Antimicrobial Activities of Indolocarbazole and Bis-indole Protein Kinase C Inhibitors

II. Substitution on Maleimide Nitrogen with Functional Groups Bearing a Labile Hydrogen

ELISABETE RODRIGUES PEREIRA, SERGE FABRE, MARTINE SANCELME and MICHELLE PRUDHOMME*

Université Blaise Pascal, Laboratoire de Chimie Organique Biologique, URA 485, 63177 Aubière, France

MARYSE RAPP

Unité INSERM U71, Rue Montalembert, 63005 Clermont-Ferrand, France

(Received for publication February 3, 1995)

New compounds, structurally related to the potent protein kinase C inhibitor staurosporine, and substituted on the imide nitrogen with a functional group bearing a labile hydrogen (hydroxymethyl, amino, hydroxy), were synthesized. Their *in vitro* inhibitory potencies towards protein kinase C and protein kinase A showed that *N*-hydroxymethyl and *N*-hydroxy substitution, unlike alkyl substitution, can provide efficient protein kinase C inhibitors. The antimicrobial activities of these new compounds against *Streptomyces chartreusis* and *Streptomyces griseus*, *Bacillus cereus*, *Escherichia coli*, *Candida albicans* and *Botrytis cinerea* were examined. They proved to be inactive against *E. coli* and two fungi. The results suggest that there is no link between *in vitro* inhibition of protein kinase C and inhibition of growth and sporulation of the two *Streptomyces* tested. Unlike indolocarbazole maleimides, bis-indole maleimides are active against the two *Streptomyces* species.

In a previous paper[1], we reported the antimicrobial activities against *Streptomyces chartreusis* and *Streptomyces griseus*, *Bacillus cereus*, *Escherichia coli*, *Candida albicans* and *Botrytis cinerea* of compounds structurally related to the protein kinase C (PKC) inhibitors staurosporine[2,3] and K-252a[4] (Fig. 1). As these two potent PKC inhibitors prevent the sporulation of different *Streptomyces* species[5], this phenomenon was also examined on structurally related analogues. No link could be found between the growth and sporulation of the two *Streptomyces* tested and the inhibitory potencies against the enzyme. No evident structure-activity relationships could be demonstrated for the antimicrobial activities.

Among the compounds exhibiting an antibacterial activity against the strains of *Streptomyces* tested, **1** (Fig. 2) substituted on the imide nitrogen with a hydroxymethyl group was found to inhibit PKC in spite of the flexibility of its framework leading usually to inactive compounds[1,6]. Moreover, while substitution on the imide nitrogen with a methyl or a benzyl group had previously been found to induce inactivity against PKC[7,8], substitution with a hydroxymethyl group led to an inhibitory effect against PKC. AKINAGA *et al.*[9] prepared K-252a derivatives. Of these, KT6124 (Fig. 1) substituted with an amino group on the imide nitrogen exhibits PKC inhibitory effect *in vitro*.

Accordingly, we investigated the syntheses, inhibitory potencies against PKC and PKA, and antimicrobial activities against the same microorganisms, of new compounds in these series, **2**~**7**, substituted on the imide nitrogen with functional groups bearing a labile hydrogen (Fig. 2). Their frameworks are stiffer than that of **1** and were thus expected to provide a stronger PKC inhibitory effect.

Results and Discussion

The starting material for the synthesis of compounds **1**~**3**, *N*-substituted with a hydroxymethyl group, was bis-indolyl-*N*-benzyloxymethylmaleimide **8** (Fig. 3) prepared from *N*-benzyloxymethyldibromomaleimide and indolyl-MgBr.[6,10] Hydrogenolysis of **8** using palladium hydroxide in ethyl acetate gave **2** in 50% yield. Photooxidation of **8** in acetonitrile with a medium pressure mercury lamp (400 W) afforded indolocarbazole **9** in 58% yield. Hydrogenolysis of **9** on Pd/C in ethanol yielded **3** (48% yield).

Fig. 1. Microbial PKC inhibitors.

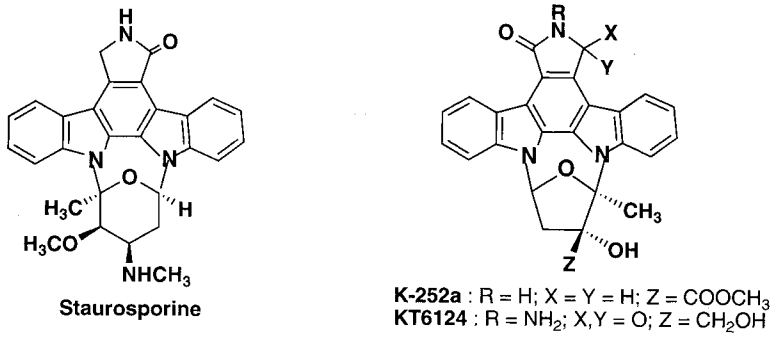


Fig. 2. Synthetic analogs of K-252c substituted on the imide nitrogen with functional groups bearing a labile hydrogen.

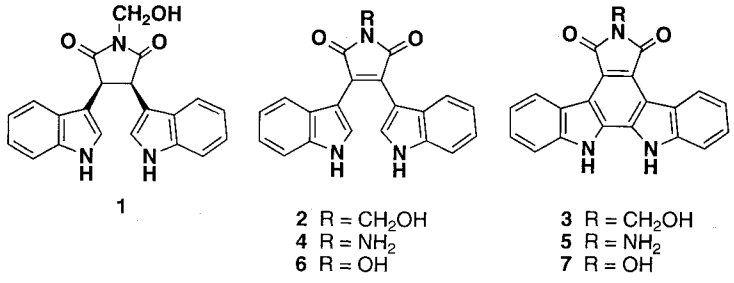


Fig. 3. Synthetic scheme for compounds **2** and **3**.

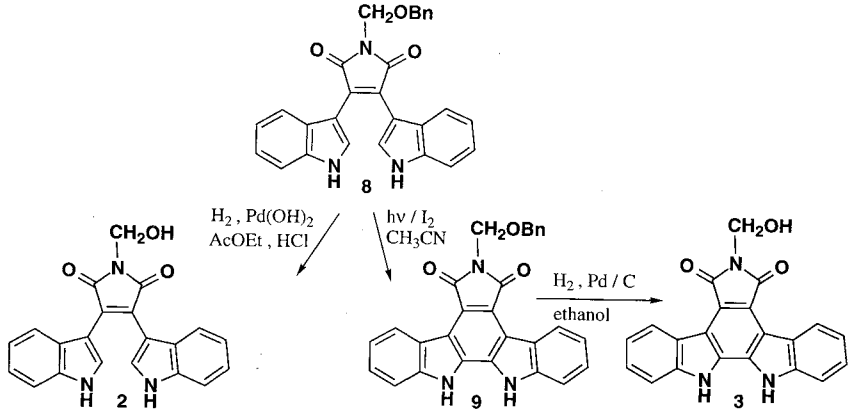
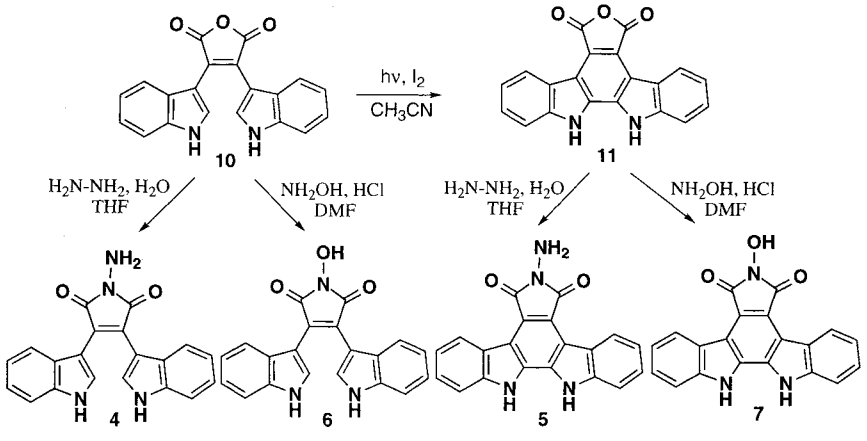


Fig. 4. Synthetic scheme for compounds **4**～**7**.



N-amino and *N*-hydroxy derivatives **4**~**7** were prepared from anhydrides **10** and **11** (Fig. 4), using hydrazine hydrate in refluxing tetrahydrofuran for the *N*-amino substituted compounds **4** and **5**, hydroxylamine hydrochloride and *N*,*N*-diisopropylethylamine in refluxing dimethylformamide for the *N*-hydroxy substituted compounds **6** and **7**. Bis-indoles **4** and **6** were obtained in quantitative yields, indolocarbazoles **5** and **7** in 60% yield.

Compounds **4** and **5** were found to be very unstable in the presence of acetone. They were readily converted to the corresponding imines (see experimental section: IR, ^{1}H and ^{13}C NMR data for imine **12** obtained from **4**). This reaction unequivocally confirms the structures of **4** and **5** as *N*-amino compounds and not cyclic 6-membered ring hydrazides sometimes claimed to be the normal products of hydrazinolysis of cyclic imides such as phtalimide[11].

The inhibitory potencies of **1**~**7** towards PKC and PKA were determined using histones IIIs and IIa respectively as substrates. Isoquinoline sulfonamide inhibitor H-7 was used as reference[12]. IC_{50} values are reported in Table 1. For the biological tests, **1**~**7** were dissolved in dimethylsulfoxide. All compounds proved to be stable in this solvent.

Of the *N*-hydroxy and *N*-amino maleimides **5**~**7**, only *N*-hydroxy bis-indole **6** was an efficient PKC inhibitor.

As expected from the preliminary results obtained for **1**[6], the more rigid maleimide **2** was much more active. Compound **3**, bearing a maleimide heterocycle and an indolocarbazole framework, was inactive towards PKC. This agrees with the weaker inhibitory properties of maleimides *versus* maleamides in the indolocarbazole series observed previously[6].

Our *N*-substituted compounds, except **1**, have lower or similar PKC inhibitory potencies compared with the parent *N*-unsubstituted compound.[6] A substitution with an alkyl group on the imide nitrogen leads to inactivity, substitution with a hydroxymethyl, a hydroxy or an amino group can afford PKC inhibitors.

The antimicrobial activities of **1**~**7** against the microorganisms previously cited were tested. The inhibitions of growth and aerial mycelium formation were examined on the two *Streptomyces* species (Table 1) and compared to the inhibitions induced by staurosporine.

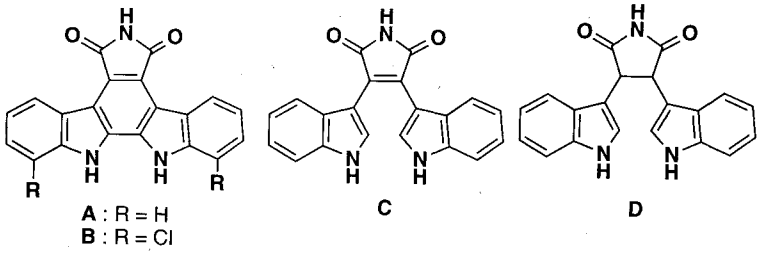
Compounds **1**~**7** had no growth inhibitory effect against two fungi (*B. cinerea* and *C. albicans*) and against *E. coli*. **2**, **6** and **1**, the strongest PKC inhibitors were active against the three Gram-positive bacteria tested. **4** (a very weak PKC inhibitor) was also active but **7**, exhibiting a stronger inhibitory effect on PKC than **4**, had no antibacterial activity.

Table 1. Inhibitory potencies of compounds **1**~**7** against protein kinase C (PKC) and protein kinase A (PKA) (IC_{50} μM); IC_{50} values for the reference H-7 were respectively 15 μM (PKC) and 7.5 μM (PKA).

Products	IC_{50} PKC (μM)	IC_{50} PKA (μM)	*S. chartreusis* NRRL 11407		*S. griseus* ATTC 23345		*E. coli* ATCC 11303	*B. cereus* ATCC 14879	*C. albicans* IP 444	*B. cinerea* DSM 877
			g.	sp.	g.	sp.				
1	16	>100	+++	+++	++	++	−	+	−	−
2	0.5	15.5	++++	+++	+++	+++	−	+++	−	−
3	>150	>150	+	−	±	−	−	±	−	±
4	108	117	++	+++	++	+	−	+	−	−
5	43.5	44.6	−	−	−	−	−	−	−	−
6	5	24.5	++	−	+++	+++	−	+	−	±
7	79.2	41.5	−	−	−	−	−	−	−	−

In vitro growth inhibitory effect against different microorganisms. For the two *Streptomyces* species: g. means inhibition of growth, sp. means inhibition of sporulation. (++++), (+++), (++), (+), (±) and (−) mean very strong, strong, medium, weak , very weak and no activity. The size of zones of growth inhibition was >15 mm (++++), 12~15 mm (+++), 10~12 mm (++), 7~9 mm (+), 6~7 mm (±). Data for staurosporine: *S. chartreusis* g. +, sp. +++, *S. griseus* g. ±, sp. ++.

Fig. 5. Structures of compounds A, B, C and D.



As previously reported[1], no evident correlation could be observed between the inhibitory potency towards PKC and the antimicrobial activity against the Gram-positive bacteria tested. Since staurosporine and K-252a inhibit the phosphorylation of several cell proteins in *S. griseus*, it seemed reasonable to correlate the inhibition of protein phosphorylation to the inhibition of sporulation[5]. Our results suggest that the inhibition of sporulation is not necessarily linked to the inhibition of *Streptomyces* protein phosphorylation by PKC. It may be induced *via* other pathways. OCHI *et al.*[13] reported a possible role of ADP-ribosyltransferase in the sporulation of *S. griseus*. ICHIMURA *et al.*[14] correlated the antitumor activity of duocarmycins to their antimicrobial activity, probably due to their ability to inhibit enzymes that damage DNA.

Some substances possessing PKC inhibitory potencies *in vitro* are inactive against the enzyme *in vivo*. This is so for KT 6124, a K-252a derivative substituted on the imide nitrogen with an amino group[9]. **1**, **2** and **6** are efficient PKC inhibitors *in vitro* and exhibit antibacterial activity against the *Streptomyces* tested, but their inhibitory effect towards PKC in intact cells remains to be investigated. Even if they prove to be active against the enzyme *in vivo*, their antibacterial activity is not necessarily due to their PKC inhibitory effect.

In conclusion, these results extend our knowledge of the structure-activity relationships in the PKC field. The bis-indoles **1**, **2**, **4** and **6**, are active against the three Gram-positive bacteria tested, while the indolocarbazoles **3**, **5** and **7** are not. This agrees with our previous findings for maleimide structures (Fig. 5). **A** and **B** have no antibacterial effects while **C** and **D** are very efficient both on growth and sporulation of the *Streptomyces* species tested.

Experimental

Chromatographic purifications were performed with flash Geduran SI 60 (Merck) 0.040~0.063 mm.

Histones IIIs and IIa, phosphatidylserine and diacylglycerol were purchased from Sigma, [γ^{32}P]ATP was from Amersham. Protein kinase A was purchased from Sigma and protein kinase C from Calbiochem.

3,4-Bis(indol-3-yl)-1-hydroxymethyl-3-pyrrolin-2,5-dione **2**

Compound **8** (100 mg; 0.22 mmol) in AcOEt (100 ml) containing 0.5 N HCl (3 ml) was hydrogenated for 3 hours (50 psi) in the presence of catalytic amounts of $Pd(OH)_2$. After filtration, the solvent was removed. Purification of the residue by flash chromatography (eluent cyclohexane-AcOEt 50:50) afforded **2** as a red solid (39 mg; 0.11 mmol; 50% yield).

IR (KBr): $\nu_{C=O}$ 1710 cm^{-1}; ν_{NH} and ν_{OH} 3250~3450 cm^{-1}; mp 112~115°C. HRMS (EI) calcd for $C_{21}H_{15}O_3N_3$: 357.1113, found 357.1092.

^{1}H NMR (400 MHz, acetone-d_6): 2.95 (1H, s, OH), 5.16 (2H, s, CH_2OH), 6.63 (2H, t, J=7.0 Hz), 6.93 (2H, d, J=7.0 Hz), 6.98 (2H, t, J=7.0 Hz), 7.41 (2H, d, J=7.0 Hz), 7.88 (2H, d, J=3.4 Hz), 10.85 (2H, s, N_{indole}-H).

^{13}C NMR (100 MHz, acetone-d_6): 60.7 (CH_2OH), 111.5, 119.6, 121.4, 121.9, 129.2 (C tert.); 106.5, 125.9, 127, 8 (C quat.); 171.3 (C=O).

6-Benzyloxymethyl-5,7-dioxo-(5*H*)-6,7,12,13-tetrahydro-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole **9**

8 (630 mg; 1.41 mmol) in acetonitrile (150 ml) in the presence of a crystal of iodine was irradiated for 5 hours with a medium pressure mercury lamp (400 W). After evaporation of the solvent, the residue was purified by flash chromatography (eluent cyclohexane-AcOEt 20:80) on silica gel previously impregnated with triethylamine, filtered off, then washed with AcOEt and dried by evaporation under reduced pressure. **9** was obtained (365 mg; 0.82 mmol; 58% yield) as a yellow fluorescent powder.

IR (KBr): $\nu_{C=O}$ 1745 cm^{-1}; ν_{NH} 3360 cm^{-1}; mp 270~275°C. HRMS (EI) calcd for $C_{28}H_{19}O_3N_3$: 445.1426, found 445.1395.

^{1}H NMR (300 MHz, DMSO-d_6): 4.68 (2H, s, CH_2), 5.19 (2H, s, CH_2), 7.20~7.40 (7H, m), 7.55 (2H, t, J=7.8 Hz), 7.80 (2H, d, J=7.8 Hz), 8.98 (2H, d, J=7.8 Hz), 11.80 (2H, s, N_{indole}-H).

^{13}C NMR (75.47 MHz, DMSO-d_6): 66.6 (CH_2); 70.3 (CH_2), 112.1, 120.3, 124.2, 126.9, 127.5 (several C), 128.2 (C tert.), 115.6, 118.4, 121.4, 125.0, 129.2, 137.8, 140.3 (C quat.), 169.4 (C=O).

6-Hydroxymethyl-5,7-dioxo-(5*H*)-6,7,12,13-tetrahydro-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole **3**

9 (0.1 g; 0.224 mmol) in ethanol (30 ml) in the presence of catalytic amounts of Pd/C was hydrogenated for 2 hours (10 psi). After filtration, the residue was washed with THF and the filtrate evaporated. Purification by flash chromatography (eluent cyclohexane-AcOEt 50:50) yielded **3** as a yellow powder (38 mg; 0.107 mmol; 48% yield).

IR (KBr): $\nu_{C=O}$ 1740 cm^{-1}; ν_{NH} and ν_{OH} 3100~3400 cm^{-1}; mp >300°C; Mass (EI) M^+ 355 (12%).

^{1}H NMR (300 MHz, DMSO-d_6): 4.96 (2H, d, J=6.4 Hz, CH_2OH), 6.28 (1 H, t, J=6,4 Hz, CH_2OH), 7.32 (2H, t , J=8 Hz), 7.51 (2H, t, J=8 Hz), 7.75 (2H, d, J=8 Hz), 8.93 (2H, d , J=8 Hz), 11.71 (2H, s, N_{indole}-H).

^{13}C NMR (75.47 MHz, DMSO-d_6): 59.7 (CH_2), 112.0, 120.2, 124.1, 126.8 (C tert.), 115.5, 118.6, 121.4, 129.0, 140.3 (C quat.), 169.1 (C=O).

1-Amino-3,4-bis(3-indolyl)-3-pyrrolin-2,5-dione **4**

To anhydride **10** (100 mg; 0.30 mmol) in THF (20 ml)

was added H_2N-NH_2, H_2O (76 mg; 1.52 mmol; 0.074 ml). The mixture was warmed to 40~50°C for 15 minutes. The solvent was removed and the residue poured into water and extracted with ether. The organic phase was washed with brine, and dried over $MgSO_4$. After removal of the solvent, **4** was obtained as a red powder (104 mg; 0.30 mmol; 100% yield). HRMS (EI) calculated for $C_{20}H_{14}N_4O_2$ 342.1116, found 342.1117.

IR (KBr): ν_{NH} and ν_{NH_2} 3400 cm^{-1} $\nu_{C=O}$ 1710 cm^{-1}; mp >310°C.

^1H NMR (400 MHz, DMSO-d_6): 4.9 (2H, s, N-NH_2), 6.68 (2H, t, J=7.7 Hz), 6.84 (2H, d, J=7.7 Hz), 7.03 (2H, t, J=7.7 Hz), 7.42 (2H, d, J=7.7 Hz), 7.81 (2H, d, J=2.3 Hz), 10.95 (2H, s, N_{indole}-H).

^{13}C NMR (100 MHz, DMSO-d_6): 105.7, 125.3 (2C), 135.9 (C quat.), 111.7, 119.3, 120.8, 121.6, 129.1 (C tert.), 170.8 (C=O).

1-Hydroxy-2,3-bis(3-indolyl)-3-pyrrolin-2,5-dione **6**

Anhydride **10** (300 mg; 0.91 mmol) was dissolved in DMF (20 ml). NH_2OH, HCl (317 mg; 4.57 mmol) and *N,N*-diisopropylethylamine (590 mg; 4.57 mmol; 0.8 ml) were added. The mixture was warmed at 90~100°C for 30 minutes. After identical work-up as for **4**, purification by flash chromatography (eluent - AcOEt) yielded **6** as a dark red powder (312 mg; 0.91 mmol; 99% yield).

IR (KBr): ν_{NH} and ν_{OH} 3390 cm^{-1}, $\nu_{C=O}$ 1710 cm^{-1}; mp 160°C. HRMS (EI) calcd for $C_{20}H_{13}N_3O_3$ 343.0957, found 343.0956.

^1H NMR (400 MHz, acetone-d_6): 6.65 (2H, t, J=7.7 Hz), 6.94 (2H, d, J=7.7 Hz), 6.99 (2H, t, J=7.7 Hz), 7.42 (2H, d, J=7.7 Hz), 7.81 (2H, d, J=2.8 Hz), 9.55 (1H, s, N-OH), 10.85 (2H, s, N_{indole}-H).

^{13}C NMR (100 MHz, acetone-d_6): 106.4, 125.1, 125.8, 136.3 (C quat.), 111.6, 119.6, 121.4, 121.9, 129.2 (C tert.), 168.5 (C=O).

6-Amino-6,7,12,13-tetrahydro-5,7-dioxo-indolo[2,3-a]-pyrrolo[3,4-c]-carbazole **5**

To anhydride **11** (100 mg; 0.30 mmol) in THF (20 ml) was added hydrazine monohydrate (0.076 g; 1.53 mmol; 0.075 ml). The mixture was refluxed for 4 hours. The solvent was removed and the residue washed with water. After filtration, the solid was washed with petroleum ether; **5** was obtained as a yellow powder (0.055 g; 0.16 mmol; 53% yield).

IR (KBr): ν_{NH} and ν_{NH_2} 3330 cm^{-1} $\nu_{C=O}$ 1700 cm^{-1}; mp >305°C. Mass (EI) M^+ 340 (50%).

^1H NMR (400 MHz, DMSO-d_6): 5.0 (2H, s, N-NH_2), 7.41 (2H, t, J=7.9 Hz), 7.60 (2H, d, J=7.9 Hz), 7.86 (2H, d, J=7.9 Hz), 9.06 (2H, d, J=7.9 Hz), 11.8 (2H, s, N_{indole}-H).

^{13}C NMR (100 MHz, DMSO-d_6): 114.9, 116.0, 122.0, 128.7, 140.4 (C quat.), 112.1, 120.3, 124.2, 126.9 (C tert.), 168.8 (C=O).

6-Hydroxy-6,7,12,13-tetrahydro-5,7-dioxo-indolo-[2,3-a]-pyrrolo[3,4-c]-carbazole **7**

To anhydride **11** (100 mg; 0.30 mmol) in DMF (20 ml) were added $NH_2OH \cdot HCl$ (106 mg; 1.53 mmol) and *N,N*-diisopropylethylamine (198 mg; 1.53 mmol; 0.26 ml). The mixture was warmed at 85°C for 1 hour then poured into water and extracted with AcOEt. The organic phase was washed with brine and dried over $MgSO_4$. After removal of the solvent and purification by flash chromatography (eluent - AcOEt) compound **7** was isolated as an orange solid (66 mg; 0.19 mmol; 63% yield).

IR (KBr): ν_{NH} and ν_{OH} 3400 cm^{-1}, $\nu_{C=O}$ 1695 cm^{-1}; mp >315°C. HRMS (EI) calcd for $C_{20}H_{11}N_3O_3$ 341.0800, found 341.0844.

^1H NMR (400 MHz, DMSO-d_6): 7.39 (2H, t, J=7 Hz), 7.59 (2H, t, J=7 Hz), 7.85 (2H, d, J=7 Hz), 8.99 (2H, d, J=7 Hz), 10.6 (1H, s, N-OH), 11.85 (2H, s, N_{indole}-H).

^{13}C NMR (100 MHz, DMSO-d_6): 115.3, 115.7, 121.3, 129.1, 140.4 (C quat.), 112.1, 120.3, 124.1, 127.0 (C tert.), 166.6 (C=O).

1-(*N*-isopropyliden-amino)-3,4-bis(3-indolyl)-3-pyrrolin-2,5-dione **12**

IR (KBr): ν_{NH} 3400 cm^{-1}, $\nu_{C=O}$ and $\nu_{C=N}$ 1690 and 1700 cm^{-1}; mp >300°C.

^1H NMR (400 MHz, DMSO-d_6): 2.05 (3H, s, CH_3), 2.40 (3H, s, CH_3), 6.73 (2H, t, J=7.5 Hz), 6.92 (2H, d, J=8 Hz), 7.06 (2H, t, J=7.5 Hz), 7.46 (2H, d, J=8 Hz), 7.88 (2H, d, J=1.4 Hz), 11.85 (2H, s, N_{indole}-H).

^{13}C NMR (100 MHz, DMSO-d_6): 20.6 and 24.8 (CH_3), 111.9, 119.5, 121.1, 121.8, 129.5 (C tert.), 105.6, 125.3, 126.4, 136.1 (C quat.), 168.1 (C=O); 178.0 (C=N).

Measurements of PKC and PKA Inhibition

Protein kinase C phosphorylation assays were performed in a reaction (80 μl) mixture containing histone IIIs (2.4 mg/ml), $MgCl_2$ (10 mM), $CaCl_2$ (0.1 mM), phosphatidylserine (10 mg/ml), diacylglycerol (10 mg/ml), ATP (10 μM), [γ^{32}P]ATP (10^6 cpm/80 μl), Tris buffer (50 mM, pH 7.5), protein kinase C (0.5 μg/ml) and inhibitors at different concentrations.

Protein kinase A phosphorylation assays were performed in a reaction (80 μl) mixture containing histone IIa (1 mg/ml), $MgCl_2$ (5 mM), ATP (10 μM), [γ^{32}P]ATP (10^6 cpm/80 μl), Tris buffer (50 mM, pH 7.0), protein kinase A (1 μg/ml) and inhibitors at different concentrations.

For each kinase, reactions were run at 30°C for 12 minutes and stopped with trichloroacetic acid (12% w/v) in the presence of bovine serum albumin (0.9 mg) as a carrier protein. After centrifugation (10 minutes at 3000 rpm), the pellet was dissolved in 1 M NaOH and precipitated a second time with trichloracetic acid. Radioactivity incorporated into histones was counted by scintillation spectrometry (Tri-Carb 4530, Packard). All experiments were carried out in triplicate.

Antimicrobial Tests

Six strains were tested: 3 Gram-positive bacteria (*B. cereus* ATCC 14879, *S. chartreusis* NRRL 11407, and *S. griseus* ATCC 23345), a Gram-negative bacterium (*E. coli* ATCC 11303), a yeast (*C. albicans* 444 from Pasteur Institute) and a filamentous fungus (*B. cinerea* DSM 877). Antibacterial activity was determined by the conventional paper disk (Durieux, N° 268, 6 mm in diameter) diffusion method using the following nutrient media: Mueller Hinton for *B. cereus* and *E. coli*, Sabouraud agar (Difco) for *C. albicans* and *B. cinerea* and Emerson agar (0.4% beef extract, 0.1% yeast extract, 0.4% peptone, 1% dextrose, 0.25% NaCl, 2% agar, pH 7.0 (Difco)) for the *Streptomyces* species. Growth inhibition was examined after incubation for 24 hours at 27°C (37°C for *E. coli*). Inhibition of sporulation was examined 3 to 5 days later. Products **1**~**7** were dissolved in DMSO and a paper disk containing each of the products (300 μg) was placed on agar plates.

References

1) SANCELME, M.; S. FABRE & M. PRUDHOMME: Antimicrobial activities of indolocarbazole and bis-indole protein kinase C inhibitors. J. Antibiotics 47: 792~798, 1994
2) TAMAOKI, T.; H. NOMOTO, I. TAKAHASHI, Y. KATO, M. MORIMOTO & F. TOMITA: Staurosporine, a potent inhibitor of phopholipid/Ca^{++} dependent protein kinase. Biochem. Biophys. Res. Commun. 135: 397~402, 1986
3) FUNATO, N.; H. TAKAYANAGI, Y. KONDA, Y. TODA, Y. HARIGAYA, Y. IWAI & S. OMURA: Absolute configuration of staurosporine by X-ray analysis. Tetrahedron Lett. 35: 1251~1254, 1994
4) KASE, H.; K. IWAHASHI & Y. MATSUDA: K-252a, A potent inhibitor of protein kinase C from microbial origin. J. Antibiotics 39: 1059~1065, 1986
5) HONG, S. K.; A. MATSUMOTO, S. HORINOUCHI & T. BEPPU: Effects of protein kinase inhibitors on *in vitro* protein phosphorylation and cellular differenciation of *Streptomyces griseus*. Mol. Gen. Genet. 236: 347~354, 1993
6) FABRE, S.; M. PRUDHOMME & M. RAPP: Protein kinase C inhibitors; Structure-activity relationships in K-252c related compounds. BioMed. Chem. 1: 193~196, 1993
7) TOULLEC, D.; P. PIANETTI, H. COSTE, P. BELLEVERGUE, T. GRAND-PERRET, M. AJAKANE, V. BAUDET, P. BOISSIN, E. BOURSIER, F. LORIOLLE, L. DUHAMEL, D. CHARON & J. KIRILOVSKY: The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. J. Biol. Chem. 266: 15771~15781, 1991
8) KLEINSCHROTH, J.; C. SCHÄCHTELE, J. HARTENSTEIN & C. RUDOLPH: European Patent, 0 410 389 A1, 1991
9) AKINAGA, S.; T. ASHIZAWA, K. GOMI, H. OHNO, M. MORIMOTO, C. MURAKATA & M. OKABE: Antitumor effect of KT 6124, a novel derivative of protein kinase C inhibitor K-252a, and its mechanism of action. Cancer Chemother. Pharmacol. 29: 266~272, 1992
10) KANEKO, T. & H. WONG: Two synthetic approaches to rebeccamycin. Tetrahedron Lett. 26: 4015~4018, 1985
11) See *Beilstein*, 24, 371
12) RICOUART, A.; J. C. GESQUIÈRE, A. TARTAR & C. SERGHERAERT: Design of potent protein kinase inhibitors using the bisubstrate approach. J. Med. Chem. 34: 73~78, 1991
13) OCHI, K.; A. PENYIGE & G. BARABAS: The possible role of ADP-ribosylation in sporulation and streptomycin production by *Streptomyces griseus*. Journal of General Microbiology 138: 1745~1750, 1992
14) ICHIMURA, M.; T. OGAWA, K. TAKAHASHI, A. MIHARA, I. TAKAHASHI & H. NAKANO: Interconversion and stability of duocarmycins, a new family of antitumor antibiotics: correlation to their cytotoxic and antimicrobial activities *in vitro*. Oncology Research 5: 165~171, 1993