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Studies on Protein Kinase C Inhibitors; Structure–Activity Relationships in Indolocarbazole and bis-Indole Series

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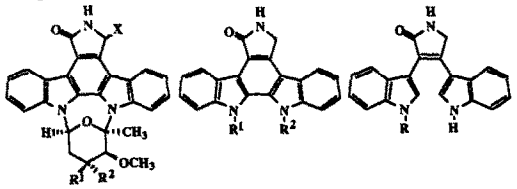
Abstract–To assess the role of the cyclic amide moiety in indolocarbazole and bis-indole protein kinase C inhibitors, five amides in these series were synthesized in which the amide group is acyclic. These new compounds have no inhibitory effect on protein kinase C, indicating that the rigid cyclic structure of the amide group is compulsory for biological activity.

Introduction

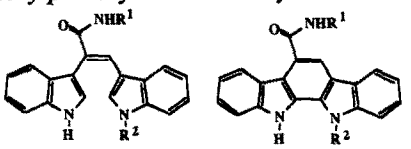
The activation of protein kinase C (PKC) is involved in signal transduction for a variety of biologically active substances that activate cell functions and proliferation.[1–4] Among the various PKC inhibitors, staurosporine, related bacterial metabolites and synthetic aglycone analogues (Scheme I) have been shown to be potent inhibitors interacting with the ATP binding site.



Staurosporine[5]: X=R2=H; R1=NHCH3 K252c[9,10] R1=R2=H B[12] R=H
TAN-1030A[6]: X=H, R1, R2= =NOH A[11] R1=-(CH2)2-CN; R2=CH3 C[12] R=-(CH2)3N(CH3)2
RK-286C[7]: X=R2=H; R1=OH
UCN-01 and 02[8] X=OH; R2=H, R1=NHCH3

Scheme I.

To investigate structure–activity relationships in PKC inhibitors, and to assess the role of the amide heterocycle, five analogues with cleaved heterocycles were synthesized. Their structures are presented in Scheme II. In compounds **1–5**, the amide function is conserved without the rigid cyclic structure of the indolocarbazole or bis-indole frameworks. In compounds **3** and **5**, one of the hydrogen atoms attached to the nitrogen atom of the indole ring is substituted by a methyl group. Since the replacement of the imide NH by NCH_3 in bis-indolyl maleimides destroys activity,[12] only primary and secondary amides were studied.

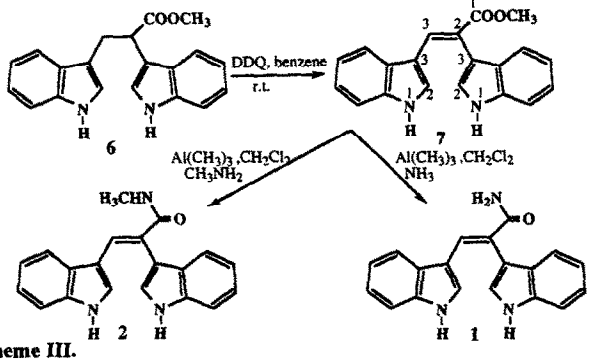


1 R^1 = H; R^2 = H
2 R^1 = CH_3; R^2 = H
3 R^1 = CH_3; R^2 = CH_3
4 R^1 = CH_3; R^2 = H
5 R^1 = CH_3; R^2 = CH_3

Scheme II.

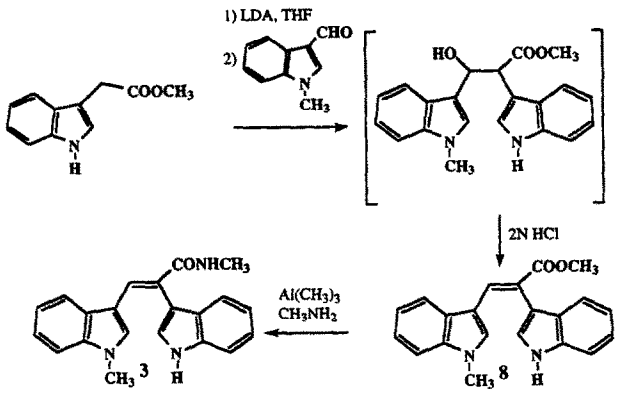
Results and Discussion

Both types of structures were prepared from methyl 2,3-bis(3-indolyl)propionate **6**, easily accessible from methyl 3-indolyl-acetate and 3-indolyl-methylene-trimethyl-ammonium iodide.[13] For the synthesis of **1** and **2**, dehydrogenation of **6** was performed in benzene using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) yielding **7**. Mild aminolysis of ester **7** to amides **1** and **2** was carried out in methylene chloride with ammonia or methylamine previously complexed with trimethylaluminium according to a method proposed by Basha *et al.*[14] for the aminolysis of a variety of esters (Scheme III).



Scheme III.

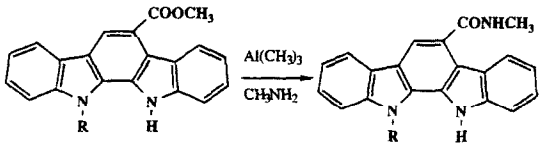
The bis indole **3** was synthesized as follows: coupling of 3-formyl-*N*-methylindole[15] and methyl 3-indoleacetate followed by dehydration of the intermediate alcohol during acid work up gave ester **8**. Aminolysis of **8** using methylamine in the same conditions as previously described yielded **3** (Scheme IV).



Scheme IV.

Aminolysis of **8** using the same procedure as employed in the synthesis of **1** failed, which could result from the deactivation of the ester by the replacement of the nitrogen proton of the indole ring with a methyl group.

Indolocarbazoles **4** and **5** were obtained respectively from **6** and **8** by aminolysis of intermediates **9** and **10** (Scheme V). **9** Was prepared from **6** by treatment with *t*-butyl hypochlorite.[13] Aromatization of **8** by heating in benzene in the presence of DDQ and *p*-toluene sulphonic acid (*p*-TsOH) afforded **10**.



9 R = H
10 R = CH_3

4 R = H
5 R = CH_3

Scheme V.

The inhibitory potencies of **1–5** towards PKC and PKA were determined using histones IIIs and IIa respectively as substrates, according to the method described by Ricouart *et al.*[16] IC_{50} values are reported in Table 1, the isoquinoline sulfonamide H-7 was tested as reference.[17]

Table 1. Inhibitory potencies for compounds **1–5** (IC_{50} μM)

Compound	PKC	PKA
H7	9.1	3.3
K252c	2.45	2.57
A	0.020	> 100
B	2.5	32
C	2.75	> 10
1	> 100	> 100
2	> 100	> 100
3	> 100	> 100
4	> 100	> 100
5	> 100	> 100

These biological tests show that the opening of the amide heterocycle leads to inactive compounds. A rigid structure in this region is therefore necessary for PKC inhibitory activity. This is not the case in the aromatic region since bis-indole systems, which are more flexible than indolocarbazole ones, retain PKC inhibitory activity.

Experimental Section

All reactions were carried out under argon atmosphere using dry solvents. The solvents were dried according to known procedures. IR spectra were recorded on a Perkin-Elmer 881 spectrometer (ν in cm^{-1}), NMR spectra on a Bruker MSL 300 (^1H: 300 MHz, ^{13}C: 75.45 MHz) (chemical shifts δ reported in ppm relative to the solvents $CHCl_3$, DMSO or acetone, the following abbreviations are used: singlet (s), doublet (d), triplet (t), pseudo-triplet (pt), multiplet (m), tertiary carbons (Ctert.), quaternary carbons (Cquat.)). Mass spectra (EI and FAB+) were determined at the Service Central d'Analyses, CNRS (Vernaison) on a VG.ZAB SEQ and at CESAMO (Talence) on a high resolution FISONS Autospec-Q spectrometer. Chromatographic purifications were performed using a flash Geduran SI 60 (Merck) 0.040–0.063 mm and silicagel plates (Kieselgel 60 F_{254} Merck).

Methyl 2,3-bis(3-indolyl)-propenoate (7)

A mixture of methyl 2,3-bis(3-indolyl)propionate (**6**) (1.50 g; 4.71 mmol) in benzene (20 mL) was added to a solution of DDQ (1.16 g; 5.1 mmol) in benzene (30 mL). After stirring at room temperature overnight, the solvent was removed. The residue was dissolved in ethyl acetate, washed with saturated aqueous $NaHSO_3$ solution and brine. Purification by flash chromatography (AcOEt/cyclohexane 80:20) led to **7** as a white powder (744 mg; 2.35 mmol; 50% yield). IR $\nu_{C=O}$ 1680, ν_{NH} 3400; m.p. 78–80 °C. Mass m/z M^+ 316 (100%); exact mass calculated for $C_{20}H_{16}N_2O_2$: 316.1252, found 316.1245.

^1H NMR ($CDCl_3$): 3.82 (3H, s, CH_3); 6.32 (1H, d, J = 2.6 Hz); 7.01 (1H, t, J = 7.8 Hz); 7.09 (1H, d, J = 2.6 Hz); 7.15–7.40 (6H, m); 7.84 (1H, t, J = 4.8 Hz); 8.05 (1H, s); 8.28 (1H, s); 8.44 (1H, s).

^{13}C NMR ($CDCl_3$): 52.2 (CH_3); 111.2; 111.3; 113.7; 118.6; 119.4; 119.8; 120.6; 120.8; 121.9; 122.4; 122.7; 124.1; 125.8; 127.0; 127.7; 133.6; 135.1; 136.2; 169.6 (C=O).

2,3-Bis(3-indolyl)-2-propenamide (1)

Ammonia was bubbled for 15 min into a solution of 2N $Al(CH_3)_3$ in hexane (3.2 mL; 6.3 mmol) in CH_2Cl_2 (20 mL). After evaporation of excess ammonia, **7** (1 g; 3.17 mmol) in CH_2Cl_2 (10 mL) was added and the solution was refluxed for 48 h. After cooling, HCl 0.7 M (5.7 mL) was added dropwise. After stirring for 30 min, the aqueous phase was separated and extracted with AcOEt (3 x 40 mL). The organic phase was washed with brine and dried over $MgSO_4$. After evaporation of the solvent and purification by flash chromatography (AcOEt/cyclohexane 7:3) **1** was obtained as a pale yellow foam (400 mg; 1.32 mmol; 42% yield). IR $\nu_{C=O}$ 1660, ν_{NH} 3180, 3400; m.p. 128–130 °C. Mass m/z M^+ 301 (100%), exact mass calculated for $C_{19}H_{15}N_{30}O$: 301.1215, found 301.1214.

^1H NMR (DMSO-d_6): 6.21 (1H, d, J = 2.2 Hz); 6.41 (1H, broad s); 6.90 (1H, pt, J = 7 Hz); 7.00–7.15 (5H, m); 7.29 (1H, dd, J = 2.6 and 6.4 Hz); 7.34 (1H, d, J = 2.6 Hz); 7.48 (1H, d, J = 7 Hz); 7.67 (1H, dd, J = 2.6 and 6.4 Hz); 8.09 (1H, s); 11.10 (1H, s, NH); 11.35 (1H, s, NH).

^{13}C NMR(DMSO-d_6): 111.6; 111.8; 117.3; 118.9; 119.2; 119.8; 121.3; 121.8; 124.3; 125.7; 128.4 (Ctert.); 110.8; 111.1; 121.1; 125.5; 127.5; 135.6; 136.5 (Cquat.); 169.5 (C=O).

2,3-Bis(3-indolyl)-N-methyl-2-propenamide (2)

CH_3NH_2 (0.11mL; 2.43 mmol) was added to a solution of 2N $Al(CH_3)_3$ in hexane (1.26 mL; 2.52 mmol) at -20 °C. The mixture was stirred for 20 min at -20 °C then allowed to reach room temperature. The intermediate **7** (700 mg; 2.21 mmol) in CH_2Cl_2 (15 mL) was added and the solution was refluxed for 24 h. A solution of 0.7 M HCl (4 mL) was added dropwise and stirring was continued for 30 min. After the same work up as for **1**, purification by flash chromatography (AcOEt/cyclohexane 7:3) yielded **2** as a white powder (412 mg; 1.31 mmol; 59% yield). IR $\nu_{C=O}$ 1660, ν_{NH} 3250, 3400; m.p. 162–163 °C. Mass m/z M^+ 315 (100%), exact mass calculated for $C_{20}H_{17}N_3O$: 315.1371, found 315.1313.

^1H NMR (acetone-d_6): 2.95 (3H, s, N–CH_3); 6.06 (1H, broad s, NH); 6.35 (1H, s); 6.91 (1H, pt, J = 7 Hz); 7.05–7.15 (4H, m); 7.19 (1H, d, J = 7 Hz); 7.34 (1H, m); 7.54 (1H, d, J = 7 Hz); 7.78 (1H, m); 8.47 (1H, s); 10.45 (1H, s, NH); 10.76 (1H, s, NH).

^{13}C NMR (methanol-d_4): 27.3 (N–CH_3); 112.5; 112.8; 119.0; 120.3; 120.7; 121.2; 123.1; 123.2; 125.5; 127.6; 131.7 (Ctert.); 112.0; 112.6; 121.8; 127.3; 129.3; 137.0; 138.5 (Cquat.); 172.4 (C=O).

Methyl 2-(3-indolyl)-3-(3-N-methylindolyl)-2-propenoate (8)

Methyl 3-indolyl-acetate (1.5 g; 7.93 mmol) in THF (25 mL) was poured into a solution of LDA (3.37g; 31.4 mmol) in THF (10 mL) at -78 °C. After stirring for 30 min, the temperature was allowed to reach -20 °C, the mixture was then cooled to -40 °C before adding *N*-methyl-3-formyl-indole (1.27g; 7.98 mmol). After warming to -20 °C, the solution was stirred at this temperature for 2 h, poured into 2N HCl and then extracted with AcOEt. The organic phase was washed with saturated aqueous $NaHCO_3$ solution and brine and dried over $MgSO_4$. After removal of the solvent and purification by flash chromatography (cyclohexane/AcOEt 60:40) **8** was obtained as a pale yellow powder (1.25g; 3.78mmol; 49% yield). IR $\nu_{C=O}$ 1680, ν_{NH} 3370: m.p. 173–175 °C. Mass m/z M^+ 330 (90%), exact mass calculated for $C_{21}H_{18}N_2O_2$: 330.1368, found 330.1368.

^1H NMR (acetone-d_6): 3.48 (3H, s, N–CH_3); 3.71 (3H, s, OCH_3); 6.52 (1H, s); 6.92 (1H, pt, J = 7 Hz); 7.15 (4H, m); 7.31 (2H, m); 7.49 (1H, d, J = 6.9 Hz); 7.72 (1H, d, J = 6.9 Hz); 8.34 (1H, s); 10.40 (1H, s, NH).

^{13}C NMR (acetone-d_6): 32.9 (NCH_3); 53.6 (OCH_3); 110.5; 112.2; 118.5; 118.9; 119.6; 120.5; 121.0; 121.8; 124.5; 129.1; 129.7 (Ctert.); 111.2; 118.8; 125.3; 126.8; 137.1; 137.3 (Cquat.); 169.3 (C=O).

2-(3-Indolyl)-3-(3-N-methylindolyl)-N-methyl-2-propenamide (3)

The same method as described for **2** was used, yielding, from **8** (700 mg; 2.12 mmol), compound **3** as a white powder (423 mg; 1.28 mmol; 60% yield). IR $\nu_{C=O}$ 1725, ν_{NH} 3200, 3420; m.p. 214–216 °C. Mass m/z M^+ 329 (100%), exact mass calculated for $C_{21}H_{19}N_3O$: 329.1528, found 329.1526.

^1H NMR (DMSO-d_6): 2.65 (3H, d, J = 4.5 Hz, N_{indole}–CH_3); 3.40 (3H, d, J = 7.4 Hz, NHCO$\underline{CH_3}$); 6.22 (1H, s); 6.90 (2H, m); 7.12 (4H, m); 7.35 (2H, m); 7.50 (1H, d, J = 7.9 Hz); 7.62 (1H, d, J = 7.9 Hz); 8.08 (1H, s, $NHCH_3$); 11.38 (1H, s, N_{indole}–H).

^{13}C NMR (DMSO-d_6): 26.6 (CONH$\underline{C}H_3$); 30.4 (N_{indole}–$\underline{C}H_3$); 110.0; 111.8; 118.1; 118.9; 119.1; 119.9; 121.3; 121.8; 124.8; 127.2; 129.5 (Ctert.); 110.2 (2C); 122.4; 125.7; 127.8; 135.8; 136.6 (Cquat.); 166.2 (C=O).

5-Methyloxycarbonyl-N-methylindolo[2,3-a]carbazole (10)

Compound **8** (200 mg; 0.60 mmol) in benzene (10 mL) was added to a solution of DDQ (175 mg; 0.77 mmol) in benzene (20 mL) and catalytic amounts of *p*-TsOH. The mixture was refluxed for 1 h. After identical work up as for **7**, chromatographic purification (cyclohexane/AcOEt 80:20) yielded **10** as an off-white powder (65 mg; 0.20 mmol; 30% yield). IR $\nu_{C=O}$ 1710, ν_{NH} 3400; m.p. 93–95 °C. Exact mass calculated for $C_{21}H_{16}N_2O_2$: 328.1212, found 328.1210.

^1H NMR (acetone-d_6): 2.98 (3H, s, N_{indole}–CH_3); 4.02 (3H, s, OCH_3); 7.22 (1H, pt, J = 6.9 Hz); 7.29 (1H, pt, J = 6.9 Hz); 7.41 (1H, pt, J = 6.9 Hz); 8.20 (1H, d, J = 6.9 Hz); 8.64 (1H, s); 9.00 (1H, d, J = 6.9 Hz); 10.98 (1H, s, NH).

^{13}C NMR (acetone-d_6): 32.2 (NCH_3); 52.0 (OCH_3); 110.1; 112.0; 117.6; 119.9; 120.7 (2C); 125.9; 126.1; 126.2 (Ctert.); 117.9; 122.6; 124.5; 141.3 (Cquat.); 169.4 (C=O).

5-(N-Methylcarbamoyl)-indolo[2,3-a]carbazole (4)

The procedure for the aminolysis was the same as described for the preparation of **2** yielding, from **9** (200 mg; 0.63 mmol), amide **4** as an off-white solid (55 mg; 0.17 mmol; 27%). IR $\nu_{C=O}$ 1660, ν_{NH} 3300; m.p. 275 °C. Mass m/z M^+ 313 (100%), exact mass calculated for $C_{20}H_{15}N_3O$: 313.1215, found 313.1214.

^1H NMR (DMSO-d_6): 2.98 (3H, d, J = 3.8 Hz, NCH_3); 7.16 (1H, pt, J = 7 Hz); 7.23 (1H, pt, J = 7 Hz); 7.38 (1H, pt, J = 7 Hz); 7.41 (1H, pt, J = 7 Hz); 7.70 (2H, pt, J = 7 Hz); 8.09 (1H, s); 8.18 (1H, d, J = 7 Hz); 8.40 (1H, d, J = 7 Hz); 8.49 (1H, d, J = 3.8 Hz, N$\underline{H}$–CH_3); 11.31 and 11.22 (2H, 2s, 2NH).

^{13}C NMR (DMSO-d_6): 30.7 (NH–CH_3); 111.6; 111.9; 112.1; 119.0; 119.6; 120.1; 123.5; 124.0; 124.9 (Ctert.); 117.4; 119.1; 123.1; 123.9; 126.1; 126.8; 128.3; 139.8; 151.8 (Cquat.); 170.5 (C=O).

5-(N-Methylcarbamoyl)-N-methylindolo-[2,3-a]carbazole (5)

Aminolysis of **10** (200 mg; 0.61 mmol) performed as described above gave **4** as an off-white solid (45 mg; 0.137 mmol; 22% yield). IR $\nu_{C=O}$ 1710, ν_{NH} 3250; m.p. 297–299 °C. Mass m/z $(M + H)^+$ 328 (100%), exact mass calculated for $C_{21}H_{17}N_3O$: 328.1450, found 328.1445.

^1H NMR (DMSO-d_6): 2.98 (3H, d, J = 4 Hz, $NHCH_3$); 3.41 (3H, s, N_{indole}–CH_3); 7.17 (1H, pt, J = 7 Hz); 7.25 (1H, pt, J = 7 Hz); 7.41 (1H, pt, J = 7 Hz); 7.46 (1H, pt, J = 7 Hz); 7.69 (2H, d, J = 7 Hz); 8.10 (1H, s); 8.21 (1H, d, J = 7 Hz); 8.34 (1H, d, J = 7 Hz); 8.10 (1H, s); 8.21 (1H, d, J = 7 Hz); 8.34 (1H, d, J = 7 Hz); 8.49 (1H, d, J = 4 Hz, $N\underline{H}CH_3$); 11.65 (1H, s, N_{indole}–H)

^{13}C NMR (DMSO-d_6): 26.3 ($NHC\underline{H}_3$); 30.4 (N_{indole}–CH_3); 109.5; 111.4; 111.6; 118.8; 119.3; 119.7; 122.9; 124.8; 124.9 (Ctert.); 118.2; 118.6; 122.1; 123.1; 123.7; 125.5; 127.6; 140.0; 140.6 (Cquat.); 170.2 (C=O).

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