Bioorganic & Medicinal Chemistry Vol. 1, No. 3, pp. 193-196, 1993
Printed in Great Britain

0968-0896/93 $6.00 + .00

Protein Kinase C Inhibitors; Structure–Activity Relationships in K252c-Related Compounds

Serge Fabre and Michelle Prudhomme*
Université Blaise Pascal, Laboratoire de Chimie Organique Biologique, URA 485, 63177 Aubière Cedex, France
Maryse Rapp
Unité INSERM U71, Rue Montalembert, 63005 Clermont-Ferrand, France

(*Received* 6 *May* 1993; *accepted* 16 *June* 1993)

Abstract–K252c-related compounds were synthesized with different framework flexibilities and different functions (imide, amide and amide–alcohol) on the non-indolic heterocycle. The inhibitory activities towards protein kinase C and protein kinase A are compared.

Introduction

In the past decade, a large number of widely diverse compounds have been found to inhibit protein kinase C (PKC) activity.[1] Staurosporine, structurally related metabolites and synthetic analogues have been shown to be potent PKC inhibitors interacting with the ATP binding site of the enzyme (Figure 1).

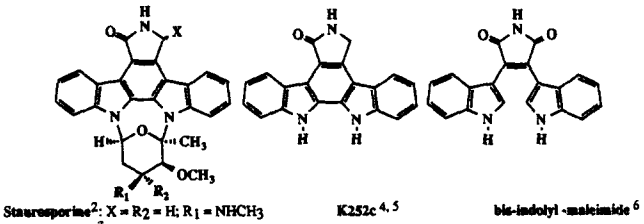
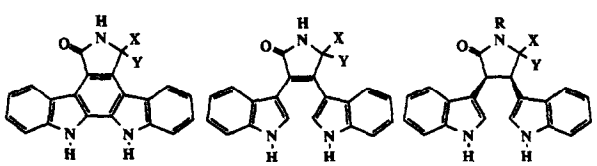


Figure 1.

In the course of structure–activity studies on PKC inhibitors in this series, we wished to determine if there was a link between the planarity of the structures and biological activity. Recent interest in this area prompted this paper.

We report here the synthesis and activity of PKC and PKA of compounds (Figure 2) in which (i) the functional groups on the non-indolic heterocyclic moiety are modified (X = Y = O; X = H, Y = OH; X = Y = H), and (ii) the rigidity of the framework is varied, from the most rigid indolocarbazoles **A** through bis-indolyl-maleimides **B** to the most flexible bis-indolyl-succinimides **C**.



A_1: X = Y = O
A_2: X = H; Y = OH
A_3: X = Y = H

B_1: X = Y = O
B_2: X = H; Y = OH
B_3: X = Y = H

C_1: X = Y = O; R = H
C_2: X = H; Y = OH; R = H
C_3: X = Y = H; R = H
C_4: X = Y = O; R = CH_2OH

Figure 2.

Results and Discussion

Brenner *et al.*[6] first synthesized the bis-indolyl-maleimide $\mathbf{B_1}$ by condensation of indolyl–MgBr on 2,3-dibromo-*N*-methyl-maleimide. Subsequently other routes were investigated by cyclization of the indole moieties via a bis Fischer indolisation.[7,8] Reduction of $\mathbf{B_1}$ using $LiAlH_4$ gave $\mathbf{B_2}$ (X = H; Y = OH) and $\mathbf{B_3}$ (X = Y = H).[9]

The indolocarbazole $\mathbf{A_1}$ was prepared from $\mathbf{B_1}$ by refluxing in toluene in the presence of *p*-toluenesulphonic acid (*p*-TsOH) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).[10] $\mathbf{A_3}$ was obtained from $\mathbf{A_1}$ by reduction with zinc amalgam.[10] $\mathbf{A_3}$ was also obtained by photocyclization of $\mathbf{B_3}$[11] and later by an intramolecular Diels–Alder reaction followed by a nitrene-mediated ring closure.[12]

We prepared $\mathbf{A_1}$ and then $\mathbf{A_2}$ and $\mathbf{A_3}$ by hemisynthesis from rebbecamycin,[5] a microbial metabolite possessing structurally related aglycone, and also via an alternative route by photocyclization of the anhydride **1**[6] to indolocarbazole anhydride **2**. Heating **2** with ammonium acetate gave $\mathbf{A_1}$ with a satisfactory yield (Figure 3).

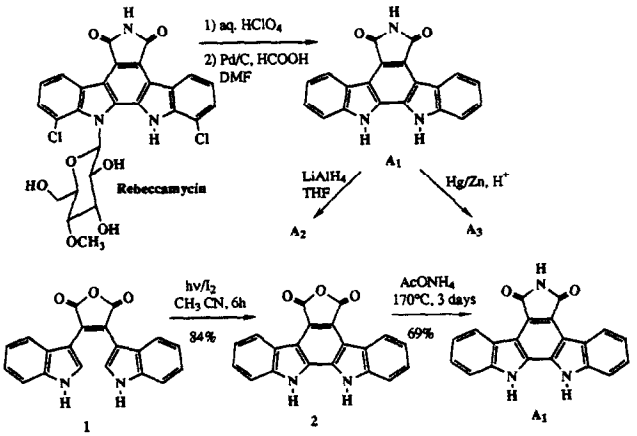


Figure 3.

The succinimide C_1 was obtained from B_1 by hydrogenation using palladium on charcoal (Pd/C) as a catalyst in dimethylformamide.[13] We prepared C_2 by reduction of C_1 with $LiAlH_4$ and C_3 from B_2 by hydrogenation using Pd/C in ethanol (Figure 4).

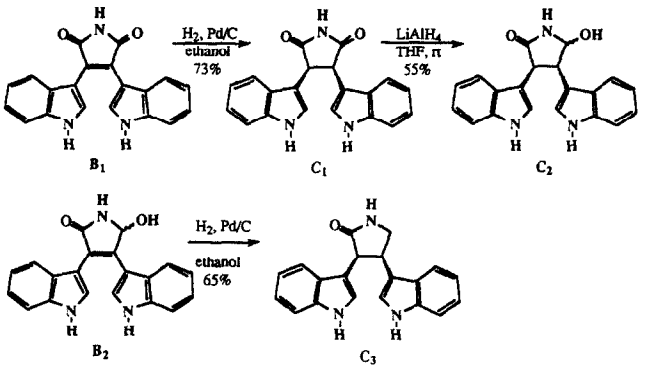


Figure 4.

Compound C_4 was synthesized to measure the effect on the biological activity of replacing the hydrogen of the imide function by a functional group, since replacing it by a methyl in B_1 is known to destroy the activity.[10] Accordingly, *N*-benzyloxymethyl-2,3-dibromomaleimide[14] was condensed with indolyl–MgBr in refluxing toluene yielding **3**. Hydrogenation of **3** on Pd/C in ethanol afforded C_4 (Figure 5).

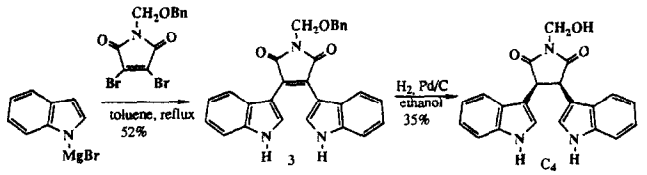


Figure 5.

The inhibitory potencies of A_{1-3}, B_{1-3}, C_{1-4} towards PKC and PKA were determined using histones IIIs and IIa respectively as substrates, according to the method described by Ricouart *et al.*[15] IC_{50} values are reported in Table 1; the isoquinoline sulfonamide **H-7** was tested as reference.[16]

Table 1. Inhibitory potencies for compounds A_{1-3}, B_{1-3}, C_{1-4} (IC_{50} μM)

Compound	PKC	PKA
H7	9.1	3.3
A_1	44.7	60
A_2	22.1	34
A_3 **(K252c)**	2.45	25.7
B_1	0.087	2.7
B_2	7.6	>50
B_3	2.5	32
C_1	>50	>100
C_2	>100	>50
C_3	> 50	>50
C_4	16	>100

All compounds exhibited more potent inhibitory activities against PKC than against PKA. The most active one was B_1. In the **A** series, A_3 (X = Y = H) had the highest activity.

The results obtained with C_1, C_2 and C_3 show that rigidity of the structures is necessary for biological activity, but, surprisingly, C_4 was found to be active.

The enantiomers **UCN 01** and **UCN 02**, which only differ in the absolute configuration of the carbon bearing the hydroxyl group, have widely different activities against PKC (**UCN 01**: IC_{50} = 4.1 nM; **UCN 02**: IC_{50} = 62 nM).[3] The structures A_2 and B_2, were obtained as mixtures of enantiomers, which might account for their relatively weak activity.

In B_1, where the hydrogen of the imide NH was replaced by a methyl group, the IC_{50} on PKC of the analogue was > 50 μM;[10] when the hydrogen of the imide NH was replaced by a hydroxy group, the IC_{50} on PKC of the analogue was 28.9 μM.[13] Unexpectedly, the flexible C_4, bearing a hydroxymethyl group on the amide nitrogen, was active.

To investigate this effect further, synthesis and testing of structures with the more active rigid frameworks in the **A** and **B** series likewise bearing a hydroxymethyl group on the amide NH, are in progress.

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 in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), 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 with flash Geduran SI 60 (Merck) 0.040–0.063 mm and silicagel plates (Kieselgel 60 F_{254} Merck).

Histone IIIs and IIa, phosphatidylserine, diacylglycerol and PKA were purchased from Sigma. [$\gamma^{32}P$] ATP was obtained from Amersham. PKC was obtained from Calbiochem. PKC phosphorylation assays were performed in a reaction mixture (80 μL) containing histone IIIs (2.4 mg/mL), $MgCl_2$ (10 mM), $CaCl_2$ (0.1 mM), phosphatidylserine (10 mg/mL), diacylglycerol (10 mg/mL), Tris/HCl buffer (pH 7.5), ATP (10 μM, 1000–2000 cpm/pmol), PKC (0.5 μg/mL). Stock solutions of inhibitors were prepared in DMSO. In each assay, data points were determined in triplicate.

6, 7, 12, 13-Tetrahydro-5,7-dioxo-5H-indolo[2,3-a] furo [3,4-c] carbazole **2**

Anhydride **1** (200 mg; 0.617 mmol) in acetonitrile (150 mL) in the presence of a crystal of iodine, was irradiated for 6 h with a medium pressure mercury lamp (400 W) until TLC indicated complete consumption of the starting material. **2** Precipitated out, was filtered off and isolated without any further purification as a bright gold–yellow powder (168 mg; 0.515 mmol; 84% yield). IR $\nu_{C=O}$ 1820, ν_{N-H} 3410; m.p. > 300°C. Exact mass (EI) calculated for $C_{20}H_{10}O_3N_2$: 326.0691, found 326.0669.

^{1}H NMR (DMSO-d_6): 7.39 (2H, t, J = 7.5 Hz); 7.59 (2H, t, J = 7.5 Hz); 7.85 (2H, d, J = 7.5 Hz); 8.78 (2H, d, J = 7.5 Hz); 12.05 (2H, s, $2N_{indole}$–H).

^{13}C NMR(DMSO-d_6): 112.5; 120.8; 123.4; 127.4 (Ctert.); 115.7; 117.7; 121.0; 129.9; 140.3 (Cquat.); 164.9 (C=O).

6, 7, 12, 13-Tetrahydro-5,7-dioxo-5H-indolo[2,3-a]pyrrolo-[3,4-c]carbazole A_1

Compound **2** was heated at 180 °C for 3 days in the presence of $AcONH_4$ (60 g). After cooling, water was poured into the mixture which was extracted with AcOEt. The organic phase was washed with water. After removal of the solvent, the residue was purified by flash chromatography (cyclohexane/AcOEt 50:50) to give A_1 as a yellow–orange powder (901 mg; 2.76 mmol; 69% yield). IR $\nu_{C=O}$ 1740, ν_{N-H} 3240, 3380; m.p. > 300 °C. Exact mass (EI) calculated for $C_{20}H_{11}O_2N_3$: 325.0851, found 325.0844.

^{1}H NMR (DMSO-d_6): 7.36 (2H, t, J = 7.3 Hz); 7.56 (2H, t, J = 7.3 Hz); 7.78 (2H, d, J = 7.3 Hz); 9.00 (2H, t, J = 7.3 Hz); 11.00 (1H, s, N_{imide}–H); 12.25 (2H, s, N_{indole}–H).

^{13}C NMR (DMSO-d_6): 111.8; 120.1; 124.3; 126.7 (Ctert.); 115.4; 119.8; 121.5; 129.1; 140.1 (Cquat.); 171.3 (C=O).

cis-*3,4-Bis(indol-3-yl)-pyrrolidin-2,5-dione* C_1

B_1 (100 mg; 0.30 mmol) prepared according to the method of Brenner *et al.*[6] was dissolved in 95% EtOH (100 mL) in the presence of catalytic amounts of Pd/C. The mixture was hydrogenated for 12 h (40 psi). After filtration, the solid was washed with THF and the solvents were removed. Purification by flash chromatography (cyclohexane/AcOEt 80:20) yielded C_1 as a white powder (72 mg; 0.22 mmol; 73% yield). IR $\nu_{C=O}$ 1750, ν_{N-H} 3270; m.p. 142–145 °C. Exact mass (EI) calculated for $C_{20}H_{15}O_2N_3$: 329.1164, found 329.1163.

^{1}H NMR (acetone-d_6): 5.00 (2H, s, C_3–H and C_4–H); 6.85–6.98 (6H, m); 7.14 (2H, d, J = 7.4 Hz); 7.51 (2H, d, J = 7.4 Hz); 9.85 (2H, s, N_{indole}–H); 10.50 (1H, s, N_{imide}–H).

^{13}C NMR (acetone-d_6): 46.0 (C_3 and C_4); 111.9; 119.3 (2C); 121.8; 125.1 (Ctert.); 109.6; 128.1; 136.6 (Cquat.); 179.5 (C=O).

(5R and S)-cis-5-Hydroxy-3,4-bis(indol-3-yl)-pyrrolidin-2-one C_2

To a solution of C_1 (164 mg; 0.498 mmol) in THF (25 mL) was added a solution of $LiAlH_4$ 1M in THF (4.2 mL; 4.2 mmol). The mixture was stirred at room temperature for 2 days, then cooled to 0 °C. Water (50 mL) was added and the mixture was acidified to pH 2 with HCl 2N. After extraction with AcOEt, the organic phase was washed with saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. The solvent was removed and the residue purified by flash chromatography (cyclohexane/AcOEt 30:40 to 20:80). The diastereomeric mixture of C_2 was obtained as a pale yellow solid (90 mg; 0.272 mmol; 55% yield). IR $\nu_{C=O}$ 1710, ν_{N-H} 3300, ν_{O-H} 3420; m.p. 114–115 °C. Exact mass (EI) calculated for $C_{20}H_{17}O_2N_3$: 331.1320 found 331.1328.

^{1}H NMR (acetone-d_6): 4.26 (1H, dd, J = 7.5 and 2 Hz); 4.78 (1H, d, J = 7.5 Hz); 5.43 and 5.58 (1H, s, OH); 7.14 (2H, d, J = 7.4 Hz); 7.51 (2H, d, J = 7.4 Hz); 9.85 (2H, s, N_{indole}–H); 10.50 (1H, s, N_{imide}–H)

^{13}C NMR (acetone-d_6): 43.1 and 47.9 (C_3 and C_4); 84.5 (C_5); 111.6; 119.2; 119.4; 121.6; 121.7; 123.1; 124.9 (Ctert.); 110.4; 133.8; 128.4; 129.0; 136.7; 136.8 (Cquat.); 178.8 (C=O).

cis-*3,4-Bis(indol-3-yl)-pyrrolidin-2-one* C_3

B_2 (94 mg; 0.282 mmol) dissolved in 95% ethanol (100 mL) was hydrogenated (40 psi) on Pd/C for 12 h. After filtration and evaporation of the solvent, the solid was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) and obtained as a white foam (58 mg; 0.184 mmol; 65% yield). IR $\nu_{C=O}$ 1680, ν_{N-H} 3200–3420; m.p. 108–110 °C. Exact mass (EI) calculated for $C_{20}H_{17}ON_3$: 315.1371, found 315.1365.

^{1}H NMR (DMSO-d_6): 3.22–3.45 (2H, m); 4.15–4.28 (2H, m); 6.29 (1H, t, J = 7 Hz); 6.31–7.05 (5H, m); 7.15 (2H, t, J = 7 Hz); 7.38 (1H, d, J = 7 Hz); 7.51 (1H, d, J = 7 Hz); 8.01 (1H, s, N_{amide}–H); 10.20 (1H, s, N_{indole}–H); 10.30 (1H, s, N_{indole}–H)

^{13}C NMR (DMSO-d_6): 37.7; 46.1; 46.3; 110.1; 111.1; 113.0; 115.7; 118.2 (2C); 118.6; 118.7; 120.5; 120.8; 122.4; 125.4; 127.3; 127.6; 135.6; 135.8; 177.8 (C=O).

(5R and S)-5-Hydroxy-3,4-bis(indol-3-yl)-3-pyrrolin-2-one B_2

To B_1 (970 mg; 2.96 mmol) in THF (150 mL) was added a solution of $LiAlH_4$ 1M in THF (25 mL; 25 mmol). The

mixture was stirred at room temperature for 18 h. After work up as for C_2, purification by flash chromatography (CH_2Cl_2/MeOH, 97:3) yielded B_2 as a pale yellow foam (534 mg; 1.63 mmol; 51% yield). IR $\nu_{C=O}$ 1680, ν_{N-H} and ν_{O-H} 3250–3400; m.p. > 250 °C. Exact mass (FAB^+, $(M - H^+)$) calculated for $C_{20}H_{14}O_2N_3$: 328.1086, found 328.1084.

^1H NMR (acetone-d_6): 5.30 (1H, d, J = 10 Hz, OH); 6.14 (1H, dd, J = 10 and 1Hz, C_5–H); 6.65 (1H, td, J = 7.5 and 1 Hz); 6.73 (1H, td, J = 7.5 and 1 Hz); 6.94 (1H, td, J = 7.5 and 1 Hz); 6.95 (1H, td, J = 7.5 and 1 Hz); 7.02 (1H, d, J = 7.5 Hz); 7.29 (1H, d, J = 7 Hz); 7.34 (1H, d, J = 7 Hz);7.38 (1H, d, J = 7 Hz); 7.51 (1H, d, J = 2.5 Hz); 7.61 (1H, d, J = 2.5 Hz); 7.83 (1H, s, N_{amide}–H); 10.50 (1H, s, N_{indole}–H); 10.61 (1H, s, N_{indole}–H).

^{13}C NMR (acetone-d_6): 80.5 (C_5); 112.2; 119.2; 120.2; 121.3; 121.8; 121.9; 122.1; 122.3; 127.4; 128.4 (Ctert.); 108.2; 110.6; 126.7 (2C); 137.0; 137.1; 137.2; 147.1 (Cquat.); 174.2 (C=O)

3,4-Bis(indol-3-yl)-N-benzyloxymethyl-3-pyrrolin-2,5-dione 3

To a suspension of magnesium (0.6 g; 25 mmol) in THF (15 mL) was added dropwise EtBr (1.9 mL; 2.77 g; 25.2 mmol). The mixture was gently warmed to initiate the reaction. A solution of indole (2.78 g; 23.8 mmol) in toluene (25 mL) was slowly added and the mixture was maintained at 45 °C for 45 min. Then *N*-benzyloxymethyldibromomaleimide (2.35 g; 6.26 mmol) prepared as described by Kaneko and Wong[14] and dissolved in toluene (50 mL) was added dropwise for 1.5 h. The mixture was refluxed for 2h, then cooled to 0 °C and acidified with a 20% aqueous solution of citric acid. After extraction with AcOEt, purification by flash chromatography on silicagel previously treated with triethylamine (eluent cyclohexane/AcOEt 50:50) afforded **3** as a red foam (1.47 g; 3.28 mmol; 52%). IR $\nu_{C=O}$ 1760, ν_{N-H} 3350, ν_{O-H} 3410; m.p. 103–106 °C. Exact mass (EI) calculated for $C_{28}H_{21}O_3N_3$: 447.1583, found 447.1590.

^1H NMR (acetone-d_6): 4.68 (2H, s); 5.16 (2H, s); 6.62 (2H, t, J = 8 Hz); 6.91 (2H, d, J = 8 Hz); 6.97 (2H, t, J = 8 Hz); 7.18–7.40 (7H, m); 7.85 (2H, d, J = 3 Hz); 10.79 (2H, s, N_{indole}–H)

^{13}C NMR (acetone-d_6): 67.9 (CH_2); 71.8 (CH_2); 112.4; 120.4; 122.3; 122.8; 128.3; 129.0; 130.1 (Ctert.); 107.3; 126.7; 128.6; 137.2; 139.3 (Cquat.); 172.4 (C=O).

cis-3,4-Bis(indol-3-yl)-N-hydroxymethyl-pyrrolidin-2,5-dione C_4

A mixture of **3** (200 mg; 0.447 mmol) in 95% ethanol (100 mL) containing catalytic amounts of Pd/C was hydrogenated for 2 days (50 psi). After filtration, the solvent was removed. Purification by flash chromatography (cyclohexane/AcOEt 50:50) led to C_4 as a pale pink foam (56 mg; 0.156 mmol; 35% yield). IR $\nu_{C=O}$ 1700, ν_{N-H} and ν_{O-H} 3250–3450; m.p. 192–195 °C. Mass (FAB^+): $(M + H)^+$ = 360. Exact mass (EI) calculated for $C_{20}H_{15}O_3N_3$ $(M–CH_2OH)^+$: 329.1164, found 329.1160.

^1H NMR (acetone-d_6): 4.98 (2H, s, C_3–H and C_4–H); 5.19 (2H, s, CH_2OH); 5.60 (1H, broad s, OH); 6.79–6.92 (6H, m); 7.11 (2H, d, J = 8 Hz); 7.44 (2H, d, J = 8 Hz); 7.83 (1H, s, N_{amide}–H); 9.90 (2H, s, N_{indole}–H)

^{13}C NMR(acetone-d_6): 45.4 (C_3 and C_4); 62.8 (CH_2OH); 111.7; 119.3 (2C); 121.9; 125.1 (Ctert.); 109.0; 128.2; 136.8 (Cquat.); 178.1 (C = O).

Acknowledgement

We are grateful to Riom-Laboratoires CERM for financial support.

References

1. Casnellie, J. E. *Advances in Pharmacology* **1991**, *22*, 167.

2. Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. *J. Antibiotics* **1977**, *30*, 275.

3. Takahashi, I.; Saitoh, Y.; Yoshida, M.; Sano, H.; Nakano, H.; Morimoto, M.; Tamaoki, T. *J. Antibiotics* **1989**, *42*, 571.

4. Nakanishi, S.; Matsuda, Y.; Iwahashi, K.; Kase, H. *J. Antibiotics* **1986**, *39*, 1066.

5. Fabre, S.; Prudhomme, M.; Rapp, M. *BioMed. Chem. Lett.* **1992**, *2*, 449.

6. Brenner, M.; Rexhausen, H.; Steffan, B.; Steglich, W. *Tetrahedron* **1988**, *44*, 2887.

7. Bergman, J.; Pelcman, B. *J. Org. Chem.* **1989**, *54*, 824.

8. Gribble, G. W.; Berthel, S. J. *Tetrahedron* **1992**, *48*, 8869.

9. Davis, P. D.; Hill, C. H.; Lawton, G. *Eur. Pat.* **1989**, A1 0328 026.

10. Toullec, D.; Pianetti, P.; Coste, H.; Bellevergue, P.; Grand-Perret, T.; Ajakne, M.; Baudet, V.; Boissin, P.; Boursier, E.; Loriolle, F.; Duhamel, L.; Charon, D.; Kirilovsky, J. *J. Biol. Chem.* **1991**, *266*, 15771.

11. Starstedt, B.; Winterfeldt, E. *Heterocycles* **1983**, *20*, 469.

12. Moody, C. J.; Rahimtoola, K. F.; Porter, B.; Ross, C. B. *J. Org. Chem.* **1992**, *57*, 2105.

13. Davis, P. D.; Hill, C. H.; Lawton, G.; Nixon, J. S.; Wilkinson, S. E.; Hurst, S. A.; Keech, E.; Turner, S. E. *J. Med. Chem.* **1992**, *35*, 177.

14. Kaneko, T.; Wong, H. *Tetrahedron Lett.* **1985**, *26*, 4015.

15. Ricouart, A.; Gesquiere, J. C.; Tartar, A.; Sergheraert, C. *J. Med. Chem.* **1991**, *34*, 73.

16. Hidaka, H.; Inagaki, M.; Kawamoto, S.; Sasaki, Y. *Biochemistry* **1984**, *23*, 5036.