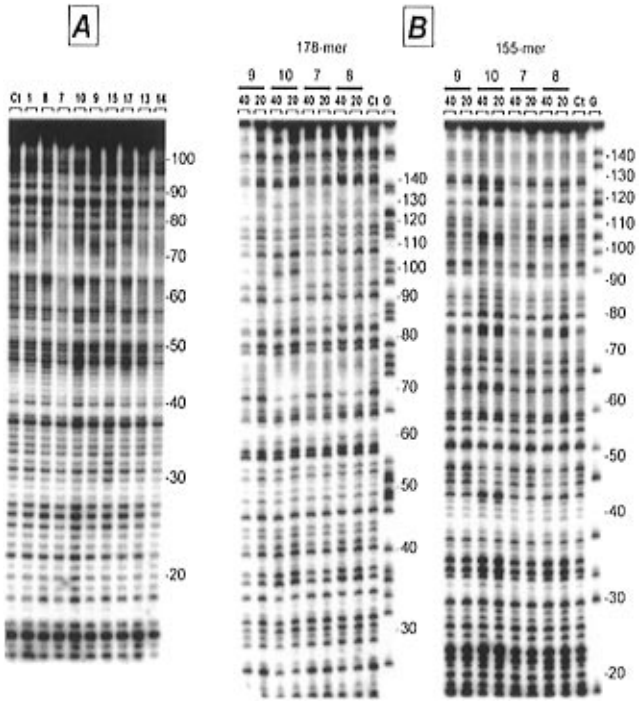


Figure 3. Sequence selective binding. Panels show DNase I footprinting with the 117-mer *Pvu*II-*Eco*RI restriction fragment of the plasmid pBS (A) and with the 155-mer *Eco*RI-*Hin*dIII fragment and 178-mer *Eco*RI-*Pvu*II fragment from plasmid pLAZ3 (B) in the presence of rebeccamycin (**1**) and/or its analogues **7**−**10**, **13**−**15**, and **17** at 20 or 40 μg/mL. In each case, the DNA was 5′-end-labeled at the *Eco*RI site with [γ-^{32}P]-ATP in the presence of T4 polynucleotide kinase. The products of nuclease digestion were resolved on an 8% polyacrylamide gel containing 7 M urea. Guanine specific sequence markers obtained by treatment of the DNA with dimethyl sulfate followed by piperidine were run in the lane marked G. Control tracks (Ct) contained no drug. Numbers on the sides of the gels refer to the standard numbering scheme for the nucleotide sequence of the DNA fragments.

of rebeccamycin analogues can be correlated with their potency against topoisomerase I (see below).

Sequence Selective Binding. Footprinting experiments were performed to investigate the nucleotide sequence selectivity of the drugs. Figure 3A shows an autoradiogram resulting from the DNase I cleavage of a 3′-end-labeled 117 base pair *Eco*RI-*Pvu*II restriction fragment from plasmid pBS in the presence and absence of drugs. Compounds **8**, **10**, and **17** affect the DNase I cleavage profile, whereas the other compounds have little, if any, effect. Densitometric analysis of this gel (not shown) showed that the sequences slightly protected by compound **10** from cleavage by DNase I mostly correspond to G·C-rich sequences (*e.g.*, 5′-CGCCAGG between positions 67 and 73). In the mean time, the drug increases the susceptibility to DNase I cleavage at A·T-rich sequences (*e.g.*, around nucleotide position 65, 5′-TTTT). Therefore, the binding of this drug to GC sequences is slightly favored over binding to AT or mixed sequences. We have recently reported similar results using the same 117-mer fragment with another series of rebeccamycin analogues[16] as well as with other intercalating drugs.[28]

Additional footprinting experiments were performed with two other DNA fragments to provide an assessment of the sequence selectivity of the tested compounds with respect to a wide variety of potential binding sites. Typical autoradiograms of footprinting gels are shown

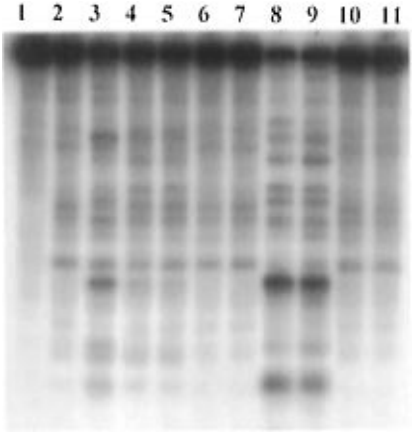


Figure 4. Topoisomerase I-mediated cleavage of DNA in the presence of rebeccamycin analogues **7** and **8**. Purified calf thymus topoisomerase I was incubated with the *Eco*RI-*Hin*dIII restriction fragment from pBR322 (^{32}P-labeled at the *Eco*RI site) in the presence and absence of the rebeccamycin analogues. Reactions were carried out for 10 min at 37 °C and then stopped with SDS−proteinase K treatment. Single-strand DNA fragments were analyzed on a 1% alkaline agarose gel in TBE buffer: Lane 1, control DNA; lane 2, DNA treated with topoisomerase I; lane 3, same as lane 2 with camptothecin (0.03 μg/mL); lanes 4−7, same as lane 2 with 10, 1, 0.1, and 0.01 mg/mL compound **7**; lanes 8−11, same as lane 2 with 10, 1, 0.1, and 0.01 mg/mL compound **8**.

in Figure 3B. Here again, it can be seen that compound **10** and, to a lesser extent, compound **8** affect the cutting of the DNA by the nuclease, whereas no footprints were detected with the α-anomers **9** and **7**. Addition of compound **10** led to a pronounced footprint around nucleotide position 70 on the 178-mer and position 110 on the 155-mer which reflects cleavage inhibition due to ligand bound to the sequences 5′-AGTGAGTCG and 5′-CCTCTAG, respectively.

Topoisomerase Inhibition. To test the topoisomerase inhibitory properties of indolocarbazole derivatives, we studied the effect of the drugs on both purified calf thymus topoisomerases I and II using the ^{32}P-labeled *Eco*RI-*Hin*dIII restriction fragment of pBR322 as a substrate. The DNA cleavage products were analyzed by alkaline (for topoisomerase I) or neutral (for topoisomerase II) agarose gel electrophoresis. In agreement with previous studies with related compounds,[15] none of the drugs has an effect on topoisomerase II. In contrast, they prove to inhibit topoisomerase I. The level of inhibition varies considerably from one congener to another depending on the nature of the sugar residue and the conformation of the glycosidic linkage between the sugar and the indolocarbazole chromophore. An autoradiograph of a typical gel obtained after treatment of the 4330 base pair DNA substrate with topoisomerase I in the presence and absence of the test drugs at concentrations ranging from 0.01 to 10 μM is shown in Figure 4. Purified topoisomerase I produces a characteristic cleavage pattern in the absence of drug. Similar electrophoretic profiles were observed in the presence of the acetylated compounds **3**−**7** at 10 μM (not shown), indicating that they exert no effect on topoisomerase I. On the contrary, almost all hydroxylated ligands stimulate topoisomerase I-mediated DNA cleavage in a dose-dependent manner. For each compound, we determined the minimum drug concentration at which topoisomerase I-mediated DNA cleavage was detected (MIC in Table 1).

Table 1. Inhibitory Activities of Compounds **1**−**17** toward PKC, Topoisomerase I, and Topoisomerase II, Antiproliferative Activities *in Vitro* against Murine B16 Melanoma and P388 Leukemia Cells, and Antimicrobial Activities against *B. cereus*

	IC_{50} (μM)			MIC (μM)		
compd	PKC	B16	P388	*B. cereus*	topoisomerase I	topoisomerase II
1	>100	0.48	0.5	10.9	1.75	>17.5
3	>100	2.5	>15	>74	>15	>15
4	nd	78	>15	>74	>15	>15
5	nd	nd	>15	>74	>15	>15
6	>100	75	>15	>74	>15	>15
7	59	0.52	6.0	6.2	>20	>20
8	>100	3.3	6.0	12.4	2.0	>20
9	62	nd	6.0	12.4	6.0	>20
10	99	5.4	6.0	6.2	0.6	>20
13	82	4.75	6.2	103	>20	>20
14	>100	3.75	6.2	3.1	2.0	>20
15	>100	0.61	0.6	>85	>17	nd
16	>100	17.5	6.8	>97	1.95	>19
17	>100	1.06	0.7	1.55	0.6	nd

In this series of rebeccamycin analogues, the methyl substitution on the imide nitrogen (compound **17**) improves the topoisomerase I inhibition (compare with compound **16**). The *N*-glycosidic bond and the sugar moiety also influence significantly the reactivity toward topoisomerase I. The data in Table 1 show that compounds **8**, **10**, and **14** bearing a β-*N*-glycosidic group are more potent inhibitors of topoisomerase I than their α-*N*-glycosidic counterparts **7**, **9**, and **13**, respectively. In addition, galactosyl **10** is more active than fucosyl **14**, glucosyl **8**, or methoxyglucosyl **16**, these last derivatives being of equal potency with MIC = 2 μM. Finally, although dechlorination of rebeccamycin seems not to affect topoisomerase I inhibition (MIC of about 2 μM for both compounds **1** and **16**), the presence of chlorine atoms in compound **15** abolishes the effect on topoisomerase I, as previously reported for other rebeccamycin analogues.[15] Our data clearly indicate that the β-*N*-glycosidic linkage represents a key element for topoisomerase I inhibition as well as for DNA intercalation as mentioned above.

Protein Kinase C Inhibition. The rebeccamycin analogues were tested for inhibition of PKC activity using protamine sulfate as a substrate. IC_{50} values are reported in Table 1. As expected for *N*-methyl-substituted derivatives, only a few compounds affect PKC activity at concentrations <100 μM (compounds **7** and **13** bearing an α-*N*-glycosidic bond are very weakly active toward PKC). Therefore, the biological activity of the drugs (see below) cannot be attributed to an interference with the functional activity of PKC as is the case with other indolocarbazoles such as the antibiotics staurosporine and K-252a.

Antimicrobial and Cytotoxic Activities. The antimicrobial activities against two Gram-positive bacteria (*Bacillus cereus* and *Streptomyces chartreusis*), a Gram-negative bacterium (*Escherichia coli*), and a yeast (*Candida albicans*) were tested for each compound. All compounds **1**−**17** were found to be inactive against *E. coli* and *C. albicans*, but some compounds are active against the Gram-positive bacterial strains. Interestingly, we found that the β-*N*-glycosidic derivatives **8**, **10** and **14** exhibit a strong antimicrobial effect against *S. chartreusis* whereas the α-*N*-glycosidic derivatives **7** and **13** are totally inactive against this microorganism. The acetylated compounds **3**−**6** are also inactive. Similar results were obtained with *B. cereus*. The MIC

values in Table 1 indicate that the acetylated derivatives **3**−**6** have no effect on the growth of *B. cereus* at a concentration <74 μM and that the α-fucose derivative **13** is 33-fold less active than the β-fucose derivative **14**, but opposite results were obtained with the glucose derivatives (**7** is slightly more active than **8**).

The antiproliferative activities were tested *in vitro* using two murine cell lines, B16 melanoma cells and P388 leukemia cells. IC_{50} values are reported in Table 1. Here again, the acetylated compounds **3**−**6** are inactive, whereas removal of the acetyl protecting groups yields active compounds. Compounds **3**−**6** cannot be used as prodrugs since the acetyl groups are apparently not cleaved inside the cells. As previously observed for other series of chlorinated and dechlorinated rebeccamycin analogues,[15] we found that compounds **15** and **17** are almost equally toxic against P388 and B16 cells whereas the dechlorinated derivative **17** inhibits topoisomerase I much more efficiently than the corresponding dechlorinated derivative **15**. The same conclusion holds true when comparing the α- and β-anomers. As mentioned above, compounds **8**, **10**, and **14** possessing a β-*N*-glycosidic linkage are much more efficient in terms of DNA binding and topoisomerase I inhibition than compounds **7**, **9**, and **13** containing an α-*N*-glycosidic bond, but there is little or no difference in terms of toxicities. Except for compounds **7** and **16**, there is a good correlation between the effect on topoisomerase I and the activity against *B. cereus*.

The capacity of the drugs to inhibit topoisomerase I *in vitro* can logically be correlated to their DNA-binding properties. From the results presented above, it is obvious that the indolocarbazole derivatives possessing a β-*N*-glycosidic bond which intercalate into DNA are potent topoisomerase I inhibitors. In contrast, the analogues containing an α-*N*-glycosidic bond do not behave as typical intercalating agents (no effect on topoisomerase I-mediated unwinding of DNA) and consequently have much less effect on topoisomerase I. The correlation with the biological data is not so direct. A correlation can be found between the drug effects on DNA and topoisomerase I and their antimicrobial activities. Indeed, the β-*N*-glycosides inhibit the growth of the two Gram-positive bacteria, while the α-*N*-glycosides are generally less active. The correlation is obvious with the fucose derivatives: compound **14** with a β-fucose residue is considerably more efficient at inhibiting topoisomerase I and *B. cereus* than its analogue **13** with an α-fucose residue. In contrast, so far as the present data go, DNA binding and topoisomerase I inhibition by this series of rebeccamycin analogues cannot be simply correlated with their potency against tumor cells. Unlike what is observed with bacteria, with eukaryotic cells there is manifestly a lack of correlation between the biochemical data and the cytotoxicities in this series of rebeccamycin analogues. With P388 leukemia cells, we found that the compounds containing an α- or a β-carbohydrate residue are equally toxic whereas they differ significantly in terms of topoisomerase I inhibition. This lack of correlation prompts the question of the exact mechanism of action of these indolocarbazole derivatives. At first sight, it could be envisaged that in cells these drugs exert little effect on topoisomerase I or that the effect has no consequence on the cell viability. However, previous studies[9−12] have clearly indicated that topoisomerase I inhibition contributes significantly to the high antitumor activity of indolocarbazole derivatives ED-110 and NB-506 referred to in the Introduction. It is worth mentioning here that we recently compared the toxicity of a rebeccamycin analogue (with a OH group on the imide nitrogen)[15] toward P388 cells sensitive or resistant to the topoisomerase I inhibitor camptothecin. Preliminary data indicate that this drug is up to 30 times more toxic to P388 cells compared to P388CPT5 cells which contain a biochemically resistant form of topoisomerase I. The fact that the P388CTP5 cell line exhibits a cross-resistance to this rebeccamycin derivative suggests that topoisomerase I is, at least partially, involved in the mechanism of cytotoxicity. Careful examination of the toxicities toward P388CPT5 cells due to the rebeccamycin analogues described here will provide interesting information on the contribution of topoisomerase I inhibition in their cytotoxicity.

On the basis of the data reported here, we must conclude that if DNA binding and topoisomerase I play a part in the biological activity of these indolocarbazole derivatives, then their pharmacological effects must demand more than specific complex formation between the drugs and topoisomerase I−DNA complexes. Their mechanism of action may involve interaction with a protein complexed to a GC-rich sequence in addition to interference with topoisomerase I. Once bound to DNA, the drug may establish contact with a second macromolecular species such as a protein (transcription factors, polymerases, etc.) to initiate the biological response. With other classes of intercalating drugs, it has been shown that in addition to interfering with topoisomerase II−DNA complexes, they can compete efficiently with various classes of DNA-binding proteins. Another plausible scenario can be envisaged, namely, that the drugs inhibit a protein kinase other than PKC. Recent work has identified topoisomerase I as a potential SR protein kinase able to phosphorylate specific serine residues in the SR domain of splicing factors.[29] Unexpectedly, topoisomerase I was found to bind ATP with a K_d similar to that described for other kinases. An ATP binding site, completely different from that of other protein kinases, was located close to the active site of the enzyme involved in the topoisomerization. These findings may explain the dual activity of indolocarbazole derivatives against topoisomerase I and certain protein kinases, and it could be hypothesized that the interaction of rebeccamycin derivatives with the ATP-binding site of topoisomerase I could mask the active site for topoisomerization leading to cleavable complex stabilization. The inhibition of the SR kinase activity of topoisomerase I may be sufficient to maintain cytotoxic properties for some rebeccamycin derivatives. The effects of the drugs on the kinase activity of topoisomerase I are currently being investigated, and the results will be reported in due course.

Experimental Section

Chemistry. IR spectra were recorded on a Perkin-Elmer 881 spectrometer (ν in cm^{-1}). NMR spectra were performed on a Bruker AC 400 spectrometer (^1H, 400 MHz; ^{13}C, 100 MHz) (chemical shifts δ in ppm, abbreviations: singlet (s), doublet (d), doubled doublet (dd), doubled doubled doublet (ddd), triplet (t), pseudotriplet (pt), doubled triplet (dt), multiplet (m), broad (br), tertiary carbons (C tert), quaternary carbons (C quat)).

Mass spectra (FAB+) were determined at CESAMO (Talence, France) on a high-resolution Fisons Autospec-Q spectrometer. Chromatographic purifications were performed by flash silica gel Geduran SI 60 (Merck) 0.040−0.063 mm or Kieselgel 60 (Merck) 0.063−0.200 mm column chromatography. For purity tests (compounds **3**−**17** except **11** and **12**), TLC was performed on fluorescent silica gel plates (60 F_{254} from Merck). Rebeccamycin was from our laboratory stock sample.

6-Methyl-12-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (3) and 6-Methyl-12-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (4). A mixture of **2** (228 mg, 0.671 mmol) in toluene (250 mL), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.379 g, 3.35 mmol), and Ag_2O (1.554 g, 6.71 mmol) was refluxed for 2.15 h and then filtered off. The filtrate was evaporated and purified by flash chromatography (eluent cyclohexane−EtOAc, 70:30) to give **3** and **4**, respectively, in 85% and 9% yields as yellow powders.

3: mp 102−104 °C; IR (KBr) ν_{NH} 3400 cm^{-1}, $\nu_{C=O}$ 1700, 1750 cm^{-1}; HRMS (FAB+) $(M + H)^+$ calcd for $C_{35}H_{32}N_3O_{11}$ 670.2037, found 670.2034; ^{1}H NMR (400 MHz, DMSO-d_6) 1.9−2.3 (12H, several s), 2.97 (3H, s, NCH_3), 4.36 (2H, br s), 4.44−4.60 (2H, m), 5.06 (1H br signal), 5.45 (1H, br signal), 6.03 (1H, d, $H_{1'}$, J = 5.3 Hz), 7.33 (1H, t, J = 7.5 Hz), 7.38 (1H, t, J = 7.6 Hz), 7.58 (2H, t, J = 7.6 Hz), 7.92 (1H, d, J = 8.2 Hz), 8.19 (1H, d, J = 7.6 Hz), 9.08 (1H, d, J = 8.0 Hz), 9.24 (1H, d, J = 8.0 Hz), 11.10 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 20.2, 20.3, 20.4, 20.5, 20.6, 20.7 (CH_3 of acetyl groups), 23.2 (NCH_3), 60.4, 61.4, 62.2 ($C_{6'}$), 65.2, 66.9, 67.6, 69.2, 70.0, 71.3, 71.7, 73.5 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 90.8, 97.3 ($C_{1'}$), 112.3 (2C), 120.2, 121.1, 124.1, 124.9, 127.0, 127.6 (C tert arom), 117.5, 117.8, 118.5, 120.7 (2C), 122.3, 128.5 (2C), 140.1 (2C) (C quat arom), 168.7, 168.9, 169.2 (2C), 170.2 (2C) (C=O).

4: mp 234 °C; IR (KBr) ν_{NH} 3400 cm^{-1}, $\nu_{C=O}$ 1750, 1700 cm^{-1}; HRMS (FAB+) (M^+) calcd for $C_{35}H_{31}N_3O_{11}$ 669.1958, found 669.1979; ^{1}H NMR (400 MHz, acetone-d_6) 1.83 (3H, s), 2.00 (3H, s), 2.05 (3H, s), 2.22 (3H, s), 3.08 (3H, s, NCH_3), 4.45 (2H, m), 4.83 (2H, m), 5.47 (1H, t, J = 8.0 Hz), 5.75 (1H, t, J = 8.0 Hz), 6.79 (1H, d, $H_{1'}$, J = 9.3 Hz), 7.38 (2H, t, J = 7.0 Hz), 7.60 (2H, t, J = 7.5 Hz), 7.80 (1H, d, J = 8.1 Hz), 7.92 (1H, d, J = 8.4 Hz), 9.17 (1H, d, J = 8.6 Hz), 9.21 (1H, d, J = 9.0 Hz), 10.17 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, acetone-d_6) 18.3, 19.7, 19.8, 20.5 (CH_3 of acetyl groups), 23.0 (NCH_3), 61.5 ($C_{6'}$), 67.7, 70.8, 72.8, 76.1, 83.5 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 110.7, 112.1, 121.3, 121.6, 125.2, 125.3, 127.2, 127.3 (C tert arom), 118.3, 119.3, 119.4, 120.9, 122.1, 122.3, 128.5, 129.8, 140.8, 141.8 (C quat arom), 167.6, 169.2, 169.4 (2C), 169.5, 170.2 (C=O).

6-Methyl-12-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (5) and 6-Methyl-12-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (6). Compounds **5** and **6** were obtained from 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl bromide as yellow powders, respectively, in 28% and 7% yields using the same procedure as described above.

5: mp 93 °C; IR (KBr) ν_{NH} 3400 cm^{-1}, $\nu_{C=O}$ 1700, 1750 cm^{-1}; HRMS (FAB+) $(M + H)^+$calcd for $C_{35}H_{32}N_3O_{11}$ 670.2037, found 670.2066; ^{1}H NMR (400 MHz, DMSO-d_6) 1.95−2.25 (12H, several s), 3.05 (3H, s, NCH_3), 4.07 (1H, m), 4.23 (1H, d, J = 6.4 Hz), 4.44−4.80 (2H, m), 5.10−5.60 (2H, m), 5.96 (1H, d, $H_{1'}$, J = 5.0 Hz), 7.37 (1H, t, J = 7.4 Hz), 7.45 (1H, t, J = 7.8 Hz), 7.61 (1H, t, J = 7.3 Hz), 7.63 (1H, t, J = 7.3 Hz), 7.92 (1H, d, J = 8.2 Hz), 8.20 (1H, d, J = 8.5 Hz), 9.13 (1H, d, J = 8.0 Hz), 9.32 (1H, d, J = 7.7 Hz), 10.90 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 20.3, 20.4, 20.5, 20.6, 20.7 (CH_3 of acetyl groups), 23.2 (NCH_3), 61.1, 61.2, 61.5 ($C_{6'}$), 63.4, 65.3, 66.2, 66.7, 67.1, 67.4, 67.6, 68.4, 69.1, 69.9, 70.8, 73.1 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 88.7, 91.2, 98.0 ($C_{1'}$), 112.3, 113.2, 120.3, 121.1, 124.2, 124.5, 127.2, 127.6 (C tert arom), 112.7, 117.4, 117.9, 118.4, 120.7, 122.3, 127.3, 128.6, 139.1, 140.2 (C quat arom), 168.8, 169.0, 169.1, 169.2, 169.4, 169.5, 169.7, 169.8, 169.9, 170.1 (C=O).

6: mp 263−265 °C; IR (KBr) ν_{NH} 3400 cm^{-1}, $\nu_{C=O}$ 1700, 1750 cm^{-1}; HRMS (FAB+) (M^+) calcd for $C_{35}H_{31}N_3O_{11}$ 669.1958, found 669.1957; ^{1}H NMR (400 MHz, acetone-d_6) 1.85−2.10 (12H, several s), 3.17 (3H, s, NCH_3), 4.55 (1H, m), 4.83 (1H, m), 5.08 (1H, m), 5.40 (1H, m), 5.61 (1H, m), 5.83 (1H, m), 6.78 (1H, d, $H_{1'}$, J = 9.2 Hz), 7.37 (1H, t, J = 7.8 Hz), 7.39 (1H, t, J = 7.6 Hz), 7.58 (1H, t, J = 7.1 Hz), 7.59 (1H, t, J = 7.6 Hz), 7.90 (1H, d, J = 8.3 Hz), 7.92 (1H, d, J = 8.4 Hz), 9.22 (1H, d, J = 8.2 Hz), 9.25 (1H, d, J = 8.5 Hz), 10.69 (1H, s, N_{indole}-H).

6-Methyl-12-(α-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (7). A mixture of **3** (124 mg, 0.184 mmol) in methanol (40 mL) and NH_4OH (18 mL) was stirred for 1 h at room temperature. The solvent was removed and the residue dissolved in EtOAc and washed with brine. The organic phase was dried over $MgSO_4$, and the solvent was removed. Purification by flash chromatography (eluent EtOAc) led to **7** as a yellow powder (73 mg, 78% yield): mp 182−185 °C; IR (KBr) $\nu_{NH,OH}$ 3300−3600 cm^{-1}, $\nu_{C=O}$ 1690, 1750 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) 2.86 (3H, s, NCH_3), 3.50 (1H, br s), 3.65 (1H, ddd, J_1 = J_2 = 9.9 Hz, J_3 = 4.1 Hz), 3.85 (2H, m), 4.05 (1H, m), 4.15 (1H, s), 4.89 (1H, dd, J_1 = J_2 = 6.3 Hz), 5.42 (1H, br s), 5.54 (1H, br s), 5.84 (1H, $H_{1'}$, d, J = 4.5 Hz), 7.31 (1H, t, J = 7.6 Hz), 7.38 (1H, t, J = 7.6 Hz), 7.55 (1H, t, J = 8.3 Hz), 7.58 (1H, t, J = 8.3 Hz), 7.85 (1H, d, J = 8.1 Hz), 8.21 (1H, d, J = 8.1 Hz), 9.03 (1H, d, J = 8.0 Hz), 9.21 (1H, d, J = 8.0 Hz), 11.30 (1H, s, $N_{indole-H}$); ^{13}C NMR (100 MHz, DMSO-d_6) 23.2 (NCH_3), 59.8, 61.4 ($C_{6'}$), 68.7, 70.6, 73.5, 74.9, 75.8, 76.3, 77.1 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 97.7 ($C_{1'}$), 112.1, 113.1, 120.2, 120.9, 124.2, 124.4, 127.0, 127.4 (C tert arom), 112.9, 117.3, 117.9, 118.2, 119.9, 120.8, 122.2, 128.5, 140.0, 140.2 (C quat arom), 169.0, 169.1 (C=O).

6-Methyl-12-(*β*-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (8). Compound **8** was obtained from **4** as an orange-yellow solid in 85% yield using the procedure described for compound **7**. **8**: mp 245−247 °C; IR (KBr) ν_{NH} 3450 cm^{-1}, $\nu_{C=O}$ 1690, 1750 cm^{-1}; HRMS (FAB$^+$) $(M + H)^+$ calcd for $C_{27}H_{24}N_3O_7$ 502.1614, found 502.1595; ^{1}H NMR (400 MHz, DMSO-d_6) 3.23 (3H, s, NCH_3), 3.65−3.69 (2H, m), 3.90 (1H, m), 4.01−4.10 (2H, m), 4.15 (1H, dd, J_1 = 10.7 Hz, J_2 = 3.1 Hz), 5.00 (1H, d, J = 4.9 Hz), 5.22 (1H, d, J = 4.4 Hz), 5.48 (1H, d, J = 4.1 Hz), 6.10 (1H, s), 6.34 (1H, $H_{1'}$, d, J = 8.4 Hz), 7.42 (2H, t, J = 7.0 Hz), 7.61 (1H, t, J = 7.1 Hz), 7.63 (1H, t, J = 6.9 Hz), 7.75 (1H, d, J = 8.1 Hz), 8.4 (1H, d, J = 8.5 Hz), 9.14 (1H, d, J = 7.9 Hz), 9.22 (1H, d, J = 7.9 Hz), 11.80 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 23.7 (NCH_3), 58.3 ($C_{6'}$), 67.5, 73.1, 76.7, 78.6, 84.5 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.8, 112.2, 120.4, 120.6, 124.4 (2C), 126.6, 126.9 (C tert arom), 117.1, 118.5, 118.6, 120.1, 121.3, 121.4, 128.2, 129.6, 140.9, 142.2 (C quat arom), 169.6, 169.7 (C=O).

6-Methyl-12-(α-D-galactopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (9). Compound **9** was obtained from **5** as a yellow solid in 75% yield using the procedure described for compound **7**. **9**: mp 187−188 °C; IR (KBr) $\nu_{NH,OH}$ 3300−3600 cm^{-1}, $\nu_{C=O}$ 1690, 1750 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) 3.02 (3H, s, NCH_3), 3.66 (2H, pt, J = 5.7 Hz), 3.93 (1H, br s), 4.02 (1H, br s), 4.11 (1H, pt, J = 6.0 Hz), 4.35 (1H, br s), 4.90 (1H, t, J = 5.4 Hz), 5.04 (1H, d, J = 4.8 Hz), 5.81 (1H, $H_{1'}$, d, J = 5.0 Hz), 5.91 (1H, br s), 7.37 (1H, t, J = 7.5 Hz), 7.43 (1H, t, J = 7.5 Hz), 7.59 (1H, t, J = 7.9 Hz), 7.62 (1H, t, J = 7.7 Hz), 7.91 (1H, d, J = 8.1 Hz), 8.25 (1H, d, J = 8.5 Hz), 9.11 (1H, d, J = 8.0 Hz), 8.31 (1H, d, J = 8.0 Hz), 11.20 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 23.5 (NCH_3), 60.3 ($C_{6'}$), 66.2, 71.4, 75.4, 77.0 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 98.9 ($C_{1'}$), 112.4, 113.3, 120.3, 120.9, 124.1, 124.5, 126.7, 127.0 (C tert arom), 117.3, 118.3, 120.0, 120.8, 121.4, 122.2, 128.6, 128.8, 140.0, 140.3 (C quat arom), 169.2, 169.8 (C=O).

6-Methyl-12-(*β*-D-galactopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (10). Compound **10** was obtained from **6** as an orange solid in 93% yield using the procedure described for compound **7**. **10**: mp 270−272 °C; IR (KBr) $\nu_{NH,OH}$ 3200−3500 cm^{-1}, $\nu_{C=O}$ 1690, 1750 cm^{-1}; HRMS (FAB+) (M^+) calcd for $C_{27}H_{24}N_3O_7$ 501.1536, found 501.1526; ^{1}H NMR (400 MHz, DMSO-d_6) 3.25 (3H, s,

NCH_3), 3.71 (1H, m), 3.77 (1H, m), 3.90 (1H, m), 4.13−4.23 (2H, m), 4.28 (1H, ddd, $J_1 = J_2 = 9.0$ Hz, $J_3 = 7.2$ Hz), 4.92 (1H, t, $J = 5.6$ Hz), 5.28 (1H, d, $J = 5.7$ Hz), 5.44 (1H, d, $J = 5.2$ Hz), 6.25 (1H, $H_{1'}$, d, $J = 9.1$ Hz), 6.90 (1H, s), 7.40 (1H, t, $J = 7.5$ Hz), 7.45 (1H, t, $J = 7.5$ Hz), 7.62 (1H, t, $J = 7.7$ Hz), 7.66 (1H, t, $J = 7.6$ Hz), 7.74 (1H, d, $J = 8.1$ Hz), 8.01 (1H, d, $J = 8.4$ Hz), 9.15 (1H, d, $J = 8.0$ Hz), 9.26 (1H, d, $J = 8.0$ Hz), 12.25 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 23.8 (NCH_3), 60.5 ($C_{6'}$), 69.2, 71.4, 74.0, 78.7 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 84.9 ($C_{1'}$), 111.2, 111.7, 120.2, 120.7, 124.4 (2C), 126.9, 127.0 (C tert arom), 116.7, 117.6, 118.2, 119.9, 120.8, 120.9, 127.7, 128.8, 140.5, 141.6 (C quat arom), 169.8, 169.9 (C═O).

6-Methyl-12-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6,7,-12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (11) and 6-Methyl-12-(2,3,4-tri-*O*-acetyl-β-L-fucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (12). A mixture of **2** (100 mg, 0.295 mmol), 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide (416 mg, 1.18 mmol), and Ag_2O (684 mg, 2.95 mmol) in benzene (80 mL) was refluxed for 2.5 h and then filtered off over Celite. The filtrate was evaporated, purified by chromatography (eluent cyclohexane−EtOAc, 80:20), and washed with hexane to give **11** and **12**, respectively, in 39% and 52% estimated yields as yellow solids. **11** and **12** were obtained with traces of sugar so their yields had to be estimated from ^{1}H NMR spectra.

11: IR (KBr) ν_{NH} 3400 cm^{-1}, $\nu_{C=O}$ 1700, 1750 cm^{-1}; HRMS (FAB+) (M^+) calcd for $C_{33}H_{29}N_3O_9$ 611.1904, found 611.1919; ^{1}H NMR (400 MHz, $CDCl_3$) 1.39 (3H, d, $J = 6.3$ Hz), 2.0−2.3 (12H, several s), 2.79 and 2.85 (3H, 2s, NCH_3), 4.45−4.75 (2H, m), 5.35−5.50 (2H, m), 6.12 and 6.23 (1H, d and br s, $H_{1'}$, $J = 4.7$ Hz), 7.31 (1H, dt, $J_1 = 7.9$ Hz, $J_2 = 2.3$ Hz), 7.40 (1H, t, $J = 7.9$ Hz), 7.43−7.57 (3H, m), 8.13 and 8.22 (1H, 2d, $J_1 = 8.7$ Hz, $J_2 = 8.3$ Hz), 9.09 (1H, d, $J = 7.9$ Hz), 9.35 (1H, d, $J = 7.1$ Hz), 10.46 and 10.51 (1H, 2s, N_{indole}-H); ^{13}C NMR (100 MHz, $CDCl_3$) 16.0, 16.4, 20.6, 20.9, 21.0, 23.3, 26.0, 30.3, 66.5, 67.3, 68.0, 68.3, 68.5, 70.0, 70.3, 71.3, 73.2, 73.3, 92.2, 99.0 ($C_{1'}$), 111.0, 112.9, 120.5, 121.7, 125.2, 125.5, 127.2, 127.8 (C tert arom), 112.7, 118.3, 118.5, 119.7, 120.8, 121.6, 123.3, 129.0, 139.3, 140.0 (C quat arom), 169.6, 169.7 (2C), 170.3, 170.5 (C═O).

12: IR (KBr) ν_{NH} 3390 cm^{-1}, $\nu_{C=O}$ 1700, 1750 cm^{-1}; HRMS (FAB+) (M^+) calcd for $C_{33}H_{29}N_3O_9$ 611.1904, found 611.1892; ^{1}H NMR (400 MHz, $CDCl_3$) 1.08 (3H, s), 1.75 (3H, d, $J = 6.2$ Hz), 1.92 (3H, s), 2.36 (3H, s), 3.25 (3H, s, NCH_3), 4.55 (1H, q, $J = 6.2$ Hz), 5.42 (1H, dd, $J_1 = 10.2$ Hz, $J_2 = 3.0$ Hz), 5.49 (1H, dd, $J_1 = 10.0$ Hz, $J_2 = 8.5$ Hz), 5.68 (1H, d, $J = 3.1$ Hz), 6.04 (1H, $H_{1'}$, d, $J = 9.0$ Hz), 7.40 (2H, dt, $J_1 = 7.5$ Hz, $J_2 = 2.0$ Hz), 7.46−7.60 (4H, m), 9.24 (1H, d, $J = 7.9$ Hz), 9.28 (1H, d, $J = 7.9$ Hz), 10.51 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, $CDCl_3$) 16.8, 19.1, 20.5, 21.3, 23.8 (CH_3), 68.3, 70.6, 71.4, 74.5 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 84.5 ($C_{1'}$), 109.9, 110.4, 121.0, 121.9, 126.0 (2C), 127.0, 127.2 (C tert arom), 118.3, 119.1, 119.6, 121.7, 122.4, 122.7, 128.3, 129.7, 140.1, 141.2 (C quat arom), 168.1, 169.9, 170.0, 170.1, 170.2 (C═O).

6-Methyl-12-(α-L-fucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (13). Compound **13** was obtained from **11** in 71% yield as a yellow solid using the procedure described for compound **7**. **13**: mp 192−195 °C; IR (KBr) $\nu_{NH,OH}$ 3400−3500 cm^{-1}, $\nu_{C=O}$ 1700, 1750 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) 1.29 (3H, d, $J = 6.3$ Hz), 3.11 (3H, s, N-CH_3), 3.40 (1H, br s), 3.69 (1H, br s), 4.02 (1H, br s), 4.28 (1H, q, $J = 6.3$ Hz), 5.05 (1H, br s), 5.72 (1H, $H_{1'}$, d, $J = 4.7$ Hz), 5.88 (1H, br s), 7.40 (1H, t, $J = 7.3$ Hz), 7.44 (1H, t, $J = 7.9$ Hz), 7.61 (1H, t, $J = 7.9$ Hz), 7.64 (1H, t, $J = 7.9$ Hz), 7.97 (1H, d, $J = 7.9$ Hz), 8.26 (1H, d, $J = 8.7$ Hz), 9.18 (1H, d, $J = 7.2$ Hz), 9.34 (1H, d, $J = 7.1$ Hz), 11.27 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 16.7, 23.1 (CH_3), 69.0, 70.4, 71.7, 76.4 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 99.2 ($C_{1'}$), 111.6, 112.0, 119.9, 120.7, 124.1, 124.4, 126.8, 127.3 (C tert arom), 117.2, 117.7, 118.2, 119.6, 120.7, 122.2, 128.6 (2C), 140.1 (2C) (C quat arom), 168.7, 168.8 (C═O).

6-Methyl-12-(β-L-fucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (14). Compound **14** was obtained from **12** in 81% yield as an orange solid using the procedure described for compound **7.** **14**: mp 256 °C; IR (KBr) $\nu_{NH,OH}$ 3350−3500 cm^{-1}, $\nu_{C=O}$ 1690, 1750 cm^{-1}; HRMS (FAB+) (M^+) calcd for $C_{27}H_{23}N_3O_6$ 485.1587, found 485.1588; ^{1}H NMR (400 MHz, DMSO-d_6) 1.40 (3H, d, $J = 6.3$ Hz), 3.22 (3H, s, NCH_3), 3.90 (1H, d, $J = 6.9$ Hz), 4.02 (1H, br s), 4.26 (1H, m), 4.35 (1H, q, $J = 6.1$ Hz), 5.26 (1H, d, $J = 6.3$ Hz), 5.40 (1H, d, $J = 4.8$ Hz), 6.22 (1H, $H_{1'}$, d, $J = 8.7$ Hz), 6.95 (1H, d, $J = 3.2$ Hz), 7.42 (1H, t, $J = 7.9$ Hz), 7.47 (1H, t, $J = 7.1$ Hz), 7.65 (1H, t, $J = 7.9$ Hz), 7.67 (1H, t, $J = 7.9$ Hz), 7.79 (1H, d, $J = 7.9$ Hz), 7.99 (1H, d, $J = 8.7$ Hz), 9.16 (1H, d, $J = 7.9$ Hz), 9.26 (1H, d, $J = 7.9$ Hz), 12.28 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 17.0, 23.7 (CH_3), 71.0, 72.0, 73.8, 74.2 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 84.8 ($C_{1'}$), 111.1, 111.7, 120.1, 120.6, 124.4 (2C), 126.8, 127.0 (C tert arom), 116.7, 117.6, 118.2, 119.9, 120.7, 120.9, 127.7, 128.8, 140.5, 141.6 (C quat arom), 169.7, 169.8 (C═O).

1,11-Dichloro-6-methyl-12-(4-*O*-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (15). Rebeccamycin (100 mg, 0.175 mmol) was dissolved in a 2 M solution of methylamine in THF (8 mL). The mixture was heated at 70 °C under pressure for 72 h. Water (250 mL) was added to allow the formation of a yellow precipitate which was filtered off and purified by flash chromatography (eluent cyclohexane−EtOAc, 60:40) to give **15** (92 mg, 0.157 mmol, 90% yield) as a yellow solid: mp >300 °C; IR (KBr) $\nu_{NH,OH}$ 3200−3600 cm^{-1}, $\nu_{C=O}$ 1690, 1750 cm^{-1}; HRMS (FAB+) (M^+) calcd for $C_{28}H_{23}N_3O_7Cl_2$ 583.0913, found 583.0937; ^{1}H NMR (400 MHz, DMSO-d_6) 3.09 (3H, s, NCH_3), 3.65 (3H, s, OCH_3), 3.58−4.15 (3H, m), 4.98 (1H, d, $J = 5.0$ Hz), 5.36 (1H, t, $J = 5.2$ Hz), 5.48 (1H, d, $J = 5.1$ Hz), 6.94 (1H, d, $H_{1'}$, $J = 8.9$ Hz), 7.38 (1H, t, $J = 7.9$ Hz), 7.42 (1H, t, $J = 7.9$ Hz), 7.67 and 7.68 (2H, 2t, $J = 7.6$ Hz), 8.90 (1H, d, $J = 8.0$ Hz), 9.08 (1H, d, $J = 8.3$ Hz), 10.58 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 23.6 (NCH_3), 60.1 ($C_{6'}$), 60.2 (OCH_3), 72.1, 77.4, 79.2, 80.3 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 84.3 ($C_{1'}$), 116.0, 116.2, 117.5, 119.2, 119.3, 121.2, 122.9, 124.9, 129.4 (2C), 137.0, 137.7 (C quat arom), 121.9, 122.5, 123.2, 123.9, 127.0, 130.0 (C tert arom), 168.6, 168.7 (C═O).

6-Methyl-12-(4-*O*-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (17). Dechlorinated rebeccamycin **16** (100 mg, 0.199 mmol) was dissolved in a 2 M solution of methylamine in THF (7 mL). The mixture was heated at 70 °C under pressure for 24 h. Water (250 mL) was added to allow the formation of a yellow precipitate which was filtered off and purified by flash chromatography (eluent cyclohexane−EtOAc, 50:50) to give **17** (70 mg, 0.136 mmol, 68% yield) as a yellow solid: mp 317−321 °C; IR (KBr) $\nu_{NH,OH}$ 3200−3600 cm^{-1}, $\nu_{C=O}$ 1690, 1750 cm^{-1}; HRMS (FAB+) (M^+) calcd for $C_{28}H_{25}N_3O_7$ 515.1692, found 515.1708; ^{1}H NMR (400 MHz, DMSO-d_6) 3.25 (3H, s, NCH_3), 3.72 (3H, s, OCH_3), 3.59−4.10 (6H, m), 5.06 (1H, d, $J = 5.3$ Hz), 5.37 (1H, d, $J = 5.6$ Hz), 6.21 (1H, s), 6.36 (1H, d, $H_{1'}$, $J = 9.0$ Hz), 7.43 (2H, t, $J = 7.5$ Hz), 7.63 and 7.64 (2H, 2t, $J = 7.2$ Hz), 7.78 (1H, d, $J = 8.2$ Hz), 8.01 (1H, d, $J = 8.5$ Hz), 9.15 (1H, d, $J = 8.0$ Hz), 9.23 (1H, d, $J = 7.9$ Hz), 11.66 (1H, s, N_{indole}-H); ^{13}C NMR (100 MHz, DMSO-d_6) 23.7 (NCH_3), 58.6 ($C_{6'}$), 60.1 (OCH_3), 73.2, 76.3, 77.2, 77.3 ($C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 84.2 ($C_{1'}$), 111.8, 112.2, 120.5, 120.7, 124.4, 124.9, 126.9, 127.1 (C tert arom), 117.1, 118.5, 118.6, 120.1, 121.1, 121.4, 128.1, 129.6, 140.9, 142.2 (C quat arom), 169.6, 169.7 (C═O).

Biological Tests. 1. DNA Unwinding, Ligation, and Footprinting Experiments. For the unwinding and ligation experiments, the plasmid pAT was used.[30] It was isolated from *E. coli* by an alkaline lysis procedure and was purified by banding twice in cesium chloride−ethidium bromide gradients. Ethidium was removed by several 2-propanol extractions followed by exhaustive dialysis against TE buffer to remove the CsCl.

DNA Unwinding Experiments:[9] Supercoiled pAT DNA (0.5 μg) was incubated with 6 units of CT-DNA topoisomerase I (TopoGen Inc.) 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 extraction with neutralized phenol. DNA samples were then added to the electrophoresis dye mixture (3 μL) and electrophoresed (35 V/cm) in a 1% agarose gel at room temperature for 15 h. Gels

were stained with ethidium bromide (1 μg/mL), washed, and photographed under UV light.

Ligation Experiments: Each sample contained 2 μL of linearized pAT DNA (0.7 μg/mL), 6 μL of water, 10 μL of drug at the desired concentration (or water in the controls), and 2 μL of 10× ligase buffer. After 20 min incubation to ensure equilibration, 2 μL (10 units) of ligase was added to each tube and the reaction was continued at room temperature for 30 min. The ligase was then denatured by heating at 65 °C for 5 min, and the samples were then added to the electrophoresis dye mixture (3 μL) prior to electrophoresis in a 1% agarose gel as described above.

Footprinting Experiments: Cleavage reactions by DNase I were performed essentially according to the previously detailed protocols.[31] Briefly, reactions were conducted in a total volume of 10 μL. Samples (3 μL) of the labeled DNA fragment were incubated with 5 μL of the buffer solution containing the desired drug concentration. After 30 min incubation at 37 °C to ensure equilibration of the binding reaction, the digestion was initiated by the addition of 2 μL of DNase I (0.01 unit/mL enzyme in 20 mM NaCl, 2 mM $MgCl_2$, 2 mM $MnCl_2$, pH 7.3). At the end of the reaction time (routinely 4 min at room temperature), the digestion was stopped by freeze-drying. After lyophilization each sample was resuspended in 4 μL of an 80% formamide solution containing tracking dyes prior to electrophoresis.

2. Topoisomerase Inhibition. Topoisomerases I and II were prepared from calf thymus as already described.[32,33] Topoisomerase I and II inhibitions were evaluated using the DNA cleavage assay carried out according to the procedure previously described.[34] Each compound was evaluated for its minimal inhibitory concentration (MIC), corresponding to the lowest concentration (μM) that produces a detectable stimulation of the DNA cleavage reaction.

3. Growth Inhibition Assay. P388 Murine Leukemia Cells: P388 murine leukemia cells were incubated at 37 °C for 96 h in the presence of various concentrations of drug and evaluated for viability by neutral red staining according to a published procedure.[35] The concentrations of drugs giving 50% growth inhibition (IC_{50}) were determined.

B16 Cell Culture: B16, a mouse melanoma cell line derived from spontaneous skin tumor in C57BI/6 mouse, was supplied by the Institut de Cancérologie et Immunogénétique, Villejuif, France. Stock cell cultures were maintained as monolayers in 25 cm^3 culture flasks in Eagle's minimum essential medium (Gibco, Paisly, Scotland) supplemented with 10% fetal calf serum (Sigma Chemical Co.), vitamin solution (100×; Gibco), 100 mM sodium pyruvate (Gibco), nonessential amino acids (100×; Gibco), 200 μM L-glutamine, and gentamycin (Schering-Plough). The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2. In these conditions, the doubling time was 15 h.

B16 Cell Cytotoxic Assay: B16 cells were plated into 60 mm Petri dishes (200 cells/dish) and allowed to adhere for 20 h before treatment. Culture medium containing increasing concentrations of drugs was added, and incubation was conducted for 24 h at 37 °C in a CO_2 incubator. After this time, the drug-containing medium was discarded, and the cells were washed with PBS. Fresh medium was added, and incubation was performed at 37 °C in a CO_2 incubator for 12 days. Dishes were then rinsed with PBS, fixed with methanol, and stained with 0.2% crystal violet solution, and colonies (>50 cells) were counted.

The antiproliferative activity is expressed as IC_{50} (50% inhibiting concentration), the drug concentration giving a 50% cloning efficiency compared to untreated cells.

4. Protein Kinase C Inhibition. Protamine sulfate was from Merck (Darmstadt, Germany). Unless specified, chemicals were from Sigma (St. Louis, MO). [γ-^{33}P]ATP (1000−3000 Ci/mmol) was obtained from Amersham. Recombinant baculoviruses from protein kinase C subtypes were supplied by Dr. Silvia Stabel, Köln, Germany.

Expression and partial purification of PKCs together with measurements of activities were carried out as previously described.[36] Stock solutions of compounds (in DMSO) were diluted in serial 10-fold dilutions using DMSO/water (v/v, 50:50) as the solvent. PKC isoenzyme activity was assayed using protamine sulfate as a substrate in the absence of phosphatidylserine and diacylglycerol.[37] Incorporation of γ-^{33}P onto protamine sulfate was determined by spotting 50 μL aliquots on P81 chromatography paper (Whatman).[38] Compounds were tested on PKC-α in two independent experiments. Data show IC_{50} values (half-maximum inhibitory concentrations) expressed in μM.

5. 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 Pasteur Institute). 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 broth (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**−**17** in DMSO (300 μg of drug/disk) were placed on Petri dishes. Growth inhibition was examined after 24 h incubation at 27 °C.

MIC values of **1**−**17** were determined classically 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.

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