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Original article

Synthesis and biological activities of indolocarbazoles bearing amino acid residues

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Abstract – Three indolocarbazole compounds bearing a tripeptide or a lysine group attached to one of the indole nitrogens via a propylamino chain and two rebeccamycin derivatives bearing a lysine residue on the sugar moiety were synthesised with the aim of improving the binding to DNA and the antiproliferative activities. Four tumour cell lines, from murine L1210 leukemia, human HT29 colon carcinoma, A549 non-small cell lung carcinoma and K-562 leukemia, were used to evaluate the cytotoxicity of the drugs. Their effects on the cell cycle of L1210 cells and their antimicrobial properties against two Gram-positive bacteria *Bacillus cereus* and *Streptomyces chartreusis*, a Gram-negative bacterium *Escherichia coli* and a yeast *Candida albicans* were also investigated.

indolocarbazole / rebeccamycin / antitumour agents / antimicrobial agents

1. Introduction

The properties of indolocarbazole compounds as protein kinase C and topoisomerase I inhibitors have been widely studied [1, 2]. Among the large number of microorganisms that produce indolocarbazole derivatives, the antitumour rebeccamycin has been isolated from *Saccharothrix aerocoloniges* (*figure 1*A) [3]. Its antitumour activity is associated at least partially, with its ability to interact with DNA and inhibit topoisomerase I [4]. In previous structure–activity relationship studies on rebeccamycin analogues, we have shown that the carbohydrate unit was a key element for both DNA-binding and topoisomerase I inhibition. Rebeccamycin derivatives lacking the sugar moiety on the indolocarbazole chromophore also bind to GC- and GT-rich sites in DNA but with a considerably lower affinity [5]. A model has been proposed for the drug-topoisomerase I-DNA ternary complex in which the planar indolocarbazole chromophore is inserted between two consecutive base pairs and the sugar residue resides into the groove of the double helix, most likely the minor groove [6] [7]. The possibilities of improving the solubility of analogues in this series were investigated by introducing various functional groups either on the indolocarbazole framework or at the imide nitrogen or on the carbohydrate moiety [4, 8–11].

Amino acids and peptides can contribute to increase the water solubility of indolocarbazoles and may increase the capacity to bind to the target. This approach has been exploited by a few groups. For example, water-soluble ester prodrugs of the microbial metabolite K-252a, an inhibitor of a number of serine–threonine kinases, were prepared with a lysine residue on the sugar unit yielding compounds that were cleaved by esterases in mammalian plasma

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(*figure 1*B) [12]. In the bisindolylmaleimide series, oligopeptide-substituted compounds were synthesised with the aim of obtaining DNA minor groove binding drugs (*figure 1*C) [13]. Pyrrole–imidazole carboxamide derivatives and peptides can confer enhanced DNA sequence recognition and they could ultimately be used to control gene expression. Different amino acid sequences have been used as DNA recognition elements. For example, the Ser-Pro-Lys-Lys (SPKK) motif was employed to favour AT-rich sites recognition by acridine intercalators (*figure 1*D) [14–17]. In the same vein, the metal-chelating peptide Gly-His-Lys (GHK) [18, 19] was coupled to a netropsin-like DNA minor groove binding unit and/or an anilinoacridine intercalator to design artificial site-specific nucleases (*figure 1*E) [20–22]. These considerations prompted us to extend this strategy to rebeccamycin derivatives.

In this paper, we report the synthesis and biological activities of maleimide–indolocarbazoles bearing a lysine or the tripeptide GHK linked to one of the indole nitrogens via a propylamino chain and rebeccamycin analogues possessing a lysine substituent on the carbohydrate unit at 2′ or 3′ positions. For each

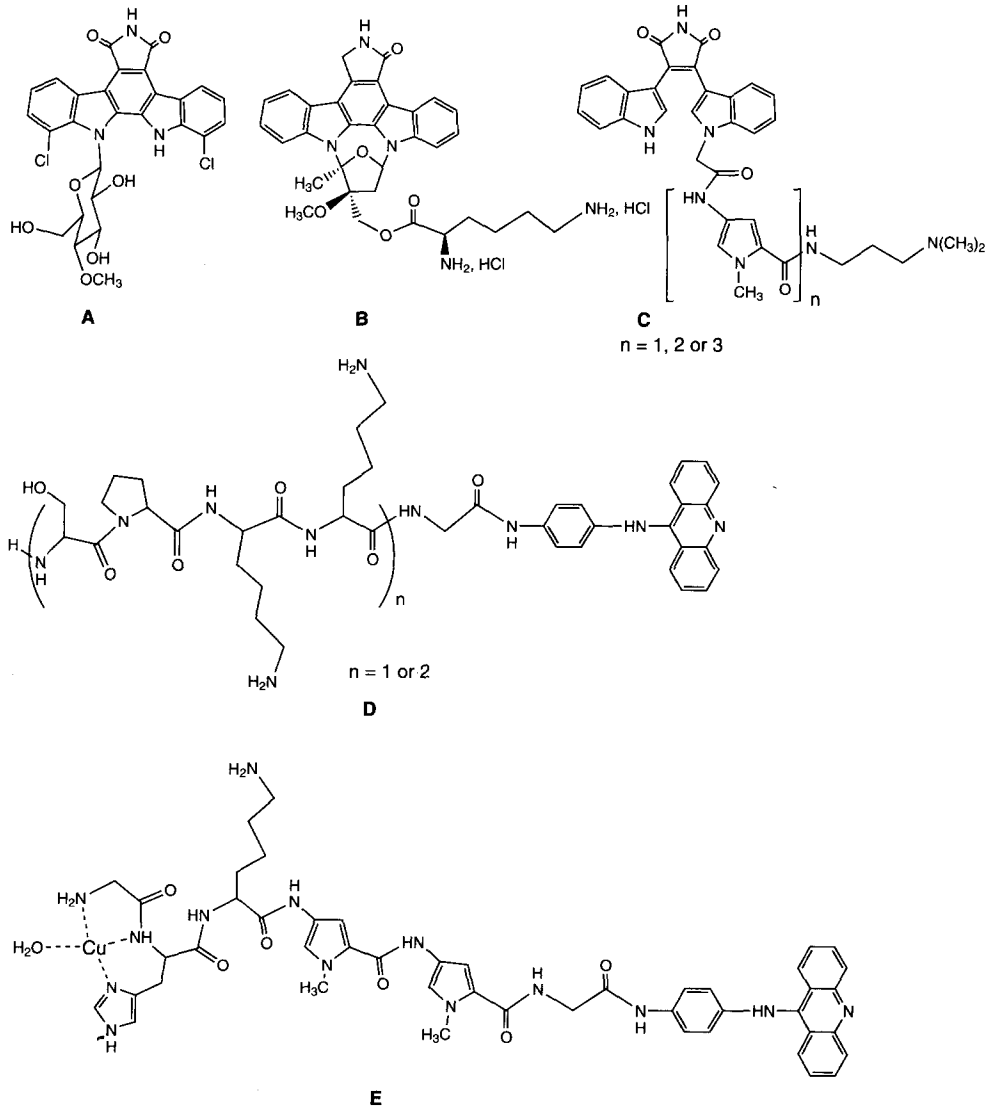

Figure 1.

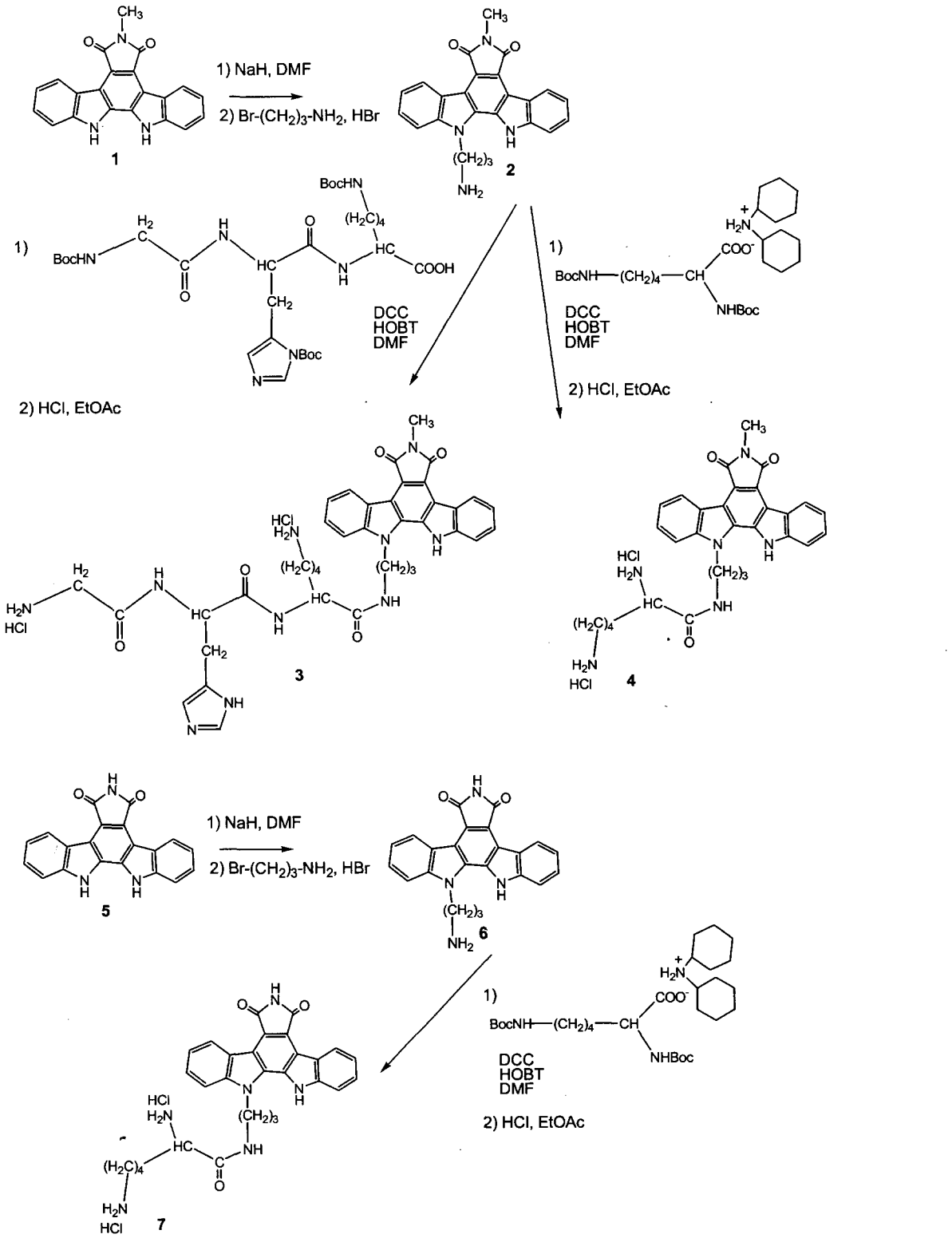

Figure 2.

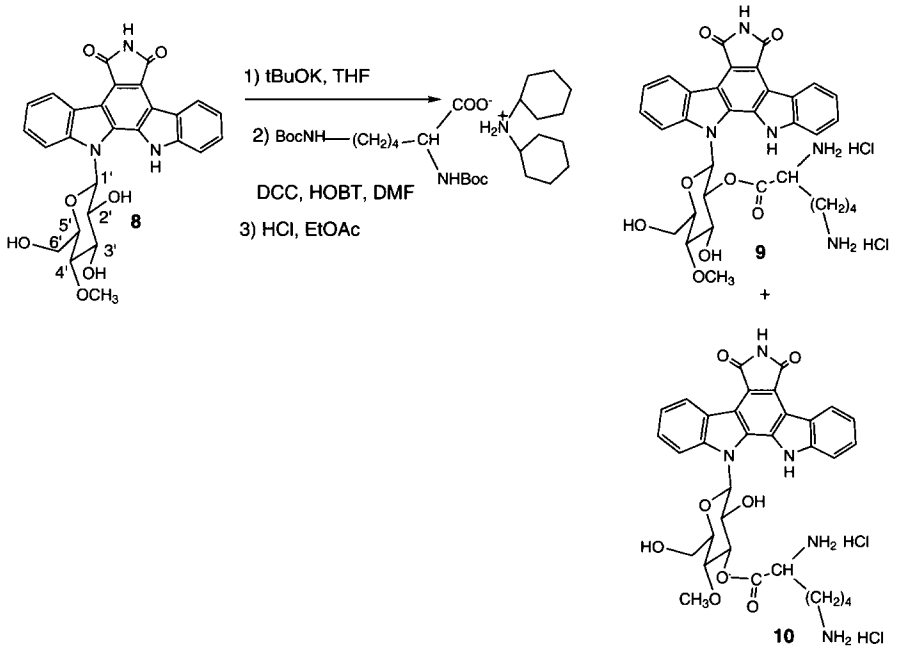

Figure 3.

compounds, we evaluated the DNA binding and topoisomerase I inhibition properties as well as their antimicrobial and antiproliferative activities. The effects of the drugs on the cell cycle of murine L1210 leukemia cells were also investigated.

2. Chemistry

Amino acid residues were coupled with the indolocarbazole aglycone substituted at one of the indole nitrogens by a propylamino chain. It was expected that the amino acid residues can reinforce the interaction of the drug with DNA, as is the case for the carbohydrate residue of rebeccamycin-type compounds. The propylamino chain was chosen as a linker to maintain a suitable distance between the aromatic framework and the positively-charged amino acid moiety.

Compounds with a methyl group on the imide nitrogen were synthesised because we have shown previously that such indolocarbazole bearing a *N*-methyl imide can inhibit topoisomerase I while maintaining a high cytotoxic potential [23]. Compound **2** was obtained from **1** by treatment with sodium hydride in DMF followed by reaction with 3-bromopropylamine hydrobromide (*figure 2*) [24]. Compound **2** was coupled with either the tripeptide *t*-Boc-L-Gly-L-His(*t*-Boc)-L-Lys(*t*-Boc)-OH or *N*α,*N*ε-di-*t*-Boc-L-lysine dicyclohexyl ammonium salt using standard peptide coupling techniques (DCC, HOBT, DMF, rt). Deprotection of the amino groups was performed in acidic medium yielding **3** and **4**, respectively, as hydrochlorides. In order to investigate the influence on the biological activity of the methyl group at the imide nitrogen, compound **6**, demethylated analogue of **2**, was prepared from aglycone **5** using the same method as for **2**. Coupling with *N*α,*N*ε-di-*t*-Boc-L-lysine dicyclohexyl ammonium salt and removal of the Boc protective groups led to compound **7**.

Analogues of K-252a derivative **B** shown in *figure 1* were prepared from dechlorinated rebeccamycin [8] **8** (*figure 3*) by deprotonation using *t*-BuOK in THF and coupling with *N*α,*N*ε-di-*t*-Boc-L-lysine dicyclohexyl ammonium salt. Compounds **9** and **10** were obtained with the amino acid residue at 2′ and 3′ positions, respectively.

3. Results and discussion

The L1210 murine leukemia cell line was used to evaluate the antiproliferative activity of the test drugs (*table I*). Compared with dechlorinated rebeccamycin

8, compounds **9** and **10** bearing a lysine group at 2′ and 3′ positions on the carbohydrate unit, respectively, exhibited a reduced cytotoxicity. Compound **7** with a lysine group on the propylamino chain of **6** was almost inactive. The cytotoxicities of compound **4**, bearing a lysine on the propylamino chain, and that of the parent compound **2** are in the same range, whereas compound **3**, bearing a tripeptide on the propylamino chain, was less cytotoxic.

Three additional tumour cell lines were tested (*table I*), human HT29 colon carcinoma, A549 non-small cell lung carcinoma and K-562 leukemia, but no significant differences were observed between compounds **3** and **4** and the parent compound **2**. Comparison of the cytotoxicities of dechlorinated rebeccamycin **8** and analogues **9** and **10** bearing a lysine at 2′ and 3′ position, respectively, showed that **9** was more efficient than **10** against K-562 cells and exhibited a profile of cytotoxicity similar to that of the parent compound **8**. The two compounds **8** and **9** inhibited at least 10 times more potently the proliferation of the two leukemia cell lines than the two solid tumour cell lines. This selectivity was less pronounced for compound **10**. The other compounds were equally cytotoxic for the four cell lines.

Cell cycle experiments with L1210 leukemia cells revealed that about 70% of the cells were recovered in the G2+M phase using **8** (1 μM), **9** and **10** (2 and 2.5 μM, respectively) (*table I*). With compound **3** (10 μM), bearing the tripeptide attached to the amino chain, 59% of the cells were recovered in the G1 phase. These results are consistent with those observed previously with other rebeccamycin analogues (unpublished results). In most cases, analogues bearing a sugar moiety induce cell cycle arrest in the G2+M phase, suggesting that these compounds could share the same mechanism of action, i.e. binding to DNA and poisoning topoisomerase I. With compound **3** (10 μM), bearing the tripeptide attached to the amino chain, 59% of the cells were recovered in the G1 phase, suggesting that, instead of topoisomerase I, unknown targets were affected in intact cells.

Drug–DNA interactions were analysed by two complementary techniques: thermal denaturation and fluorescence. In most cases, the fluorescence of rebeccamycin analogues increases upon binding of the drug to DNA, providing thus a convenient approach to evaluate the strength of the interaction [6]. Fluorescence titration experiments performed with the polynucleotide poly(dAC·dGT) revealed that, as expected, the binding of the drug to DNA is increased when a positively-charged lysine residue is linked to the indolocarbazole chromophore via a propylamino linker. A much higher binding constant was measured with compound **4** compared to **2** showing thus that, as expected, the incorporation of the lysine residue enhances significantly the DNA interaction (*table II*). The GHK-containing derivative was also found to bind tightly to DNA but surprisingly, the lysine derivative **7** showed little interaction with DNA. Roughly equal binding constants were measured for compounds **8** and **9**–**10**, indicating that the addition of a lysine residue on the sugar unit of rebeccamycin does not necessarily confer enhanced binding to DNA. The fact that compounds **3** and **4** exhibit a much higher affinity for DNA was also evidenced by melting temperature (T_m) measurements performed

Table I. In vitro antiproliferative activities against four tumour cell lines: murine leukemia L1210, and human HT29 colon carcinoma, A549 non-small cell lung carcinoma and K-562 leukemia (IC_{50} μM).

Cpd	L1210	L1210 cell cycle	HT29	A549	K-562
1	5.1	G1 66% at 25 μM	ne	ne	ne
2	2	NS, tox at 10 μM	5.1	5.5	4.5
3	3.8	G1 59% at 10 μM	15.0	8.1	5.6
4	1.9	NS, tox at 10 μM	6.4	5.5	5.4
7	36.4	ne	>10	>10	>10
8	0.11	G2M 71% at 1 μM	4.0	3.4	<0.1
9	0.31	G2M 71% at 2 μM	2.3	2.8	<0.1
10	1	G2M 68% at 2.5 μM	6.2	5.6	0.25

Effect on L1210 cell cycle: percentage of cells recovered in G1 or G2+M phases with a concentration of drug expressed in μM. Twenty-four percent of untreated control cells were in the G2+M phase of the cell cycle, 44% in the G1 phase and 28% in the S phase. NS, not specific, i.e. no modification of the cell cycle; ne not evaluated.

Table II. DNA binding constants.

Cpd	K ($\times 10^5$ M^{-1})
2	3.8
3	15
4	17
7	1.7
8	2.7
9–10	4

Binding constants were calculated from fluorescence measurements performed in BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 1 mM Na_2EDTA, pH 7.0). Excitation was at 320 nm and fluorescence emission was monitored over the range 340–620 nm [6].

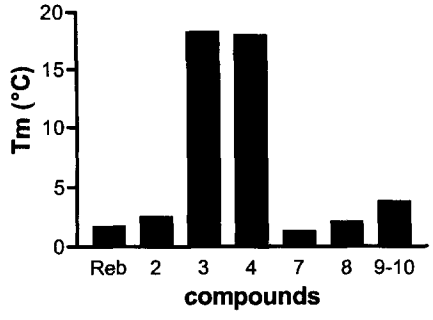

Figure 4. Variation of the ΔT_m ($T_m^{drug-DNA\ complex} - T_m^{DNA\ alone}$, in °C) of the complexes between calf thymus DNA and the test compounds. Melting temperature measurements were performed in BPE buffer at pH 7.1 with a drug/DNA ratio of 0.1.

with calf thymus DNA. As shown in *figure 4*, compounds **2**, **7–10**, as well as rebeccamycin itself, only slightly increase the T_m of DNA by 1–4 °C whereas the T_m shifted from 58 to 76 °C ($\Delta T_m = 18$ °C) with both compounds **3** and **4**. But the increased DNA affinity is at the expense of sequence recognition and topoisomerase I inhibition since these two compounds showed no preferential binding sites in footprinting experiments and do stabilise topoisomerase I-DNA covalent complexes as is the case for camptothecin and certain rebeccamycin derivatives.

3.1. Antimicrobial properties

The antimicrobial activities of compounds **2–10** were tested against two Gram-positive bacteria (*Bacillus cereus* and *Streptomyces chartreusis*), a Gram-negative bacterium (*Escherichia coli*) and a yeast (*Candida albicans*) (*table III*). Compared with the parent compound **2**, compounds **3** and **4** bearing a tripeptide chain and a lysine residue, respectively are more efficient against *B. cereus* and *S. chartreusis*. Compound **4** inhibited the growth of *C. albicans*. Compound **7**, analogue of **4** without substitution at the imide nitrogen by a methyl group, was inactive against the microorganisms tested. Rebeccamycin analogues **9** and **10** inhibited strongly the growth of the Gram positive bacteria tested, and **9** inhibited strongly the sporulation of *S. Chartreusis* whilst the parent compound **8** had no effect. Moreover, compound **9** inhibited the growth of the yeast *C. albicans* that was never observed with the rebeccamycin analogues previously studied.

In conclusion, this novel series of indolocarbazoles bearing amino-acid residues provided compounds with improved water solubility and varied biological activities. The aim of improving the capacity of the drugs to interact with DNA has been partially achieved but this is at the expense of sequence recognition and topoisomerase I inhibition. As a result, we observed that the introduction of a lysine residue or the GHK tripeptide in the place of the sugar moiety on the indolocarbazole framework or on the sugar moiety did not improve the

Table III. Antimicrobial activities of compounds **2–10** against two Gram-positive bacteria, *B. cereus* and *S. chartreusis*, a Gram-negative bacterium *E. coli*, and a yeast *C. albicans*.

Cpd	*B. cereus* ATCC 14579	*S. Chartreusis* NRRL 11407	*E. coli* ATCC 11303	*C. albicans* IP 444
2	±	±	–	–
3	+	+, sp –	–	±
4	++	+, sp –	–	+
7	–	–	–	–
8	±	–	–	–
9	++++	+++, sp ++++	–	+
10	+++	±, sp +	–	–

The size of zones of growth inhibition was 13–16 mm (++++), 10–12 mm (+++), 8–9 mm (++), 7–8 mm (+), 6–7 mm (±); sp: inhibition of sporulation.

antiproliferative activities in vitro. However, interestingly compounds **9** and **10** bearing a lysine at 2′ and 3′ positions, respectively, were found to exhibit strong antimicrobial activities against the two Gram-positive bacteria tested. Compounds **4** and **9** exerted antimicrobial properties against the fungus *C. albicans*. Such an effect had never been observed before for the hundred of rebeccamycin analogues we have synthesised so far, providing thus a new challenge for the development of antimicrobial agents. The studies reported in this paper are part of large structure–activity relationships program aimed at developing indolocarbazoles of therapeutic values. The data contribute to extend the knowledge in the field.

4. Experimental

4.1. 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), doublet (d), triplet (t), pseudo-triplet (pt), doubled triplet (dt), multiplet (m), br s (broad signal), 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 on a high resolution Fisons Autospec-Q spectrometer at CESAMO (Talence, France). Chromatographic purifications were performed by flash silicagel Geduran SI 60 (Merck) 0.040–0.063 mm or Kieselgel 60 (Merck) 0.063–0.200 mm column chromatography. For purity tests, TLC were performed on fluorescent silica gel plates (60 F_{254} from Merck). Rebeccamycin was from our laboratory stock sample. The tripeptide *N*-Boc-L-Gly-*N*-Boc-L-His-L-Lys-OH has been furnished by Riom-Laboratoires CERM, France.

4.1.1. 6-Methyl-12-(N-propylamino)-6,7,12,13-tetrahydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7-dione (2)

To a solution of *N*-methylated aglycone **1** (400 mg, 1.16 mmol) in DMF (80 mL) was added NaH (60% dispersion in mineral oil, 140 mg). The mixture was stirred at room temperature (r.t.) for 30 min before the addition of 3-bromopropylamine hydrobromide (260 mg, 1.18 mmol). The mixture was stirred at r.t. for 24 h. After addition of saturated aq. $NaHCO_3$ and extraction with EtOAc then CH_2Cl_2, the organic phases were dried over $MgSO_4$. The solvent was removed and the residue purified by flash chromatography (silicagel, eluent: CH_2Cl_2–MeOH, 95:5) to give the amine **2** (217 mg, 0.55 mmol, 47% yield) as a yellow solid: m.p. >300 °C. IR (KBr) ν_{CO} 1695, 1745 cm^{-1}, ν_{NH} 3100–3600 cm^{-1}; HRMS (FAB+) $[M+H]^+$ Calc. for $C_{24}H_{21}N_4O_2$ 397.1664, Found: 397.1647; ^1H-NMR (400 MHz, DMSO-d_6): 2.15 (2H, m), 2.75 (2H, t, $J = 5.4$ Hz), 3.16 (3H, s, NCH_3), 3.39–4.01 (3H, br s), 4.87 (2H, t, $J = 6.4$ Hz), 7.37 (1H, dt, $J_1 = 7.9$ Hz, $J_2 = 1.0$ Hz), 7.41 (1H, t, $J = 7.9$ Hz), 7.58 (1H, dt, $J_1 = 7.9$ Hz, $J_2 = 1.0$ Hz), 7.63 (1H, t, $J_1 = 7.4$ Hz, $J_2 = 0.9$ Hz), 7.75 (1H, d, $J = 7.9$ Hz), 7.83 (1H, d, $J = 8.3$ Hz), 9.08 (1H, d, $J = 7.9$ Hz), 9.11 (1H, d, $J = 7.9$ Hz). ^{13}C-NMR (100 MHz, DMSO-d_6): 23.4 (NCH_3), 31.4, 36.7, 41.3 (CH_2), 109.7, 112.2, 119.7, 120.1, 124.1, 124.3, 126.5, 126.6 (C tert arom), 115.2, 116.8, 118.2, 118.6, 121.0, 121.1, 129.3, 130.1, 141.0, 141.9 (C quat. arom.), 169.6 (2C=O).

4.1.2. 6-Methyl-12-N-(L-Glycyl-L-Histidyl-L-Lysyl-propylamino)-6,7,12,13-tetrahydro-5H-indolo[2,3-a]-pyrrolo[3,4-c]carbazole-5,7-dione, dihydrochloride (3)

A solution of the tripeptide *N*-Boc-Gly-*N*-Boc-His-*N*-Boc-Lys-OH (323 mg, 0.5 mmol) in DMF (10 mL) was cooled to 0 °C before addition of *N*-methyl-aglycone **2** (200 mg, 0.5 mmol), HOBT (68 mg, 0.5 mmol) and DCC (114 mg, 0.56 mmol). The mixture was stirred at r.t. for 26 h. After filtration over Celite and washing with CH_2Cl_2, the solvents were removed and the residue purified by column chromatography (eluent: MeOH–EtOAc, 5:95) to give tri-*N*-Boc-**3** which was then treated at r.t. with a 3 M solution of HCl in EtOAc (5 mL) for 2 h. The solid was filtered off and washed with EtOAc and toluene to yield **3** (93 mg, 0.12 mmol, 24% yield) as red–orange crystals; m.p. >300 °C. IR (KBr): $\nu_{C=O}$ 1750, 1690, 1640 cm^{-1}, $\nu_{NH,OH}$ 3160–3680 cm^{-1}. HRMS (FAB+) $[M+H]^+$: Calc. for $C_{38}H_{43}N_{10}O_5$, 719.3418, Found: 719.3434%. ^1H-NMR (400 MHz, CH_3OD): 1.55 (1H, m), 1.64 (1H, m), 1.76–1.90 (4H, m), 2.21 (2H, m), 3.01 (3H, s, NCH_3), 3.06 (2H, pt, $J = 7.5$ Hz), 3.20–3.46 (4H, m), 3.87 (2H, s), 4.34 (1H, m), 4.65 (2H, t, $J = 7.2$ Hz), 4.91 (1H, t, $J = 6.1$ Hz), 7.46 (1H, dt, $J_1 = 6.9$ Hz, $J_2 = 0.7$ Hz), 7.47 (1H, s), 7.48 (1H, t, $J = 6.4$ Hz), 7.65 (1H, t, $J = 8.2$ Hz), 7.67 (1H, d, $J = 8.5$ Hz), 7.70 (1H, t, $J = 8.1$ Hz), 7.87 (1H, d, $J = 8.2$ Hz), 8.74 (1H, d, $J = 1.2$ Hz), 9.11 (1H, d, $J = 8.0$ Hz), 9.13 (1H, d, $J = 8.1$ Hz). ^{13}C-NMR (100 MHz, DMSO-d_6): 23.9 (NCH_3), 24.2, 28.4, 28.7, 31.7, 32.4, 38.4, 40.7, 41.9, 43.5 (CH_2), 53.8, 55.7 (CH), 110.1, 112.4, 117.0, 120.3, 120.4,

124.1, 124.4, 126.9, 127.0,133.5 (C tert arom), 116.0, 116.9, 118.8 (2C), 120.8, 121.0, 127.9, 128.8, 129.3, 141.1, 141.3 (C quat. arom.), 166.1, 169.3, 169.7 (2C), 171.8 (C=O).

4.1.3. 6-Methyl-12-N-(L-Lysyl-propylamino)-6,7,12,13-tetrahydro-5H-indolo[2,3-a]pyrrolo[3,4-c]-carbazole-5,7-dione, dihydrochloride (4)

A solution of 1 M H_2SO_4 (0.6 mL, 0.6 mmol) was added to a suspension of $N\alpha$,$N\varepsilon$-di-*t*-Boc-L-lysine dicyclohexyl ammonium salt (264 mg, 0.5 mmol) in EtOAc (1 mL) cooled at 0 °C. The mixture was stirred at r.t. for 30 min. Water was added, the aqueous phase was extracted twice with EtOAc, the organic phase was dried over $MgSO_4$ and evaporated to give free di-*t*-Boc-Lys which was used directly for the coupling step. The solution of di-*t*-Boc-Lys in DMF (10 mL) was cooled to 0 °C before addition of **2** (200 mg, 0.5 mmol), HOBT (68 mg, 0.5 mmol) and DCC (114 mg, 0.56 mmol). The reaction mixture was stirred at r.t. for 18 h. After filtration over Celite and washing with CH_2Cl_2, the solvents were removed and the residue was purified by column chromatography (eluent: EtOAc–cyclohexane, 70:30) to give the coupling product which was then treated at r.t. with a 3 M solution of HCl in EtOAc (5 mL) for 2 h. The solid was filtered off and washed with EtOAc and toluene to yield **4** (143 mg, 0.24 mmol, 49% yield) as a dark red solid: m.p. 228 °C; IR (KBr) $\nu_{C=O}$ 1670, 1675, 1680 cm^{-1}, ν_{NH} 2700–3300 cm^{-1}; HRMS (FAB+) $[M+H]^+$: Calc. for $C_{30}H_{33}N_6O_3$, 525.2614, Found: 525.2622. ^{1}H-NMR (400 MHz, DMSO-d_6): 1.38 (2H, m), 1.59 (2H, m), 1.75 (2H, m), 2.04 (2H, m), 2.74 (2H, m), 3.10 (3H, s, NCH_3), 3.27 (2H, m), 3.83 (1H, m), 5.04 (2H, t, $J = 6.58$), 7.36 (1H, t, $J = 7.1$ Hz), 7.37 (1H, t, $J = 7.1$ Hz), 7.58 (1H, t, $J = 7.7$ Hz), 7.61 (1H, t, $J = 7.5$ Hz), 7.83 (1H, d, $J = 8.3$ Hz), 7.96 (1H, d, $J = 8.1$ Hz), 8.10 (3H, br s), 8.34 (3H, br s), 9.05 (1H, d, $J = 7.5$ Hz), 9.06 (1H, s, NH), 9.07 (1H, d, $J = 7.6$ Hz), 12.04 (1H, s, $N_{indole}H$). ^{13}C-NMR (100 MHz, DMSO-d_6): 21.2, 26.2, 30.1, 30.3, 36.4, 38.2, 42.1 (CH_2), 23.5 (NCH_3), 51.9 (CH), 110.0, 112.4, 120.0, 120.2, 120.3, 124.1, 124.4, 126.8 (C tert arom), 116.0, 116.9, 118.6, 118.7, 120.8, 121.0, 127.8, 129.1, 141.0, 141.3 (C quat. arom.), 168.4 (2C), 169.6 (C=O).

4.1.4. 12-N-Propylamino-6,7,12,13-tetrahydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7-dione (6)

To a solution of **5** [25](231 mg, 0.71 mmol) in DMF (40 mL) was added NaH (60% w/w dispersion in mineral oil, 113 mg, 2.84 mmol). The mixture was stirred at r.t. for 30 min before addition of 3-bromopropylamine hydrobromide (155 mg, 0.71 mmol). The reaction mixture was stirred at r.t. for 24 h before adding saturated aq. $NaHCO_3$. After extraction with CH_2Cl_2, the organic phase was dried over $MgSO_4$. The solvent was removed and the residue purified by chromatography (eluent: MeOH–CH_2Cl_2 10:90) to yield **6** (74 mg, 0.19 mmol, 27% yield) as yellow crystals which were used directly for the coupling step. ^{1}H-NMR (400 MHz, DMSO-d_6): 2.17 (2H, quint, $J = 6.1$ Hz), 2.79 (2H, t, $J = 5.8$ Hz), 3.80 (4H, br s), 4.95 (2H, t, $J = 5.9$ Hz), 7.37 (1H, t, $J = 7.8$ Hz), 7.42 (1H, t, $J = 7.7$ Hz), 7.59 (1H, dt, $J_1 = 8.2$ Hz, $J_2 = 1.2$ Hz), 7.64 (1H, dt, $J_1 = 7.4$ Hz, $J_2 = 1.2$ Hz), 7.80 (1H, d, $J = 8.2$ Hz), 7.88 (1H, d, $J = 8.3$ Hz), 9.10 (1H, d, $J = 7.9$ Hz), 9.14 (1H, d, $J = 7.8$ Hz).

4.1.5. 12-N-(L-Lysyl-propylamino)-6,7,12,13-tetrahydro(5H)-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole-5,7-dione (7)

The same procedure as described for **4** afforded, from **6** (74 mg, 0.19 mmol), compound **7** (20 mg, 0.03 mmol, 16% yield) as red–orange crystals; m.p. >300 °C. IR (KBr); $\nu_{C=O}$ 1715, 1650 cm^{-1}, $\nu_{NH,OH}$ 3160–3660 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): 1.37 (2H, m), 1.58 (2H, m), 1.75 (2H, m), 2.08 (2H, m), 2.74 (2H, m), 3.31 (2H, m), 3.83 (1H, m), 5.16 (2H, m), 7.39 (1H, t, $J = 7.5$ Hz), 7.42 (1H, t, $J = 7.5$ Hz), 7.60 (1H, t, $J = 7.5$ Hz), 7.65 (1H, t, $J = 7.5$ Hz), 7.93 (1H, d, $J = 8.3$ Hz), 8.00 (1H, d, $J = 8.1$ Hz), 8.18 (6H, br s), 9.04 (1H, s, NH), 9.13 (1H, d, $J = 7.9$ Hz), 9.17 (1H, d, $J = 7.9$ Hz), 11.08 (1H, s, NH), 12.51 (1H, s, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 21.2, 26.2, 30.1, 30.3, 36.5, 38.2, 42.2 (CH_2), 51.9 (CH), 110.2, 112.5, 120.3, 120.4, 124.2, 124.6, 126.8, 126.9 (C tert arom), 116.1, 116.9, 119.9, 120.0, 120.9, 121.2, 128.1, 129.5, 141.1, 141.3 (C quat. arom.), 168.4, 171.1 (2C) (C=O).

4.1.6. 12-(2-O-L-Lysyl-4-O-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro(5H)-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole-5,7-dione dihydrochloride (9) and 12-(3-O-L-Lysyl-4-O-methyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydro(5H)-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole-5,7-dione dihydrochloride (10)

A solution of H_2SO_4 1 M (1.29 mL, 1.29 mmol) was added to a suspension of $N\alpha$,$N\varepsilon$-di-*t*-Boc-L-lysine dicyclohexyl ammonium salt (559 mg, 1.06 mmol) in

EtOAc (2.15 mL) cooled with an ice bath. The reaction mixture was stirred at r.t. for 30 min. Water was added, the aqueous layer was extracted twice with EtOAc, the organic phase was dried over $MgSO_4$ and evaporated to give the free di-*N*-*t*-Boc-L-lysine which was used directly in the coupling step. The solution of di-*N*-*t*-Boc-L-lysine in DMF (22 mL) was cooled to 0 °C before addition of HOBT (144 mg, 1.06 mmol) and DCC (241 mg, 1.17 mmol). The mixture was stirred at r.t. for 30 min before addition to a solution of dechlorinated rebeccamycin **8** (530 mg, 1.06 mmol) in THF (11 mL) treated before with *t*BuOK (119 mg, 1.06 mmol) for 30 min. The reaction mixture was then stirred at 60 °C for 24 h. After cooling, saturated aq. NaCl was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, evaporated and the residue purified by chromatography (first column: eluent: THF–toluene, 20:80). A second column (eluent, EtOAc–CH_2Cl_2, 35:65) gave **Boc-protected-10** (88 mg). **Boc-protected-9** (54 mg) was obtained after a second purification by PLC plate (eluent, EtOAc–CH_2Cl_2 20:80).

Boc-protected-9 was treated at r.t. with a 3 M solution of HCl in EtOAc (1 mL) for 5 h. The evaporation of the reaction mixture gave red crystals which were washed with EtOAc to yield **9** (45 mg, 0.06 mmol, 5% yield); m.p. >300 °C; IR (KBr): $\nu_{C=O}$ 1700, 1740, 1750 cm^{-1}, $\nu_{NH,OH}$ 3100–3600 cm^{-1}; HRMS (FAB+) $[M+H]^+$: Calc. for $C_{33}H_{36}N_5O_8$, 630.2564, Found: 630.2550%. ^{1}H-NMR (400 MHz, DMSO-d_6): 0.7–1.1 (4H, m), 1.32 (2H, m), 2.66 (2H, m), 2.79 (1H, m), 3.73 (3H, s, OCH_3), 3.96 (2H, m), 4.05 (1H, m), 4.17 (1H, m, $H_{3'}$), 4.25 (1H, d, $J=8.9$ Hz), 5.10 (1H, t, $J=8.9$ Hz, $H_{2'}$), 5.92 (1H, d, $J=4.9$ Hz, $OH_{3'}$), 6.42 (1H, t, $J=3.4$ Hz, $OH_{6'}$), 6.78 (1H, d, $J=8.9$ Hz, $H_{1'}$), 7.43 (1H, t, $J=8.0$ Hz), 7.49 (1H, t, $J=7.9$ Hz), 7.63 (1H, t, $J=8.4$ Hz), 7.67 (1H, t, $J=7.9$ Hz), 7.81 (1H, d, $J=7.9$ Hz), 8.05 (4H, br s), 8.10 (1H, d, $J=7.9$ Hz), 9.14 (1H, d, $J=8.4$ Hz), 9.19 (1H, d, $J=7.9$ Hz), 11.22 (1H, s, NH), 11.68 (1H, s, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 21.2, 26.1, 28.8, 38.1 (CH_2 lys), 51.3 (CH lys), 58.2 ($C_{6'}$), 60.3 (OCH_3), 73.3, 75.5, 76.8, 77.2, 80.9 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.5, 112.3, 120.6, 121.4, 124.5, 124.7, 127.2 (2C) (C tert arom), 117.3, 118.3, 119.4, 121.2, 121.4, 121.6, 128.3, 129.6, 140.8, 140.9 (C quat. arom.), 170.9 (C=O).

Boc-10 was treated at 50 °C with a 3 M solution of HCl in EtOAc (4.5 mL) for 2 h. After evaporation, a mixture of MeOH–EtOAc (10:90, v/v) was added to the residue to give crystals which were filtered off and washed with EtOAc and THF yielding **10** as green crystals (52 mg, 0.07 mmol, 6% yield); m.p. >300 °C. IR (KBr): $\nu_{C=O}$ 1700, 1750, 1760 cm^{-1}, $\nu_{NH,OH}$ 2800–3600 cm^{-1}. HRMS (FAB+) $[M+H]^+$: Calc. for $C_{33}H_{36}N_5O_8$, 630.2564, Found: 630.2572%. ^{1}H-NMR (400 MHz, DMSO-d_6): 1.31–1.98 (6H, 3m), 2.65 (1H, m), 2.80 (2H, br s), 3.62 (3H, s, OCH_3), 3.65 (1H, m), 3.83 (1H, m), 4.03 (2H, m), 4.28 (1H, m), 5.48 (1H, d, $J=5.6$ Hz, $OH_{2'}$), 5.51 (1H, t, $J=9.7$ Hz, $H_{3'}$), 6.50 (1H, br s, $OH_{6'}$), 6.66 (1H, d, $J=8.9$ Hz), 7.43 (2H, t, $J=7.41$ Hz), 7.62 (1H, t, $J=7.0$ Hz), 7.45 (1H, t, $J=7.0$ Hz), 7.78 (1H, d, $J=7.9$ Hz), 7.93 (1H, br s), 8.05 (3H, br s), 8.67 (3H, br s), 9.14 (1H, d, $J=7.9$ Hz), 9.20 (1H, d, $J=7.8$ Hz), 11.20 (1H, s, NH), 11.65 (1H, s, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 21.1 and 21.3, 26.0 and 26.2, 29.2, 38.0 and 38.2 (CH_2 lys), 51.6 and 51.8 (CH lys), 58.1 ($C_{6'}$), 60.0 (OCH_3), 70.6, 75.1, 76.7, 78.4, 83.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.7, 112.3, 120.1, 120.6, 121.0, 124.5, 126.9, 128.1 (C tert arom), 117.1, 118.6, 119.5, 121.1, 121.3, 121.5, 128.0, 129.6, 140.7, 142.1 (C quat. arom.), 168.4, 170.9 (C=O).

4.2. 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. 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^{-1}. The 'melting' temperature T_m was taken as the mid-point of the hyperchromic transition. The fluorescence measurements were performed as previously described [6].

4.3. Growth inhibition assay

Tumour cells were provided by American Type Culture Collection (Frederik, MD, USA). They were cultivated in RPMI 1640 medium (Life Science technologies, Cergy-Pontoise, France) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 10 mM HEPES buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described [26]. Cells were

continuously exposed to graded concentrations of the compounds for four doubling times, then 15 μL of 5 mg mL^{-1} 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were added to each well and the plates were incubated for 4 h at 37 °C. The medium was then aspirated and the formazan solubilised by 100 μL of DMSO. Results are expressed as IC_{50}, concentration which reduced by 50% the optical density of treated cells with respect to untreated controls.

4.4. Cell cycle analysis

For the cell cycle analysis, L1210 cells (2.5×10^5 cells mL^{-1}) were incubated for 21 h with various concentrations of the compounds, then fixed by 70% EtOH (v/v), washed and incubated in PBS containing 100 μg mL^{-1} RNAse and 25 μg mL^{-1} propidium iodide for 30 min at 20 °C. For each sample, 10^4 cells were analysed on a XL/MCL flow cytometre (Beckman Coulter). The fluorescence of propidium iodide was collected through a 615 nm long-pass filter.

4.5. Antibiogram tests

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). 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 **2**–**10** in DMSO (300 μg of drug per disk) were placed on Petri dishes. Growth inhibition was examined after 24 h incubation at 27 °C.

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