Inhibitors of Protein Kinase C. 2. Substituted Bisindolylmaleimides with Improved Potency and Selectivity

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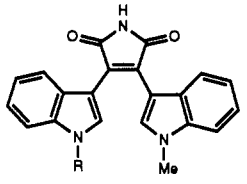
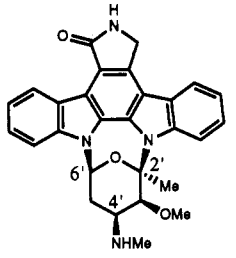
A hypothetical mode of inhibition of protein kinase C (PKC) by the natural product staurosporine has been used as a basis for the design of substituted bisindolylmaleimides with improved potency over the parent compound. Structure–activity relationships were consistent with the interaction of a cationic group in the inhibitor with a carboxylate group in the enzyme, and the most potent compound had a K_i of 3 nM. The inhibitors were competitive with ATP but inhibited cAMP-dependent protein kinase (PKA) only at much higher concentrations despite the extensive sequence homology between the ATP-binding regions of PKA and PKC. Three compounds were evaluated further and found to inhibit a human allogeneic mixed lymphocyte reaction pointing to the potential utility of PKC inhibitors in immunosuppressive therapy. One of these compounds was orally absorbed in the rat and represents an attractive lead in the development of PKC inhibitors as drugs.

Introduction

Protein kinase C (PKC) is an important element in the signal transduction pathways of a variety of hormones, cytokines, and growth factors.[1] Stimulation of PKC following receptor occupation can lead to cellular activation, secretion, and proliferation and thus the enzyme may be involved in the pathogenesis of diseases such as cancer,[2] asthma,[3] AIDS,[4] hypertension,[5] psoriasis,[6] and rheumatoid arthritis (through T-cell activation[7]). We have described a series of bisindolylmaleimide PKC inhibitors (e.g. **1a**) which are more selective than the general protein kinase inhibitor staurosporine **2**, the natural product on which they were based.[8] These earlier inhibitors are less potent than staurosporine and only modestly selective for PKC over phosphorylase kinase.



1a R=Me
1b R=^{n}Pr



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To improve upon the potency of the bisindolylmaleimides the structure of staurosporine has again been used as a guide. Structure–activity relationships (SARs) of staurosporine analogues point to a significant role for the 4′-methylamino substituent; benzoylation of this moiety reduces activity 8-fold[9] and replacement with hydroxyl results in a compound 3 orders of magnitude less active.[10] Methylation of the amino nitrogen, however, does not reduce potency.[11] These SARs are consistent with a requirement for a potentially cationic 4′-substituent which might interact electrostatically with the enzyme.

To take advantage of the putative amine or cation binding site a common mode of binding for staurosporine and the bisindolylmaleimides was postulated (Figure 1). In both types of inhibitor a free NH in the lactam or imide ring appears to be essential, alkylation in both cases abolishing activity,[8,9] and therefore this ring was chosen as a likely area of common interaction with the enzyme. Bisindolylmaleimide **1a** was modeled in a low-energy conformation which best approximates the structure of the staurosporine indolocarbazole since this conformation appeared more likely to fit into a staurosporine binding site than did a conformation corresponding to the observed solid-state structure of a closely related compound.[8] Two conformations of staurosporine were considered since it has been shown[12] that the bioactive conformation of the sugar moiety may be either the chair (the solution conformation of the amino sugar free base) or the boat (the solution conformation of the protonated form). The chair conformer shown (Figure 1a) is an X-ray crystal structure[13] and the boat (Figure 1b) is a modeled structure obtained by MOPAC[14] geometry optimization with the AM1 method.

We have shown previously[8] that groups larger than methyl on one indole nitrogen of the bisindolylmaleimides

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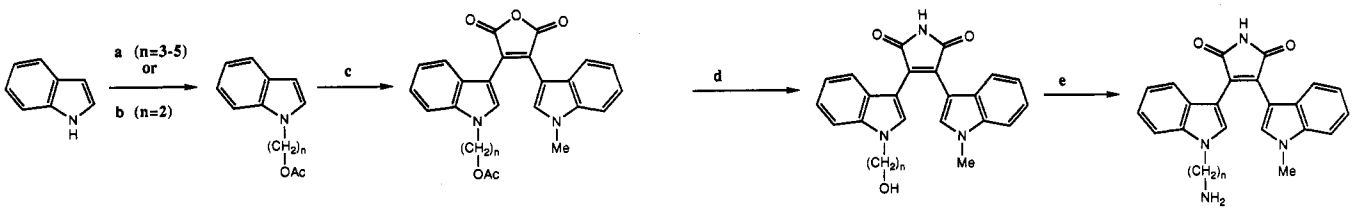
Scheme I[a]



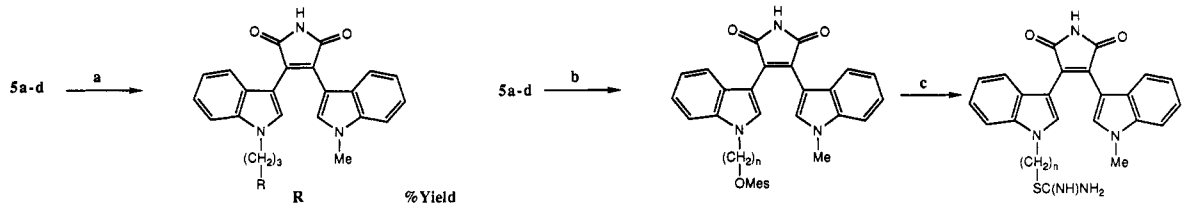
	n	% Yield		n	% Yield
3a	2	46	4a	2	20
3b	3	86	4b	3	16
3c	4	74	4c	4	15
3d	5	80	4d	5	10

	n	% Yield
5a	2	72
5b	3	94
5c	4	53
5d	5	43

	n	% Yield
6a	2	49
6b	3	51
6c	4	75
6d	5	50

[a] Conditions: (a) i, NaH; DMF; ii, $Br(CH_2)_nOAc$. (b) i, NaH, DMF; ii, ethylene oxide; iii, Ac_2O, pyridine. (c) i, $(COCl)_2$, CH_2Cl_2, 0 °C; ii, *N*-methyl-3-indoleacetic acid, Et_3N, CH_2Cl_2. (d) NH_3 aq DMF, 140 °C, 4 h. (e) i, Tf_2O, 2,6-lutidine, CH_2Cl_2, 0 °C; ii, NH_3 aq room temperature.

Scheme II[a]



	R	% Yield
7	NHMe	50
8	NMe_2	54
9	NMe_3^+	59

	n	% Yield
10a	2	100
10b	3	73
10c	4	67
10d	5	93

	n	% Yield
11a	2	43
11b	3	79
11c	4	57
11d	5	48

[a] Conditions: (a) i, Tf_2O, lutidine, CH_2Cl_2, 0 °C; ii, amine. (b) Mes_2O, pyridine, CH_2Cl_2 rt. (c) Thiourea, EtOH, reflux.

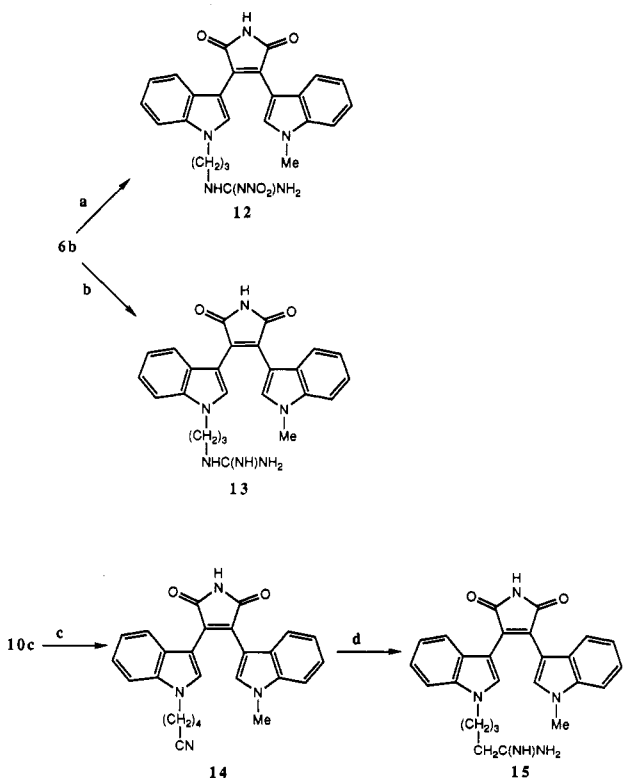
do not abrogate inhibitory activity. Side chains were therefore modeled on this position in low-energy conformations that placed a cationic end group at a position in space corresponding to the position of the amine group in either staurosporine conformer. An example of this is shown in Figure 1d where a methylene chain attached to indole nitrogen permits an appended amino group to approximate either of the target spatial positions. For the chair amine position, amines could be brought within 1 Å by methylene chains of two to five carbon atoms in conformations with calculated enthalpies less than 5 kcal mol^{-1} above the global minimum. The best fit was given by the three-carbon chain (e.g. 0.3 Å with 2.7 kcal mol^{-1} enthalpy penalty). For the boat amine position the best fit was with the four-carbon chain (e.g. 0.3 Å with 0.7 kcal mol^{-1} enthalpy penalty) but the three-carbon chain was nearly as good (0.4 Å, 0.8 kcal mol^{-1}). All but the two-carbon compound fitted better to the boat amine position than to the chair.

Compounds were thus designed to interact with the putative amine binding site and therefore to have potentially greater potency than the parent bisindolylmaleimide.

Chemistry

The required (aminoalkyl)bisindolylmaleimides **6** were prepared by a route which also provided the corresponding alcohols for evaluation (Scheme I). For methylene chains containing three to five carbon atoms the acetates **3** were obtained directly by alkylation of indole. The two-carbon atom chain was introduced by reaction of indole with ethylene oxide followed by acetylation. Acetates **3** were used as substrates in our glyoxylation–intramolecular Perkin reaction procedure[15] to afford the maleic anhydrides **4** which, in turn, gave the alcohols **5** on treatment with ammonia. The alcohols were converted into the amines **6** via the corresponding triflates and also into

Scheme III[a]



[a] Conditions: (a) 3,5-Dimethyl-N^2-nitro-1-pyrazole-1-carboxamidine, $NaHCO_3$, EtOH, reflux, 16 h (94%). (b) 3,5-Dimethylpyrazole-1-carboxamidine nitrate, $NaHCO_3$, EtOH, reflux, 4 h (56%). (c) NaCN, DMSO, 50 °C, 8 h (11%). (d) i, HCl/EtOH, 18 h; ii, NH_3/EtOH, 18 h (73%).

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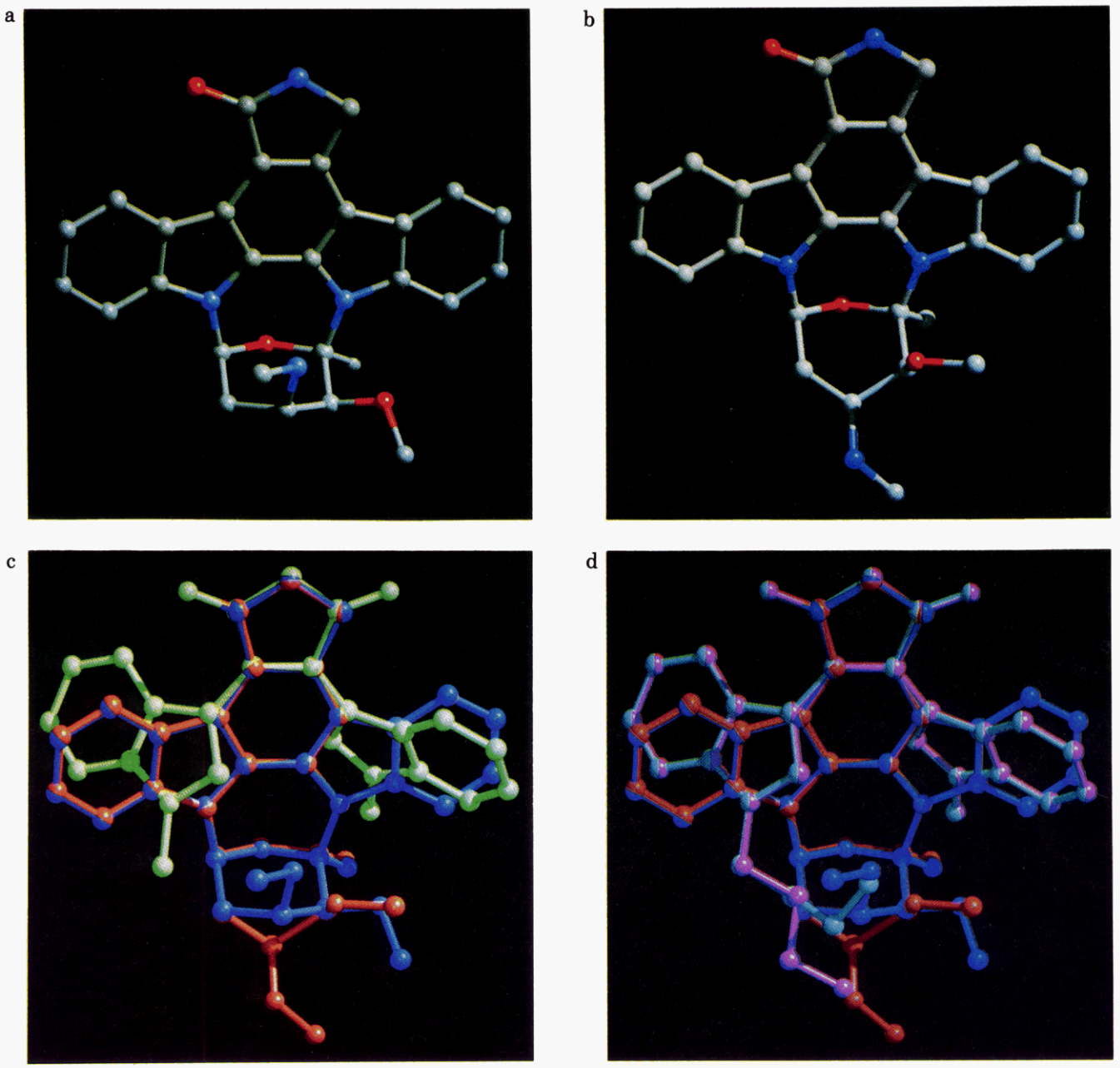

Figure 1. (a) X-ray structure of staurosporine; (b) modeled structure of the alternative conformer of staurosporine, obtained by geometry optimization (MOPACv5/AM1) of a boat conformation of the sugar moiety; (c) both conformers of staurosporine (blue, chair and orange, boat) overlayed with a modeled conformation of bisindolylmaleimide 1 (green) by matching lactam and imide rings; and (d) three-carbon methylene chains (pink and cyan) in low-energy conformations connect an indole nitrogen of a bisindolylmaleimide with an amino group near to the corresponding position in space of the staurosporine chair and staurosporine boat amine, respectively.

amines **7–9** by a similar procedure (Scheme II). The alcohols also afforded mesylates **10** which were converted into the isothioureas **11**. Standard reagents were used to obtain both nitroguanidine **12** and guanidine **13** from amine **6b** (Scheme III) and amidine **15** was prepared from mesylate **10c** via nitrile **14**.

Results and Discussion

Amines **6a–d** all showed improved inhibitory potency (Table I) over both the parent bisindolylmaleimide **1a** (IC_{50} 300 nM) and the propyl compound **1b** (IC_{50} 480 nM),[1] suggesting that the amino groups are interacting favorably with the enzyme. This is probably an electrostatic interaction involving protonated amines since the alcohols were less active and the quaternary amine **9** retained potency. The optimum interaction between ammonium and carboxylate in the gas phase has been calculated to involve neutral hydrogen bonds rather than electrostatic attraction,[16] but this description applies only to a perfect geometry that is unlikely to occur in constrained systems and the effect of solvent has not been calculated.

The lack of a strong dependency of the amine IC_{50} values on the chain length is also in accord with an amine binding site hypothesis and reflects the flexibility of the methylene chains and the fact that the amine binding site is probably not a single point but an area on the enzyme surface. For example the amino groups of the modeled low-energy conformers are all close enough to interact with a common carboxylate, especially given that ion/ion electrostatic interactions are the least distance dependent of intermo-

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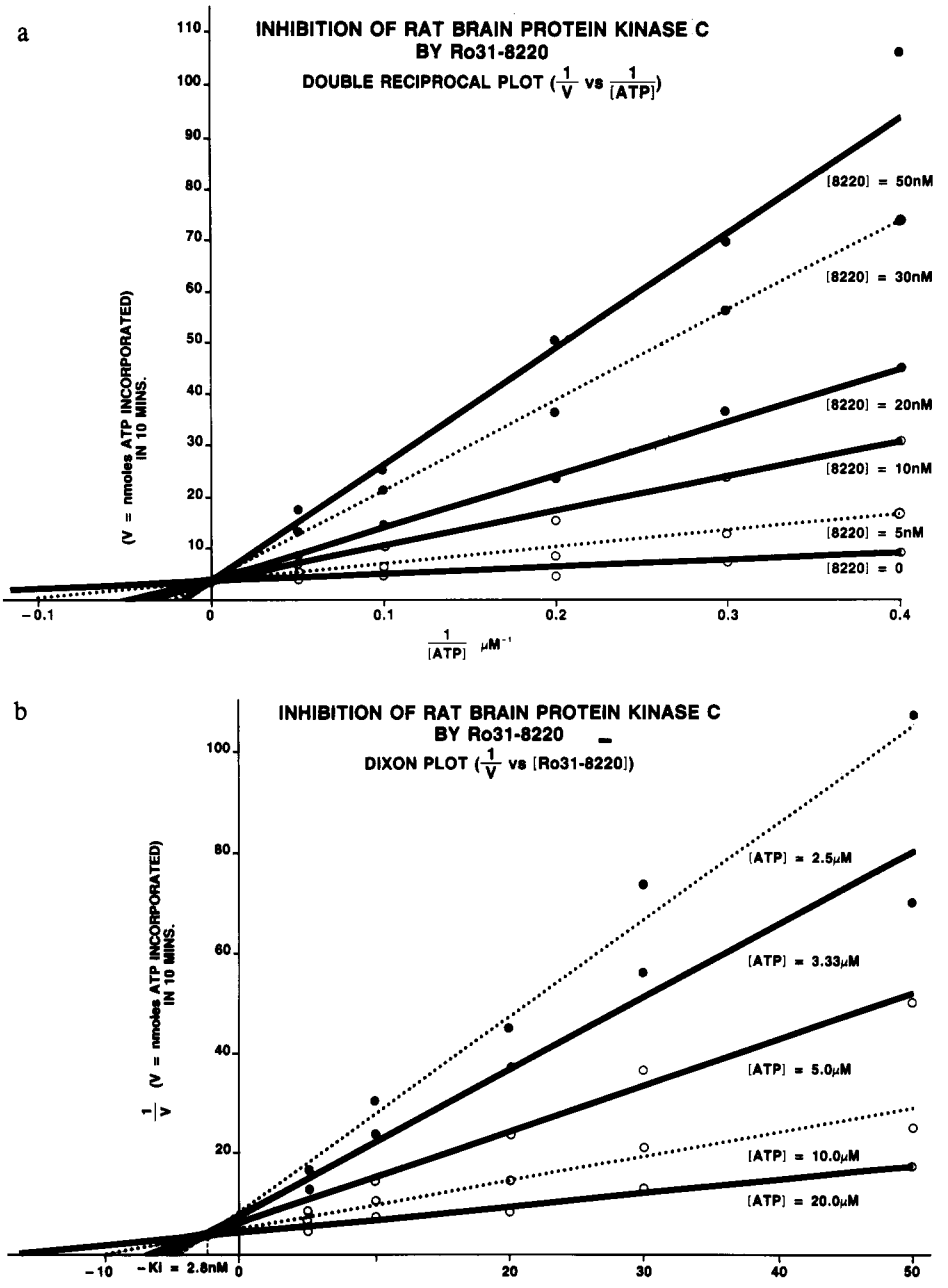

Figure 2. Lineweaver-Burk (a) and Dixon (b) plots for inhibition of PKC by **11b**.

lecular attractive forces.[17] The longer chain compounds appear to fit the binding site better since the extra degrees of freedom lost on binding do not result in markedly higher IC_{50} values (i.e. the less favorable entropy change is offset by a more favorable enthalpy of binding). This is more consistent with an amine binding site in general proximity to the "boat" amine position than with a site near the "chair" amine.

The ideal recognition partner for a carboxylate is a bifurcated cation such as amidinium or guanidinium which form strong cyclic H-bonded complexes with the anion.[16,18,19] Formal replacement of the amino group in **6** with amidine (compound **15**), guanidine (compound **13**), or an isothiourea (**11**) indeed increased potency and, in the isothiourea series, the potency was dependent on chain length, in contrast to the amine series. This is consistent with a carboxylate/bifurcated cation interaction which would be expected to be more geometry dependent than a carboxylate/ammonium interaction.

The activity of **12** is interesting since nitroguanidine has a low pK_a (−0.5[20]) and consequently **12** would not be significantly protonated at the pH of the assay (7.4). The nitroguanidine group has, however, a large dipole moment,[21] and it may be this property which results in the high potency of **12**.

The most potent compound, isothiourea **11b**, was found to inhibit PKC competitively with respect to ATP with

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Table I. Inhibition of PKC

number	formula	analysis	IC_{50} vs PKC, μM	IC_{50} vs PKA, μM
5a	$C_{23}H_{19}N_3O_3$	C,H,N	0.34 ± 0.01 (2)	22.1 ± 3.9 (2)
5b	$C_{24}H_{21}N_3O_3$	C,H,N	0.13 ± 0.03 (2)	14.2 ± 2.6 (3)
5c	$C_{25}H_{23}N_3O_3$	C,H,N	0.17 ± 0.05 (3)	18.4 ± 2.2 (2)
5d	$C_{26}H_{25}N_3O_3$	C,H,N	0.28 ± 0.03 (2)	21.3 ± 0.8 (2)
6a	$C_{23}H_{20}N_4O_2 \cdot HCl$	C,H,N	0.064 ± 0.001 (2)	8.9 ± 1.9 (2)
6b	$C_{24}H_{22}N_4O_2 \cdot C_2H_4O_2$	C,H,N	0.075 ± 0.019 (17)	5.2 ± 1.2 (12)
6c	$C_{25}H_{24}N_4O_2 \cdot 0.5C_2H_4O_2$	C,H,N	0.055 ± 0.019 (3)	9.3 ± 0.7 (2)
6d	$C_{26}H_{26}N_4O_2 \cdot HCl$	C,H,N	0.076 ± 0.02 (2)	10.4 ± 0.8 (2)
7	$C_{25}H_{24}N_4O_2 \cdot HCl$	C,H,N	0.035 ± 0.02 (2)	11.9 ± 1.2 (2)
8	$C_{26}H_{26}N_4O_2 \cdot HCl$	C,H,N	0.045 ± 0.032 (2)	25.6 ± 5.0 (2)
9	$C_{27}H_{29}N_4O_2^+CF_3SO_2^-$	*a*	0.093 ± 0.004 (2)	64.5 ± 0.2 (2)
11a	$C_{24}H_{21}N_5O_2S \cdot CH_4O_3S$	*a*	0.041 ± 0.001 (2)	12.1 ± 2.8 (2)
11b	$C_{25}H_{23}N_5O_2S \cdot CH_4O_3S$	C,H,N	0.010 ± 0.003 (2)	0.9 ± 0.4 (2)
11c	$C_{26}H_{25}N_5O_2S \cdot CH_4O_3S$	C,H,N	0.028 ± 0.009 (2)	2.7 ± 0.5 (2)
11d	$C_{27}H_{27}N_5O_2S \cdot CH_4O_3S$	C,H,N	0.047 ± 0.03 (3)	4.6 ± 0.4 (2)
12	$C_{25}H_{23}N_7O_4 \cdot 0.3CH_4O$	C,H,N	0.026 ± 0.002 (2)	4.9 ± 0.9 (3)
13	$C_{25}H_{24}N_6O_2 \cdot HNO_3$	C,H,N	0.019 ± 0.005 (2)	2.9 ± 0.6 (2)
15	$C_{26}H_{25}N_5O_2 \cdot 1.2HCl$	C,H,N	0.031 ± 0.001 (2)	7.4 ± 1.3 (2)

[a] Characterized by high-resolution mass spectrometry; homogeneous by thin-layer chromatography.

a K_i of 3 nM (Figure 2). For comparison, the K_i of staurosporine is 141 nM.[22] Compounds **6b**, **9**, and **12** also competed with ATP as shown by the 6–8-fold increase in IC_{50} values on increasing the ATP concentration 10-fold.[23]

Despite the strong sequence homology between ATP-binding regions of protein kinases,[24] these ATP-competitive inhibitors are selective for PKC over the closely related cAMP-dependent protein kinase, PKA (Table I). Compounds **6b** (Ro 31-7549), **11b** (Ro 31-8220), and **12** (Ro 31-8161) are also highly selective for PKC over a calcium/calmodulin dependent protein kinase[23] and phosphorylase kinase.[25]

Since PKC is an intracellular enzyme, a therapeutically useful inhibitor must be able to cross the cell membrane and to inhibit the enzyme under the conditions prevailing inside the cell. In the case of ATP-competitive inhibitors the high intracellular concentration of ATP must be overcome. Compounds **6b**, **11b**, and **12** have all been shown[23] to effectively inhibit PKC within intact platelets and T-cells. We wished particularly to investigate the potential utility of these inhibitors in the treatment of T-cell-dependent diseases such as autoimmune disorders and tissue rejection following transplant therapy. Compounds **6b**, **11b**, and **12** were therefore evaluated in an in vitro model of antigen-driven T-cell proliferation, the mixed lymphocyte reaction[26] (MLR), and were found to inhibit proliferation with IC_{50} values (2.0, 0.2, and 0.5 μM, respectively) similar to those required for inhibition of intracellular PKC.

To further evaluate these three compounds as potential drugs the levels of PKC inhibitory activity in rat plasma was measured after both oral and intravenous dosing. Very little inhibitory activity was present at 2 h after administration of **11b** or **12** by either route but **6b** had a better pharmacokinetic profile (Figure 3) and, despite its lower in vitro potency, appears a more promising lead in the development of a PKC inhibitor for therapeutic use.

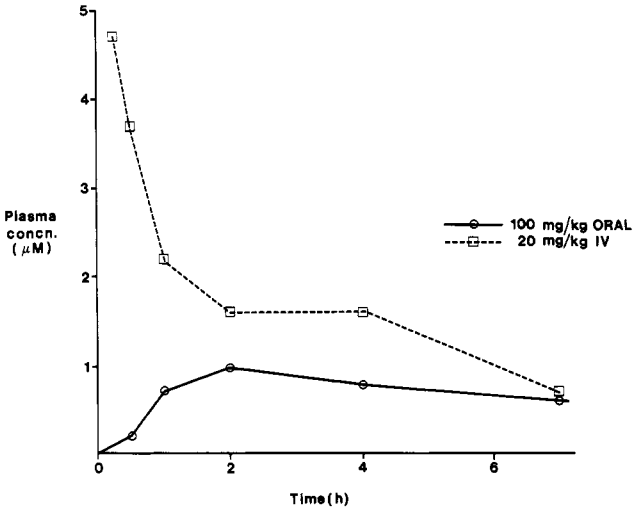

Figure 3. Plasma profile of PKC inhibition following a single administration of **6b** po and iv to female AP1 rats.

In conclusion a series of PKC-inhibitory-substituted bisindolylmaleimides has been designed by analogy with the structure of staurosporine. Potentially cationic groups in these compounds interact favorably with the enzyme and SARs are consistent with the site of interaction being a carboxylate group. The inhibitors are among the most potent and selective known and inhibit PKC within intact cells. Amine **6b** is orally absorbed in rats and represents an attractive lead in the development of even more potent inhibitors, possibly by a conformational restriction approach, which may provide therapy for a variety of poorly-treated diseases.

Experimental Section

General. Melting points were determined on a Buchi apparatus in glass capillary tubes and are uncorrected. Thin-layer chromatography was performed on silica gel aluminum-backed plates (5554) and glass-backed plates (5719) purchased from E. Merck & Co., and flash chromatography was performed on Sorbisil C60 40/60 A silica gel (Crosfield Chemicals). Mass spectra were obtained with either a Kratos MS902 mass spectrometer in the electron-impact mode or a Finnigan 8430 instrument in chemical-ionization mode. ^1H NMR spectra were recorded on either

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a Bruker AC-250 or a Bruker WM-300 spectrometer and chemical shifts are given in ppm (δ) from tetramethylsilane as internal standard. IR spectra were recorded on a Perkin-Elmer Model 782 spectrophotometer.

1-(2-Acetoxyethyl)indole (3a). A stirred solution of indole (11.7 g, 100 mmol) in DMF (500 mL) was treated with sodium hydride (4 g of a 60% dispersion in mineral oil, 100 mmol). After 1 h the reaction mixture was cooled to 3 °C and ethylene oxide (10 mL) was added. The mixture obtained was allowed to warm to room temperature, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (ether/petrol, 1:1) to give 1-(2-hydroxyethyl)indole (7.63 g) as a colorless oil. A stirred solution of this material (4.6 g, 28.6 mmol) in ether (10 mL) and acetic anhydride (5 mL) was treated with pyridine (1 mL) at 0 °C. After 2 h the reaction mixture was diluted with water (100 mL) and extracted with dichloromethane (150 mL). The organic extract was dried (Na_2SO_4) and evaporated to give the title compound (5.7 g, 46%) as a colorless oil: ^{1}H NMR ($CDCl_3$) 2.04 (3 H, s, OAc), 4.33–4.50 (4 H, m, CH_2CH_2 OAc), 6.56 (1 H, d, J = 3.5 Hz, 3-H), 7.10–7.32 (3 H, m, indole-H), 7.40 (1 H, d, J = 8 Hz, indole-H), 7.67 (1 H, d, J = 8 Hz, indole-H); IR ϑ_{max} (film) 1740 cm^{-1}; MS m/z 203 (M^+); exact mass, 203.0939 ($C_{12}H_{13}NO_2$ requires 203.0946).

1-(3-Acetoxypropyl)indole (3b). A stirred solution of indole (1.17 g, 10 mmol) in DMF (20 mL) was treated with sodium hydride (800 mg of a 60% dispersion in mineral oil, 20 mmol). After 0.5 h 3-bromopropyl acetate (3.62 g, 20 mmol) was added and the mixture obtained was stirred at room temperature under a nitrogen atmosphere for 18 h. The reaction mixture was acidified with 2 M HCl and extracted with dichloromethane (150 mL). The organic extract was washed with water (3 × 100 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified by flash chromatography on silica gel (ether/petrol, 1:2) to give the title compound (1.87 g, 86%) as a colorless oil: ^{1}H NMR ($CDCl_3$) 2.11 (3 H, s, OAc), 2.14–2.30 (2 H, m, $CH_2CH_2CH_2OAc$), 4.08 (2 H, t, J = 6.5 Hz, CH_2 OAc), 4.27 (2 H, t, J = 6.5 Hz, CH_2N), 6.53 (1 H, d, J = 3.5 Hz, 3-H), 7.07–7.45 (4 H, m, indole-H), 7.67 (1 H, d, J = 8 Hz, indole-H); IR ϑ_{max} (nujol) 1735 cm^{-1}; MS m/z 217 (M^+). Analysis ($C_{13}H_{15}NO$) C, H, N.

3-[1-(3-Acetoxypropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-2,5-furandione (4b). A solution of 1-(3-acetoxypropyl)indole (**3b**) (868 mg, 4 mmol) in dichloromethane (10 mL) was treated with oxalyl chloride (370 μL, 4.2 mmol) at 0 °C. The solution obtained was stirred at room temperature for 3 h and then evaporated to dryness. The residue was dissolved in dichloromethane (30 mL), and this solution was cooled to 0 °C under nitrogen. 1-Methylindole-3-acetic acid (756 mg, 4 mmol) and triethylamine (1.1 mL, 8 mmol) were added, and the mixture was stirred for 18 h at room temperature. The solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel (ethyl acetate/petrol, 1:2) to give the title compound (290 mg, 16%) as a red solid: mp (EtOAc/petrol) 94–6 °C; ^{1}H NMR ($CDCl_3$); 2.08 (3 H, s, OAc), 2.16 (2 H, quintet, J = 6.5 Hz, $CH_2CH_2CH_2OAc$), 3.88 (3 H, s, NCH_3), 4.05 (2 H, t, J = 6.5 Hz, CH_2OAc), 4.28 (2 H, t, J = 6.5 Hz, CH_2N), 6.70–6.90 (3 H, m, indole-H), 7.28–7.36 (2 H, m, indole-H), 7.70 (1 H, s, indole 2-H), 7.81 (1 H, s, indole 2-H); IR ϑ_{max} (nujol) 1745 and 1815 cm^{-1}; MS m/z 442 (M^+). Analysis ($C_{26}H_{22}N_2O_5$) C, H, N.

3-[1-(3-Hydroxypropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (5b). A solution of 3-[1-(3-acetoxypropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-2,5-furandione (**4b**) (100 mg, 0.23 mmol) in DMF (2 mL) and 33% aqueous ammonia (2 mL) was heated to 140 °C for 4 h in a sealed vessel. The cooled solution was evaporated to dryness, and the residue was crystallized from ethyl acetate to give 85 mg (94%) of **5b** as a red solid: mp 185–7 °C; ^{1}H NMR (DMSO-d_6) 1.77 (2 H, quintet, J = 6.5 Hz, $CH_2CH_2CH_2OH$), 3.85 (3 H, s, NCH_3), 4.28 (2 H, t, J = 6.5 Hz, CH_2N), 4.64 (1 H, t, J = 5 Hz, OH), 6.55–6.74 (3 H, m, indole-H), 6.90 (1 H, d, J = 8 Hz, indole-H), 6.97–7.08 (2 H, m, indole-H), 7.37–7.52 (2 H, m, indole-H), 7.72 (1 H, s, indole 2-H), 7.86 (1 H, s, indole 2-H), 10.93 (1 H, bs, NH); IR ϑ_{max} (nujol) 1695 and 1745 cm^{-1}; MS m/z 399 (M^+); exact mass 399.1581 ($C_{24}H_{21}N_3O_3$ requires 399.1600). Analysis ($C_{24}H_{21}N_3O_3$) C, H, N.

3-[1-(3-Aminopropyl)indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Acetate (6b). A mixture of alcohol **5b** (1 g, 2.5 mmol) and 2,6-lutidine (550 mg, 5.2 mmol) in dichloromethane (50 mL) was added dropwise to a solution of trifluoromethanesulfonic anhydride (1.88 g, 6.7 mmol) in dichloromethane (50 mL) at 0 °C. After 2 h 33% aqueous ammonia (50 mL) was added and the mixture stirred vigorously overnight. The organic phase was washed well with water, dried ($MgSO_4$), and concentrated. The residue was chromatographed once on silica gel with 5% methanol in dichloromethane and again with chloroform/methanol/acetic acid/water (60:15:2:3) to give **6b** as a red solid: mp 179–181 °C; yield 580 mg, 51%; ^{1}H NMR (DMSO-d_6) 1.82 (3 H, s, acetate), 1.88 (2 H, m, $CH_2CH_2CH_2$), 2.57 (2 H, t, J = 5 Hz), 4.31 (2 H, t, J = 5 Hz), 6.55–6.70 (3 H, complex signal, indole-H), 6.88 (1 H, d, J = 7.5 Hz, indole-H), 7.03 (2 H, overlapping t, J = 7.5 Hz for both, indole-H), 7.42 (1 H, d, J = 7.5 Hz, indole-H), 7.50 (1 H, d, J = 7.5 Hz, indole-H), 7.77 (1 H, s, indole 2-H), 7.85 (1 H, s, indole 2-H), (exchangeables not observed); IR ϑ_{max} (nujol) 1705, 1755, and 2500–3300 cm^{-1}; exact mass 398.1730 ($C_{24}H_{22}N_4O_2$ requires 398.1740). Analysis ($C_{24}H_{22}N_4O_2 \cdot C_2H_4O_2$) C, H, N.

3-[1-[3-(Methylamino)propyl]-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Hydrochloride (7). A suspension of 3-[1-(3-hydroxypropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (**5b**) (250 mg, 0.63 mmol) and 2,6-lutidine (134 mg, 1.25 mmol) in dichloromethane (50 mL) was added to a stirred solution of trifluoromethanesulfonic anhydride (263 mg, 0.93 mmol) in dichloromethane (40 mL) at 0 °C under nitrogen. After 0.5 h a 33% solution of methylamine in ethanol (1 mL, 8 mmol) was added and the mixture was allowed to warm to room temperature. After 1 h the solvent was removed in vacuo and the residue was purified by flash chromatography (dichloromethane/methanol/acetic acid/water, 90:18:3:2). The product was partitioned between ethyl acetate (200 mL) and 10% sodium bicarbonate (150 mL), and the organic phase was dried (Na_2SO_4) and concentrated. The residue was dissolved in ethyl acetate (25 mL) and treated with a saturated solution of HCl in ethyl acetate (3 mL). The precipitate was filtered off and dried to give 140 mg (50%) of the title compound as a red solid: mp 173–175 °C; ^{1}H NMR (DMSO-d_6) 2.00–2.12 (2 H, m, $CH_2CH_2CH_2NHCH_3$), 2.46 (3 H, s, $NHCH_3$), 2.70–2.84 (2 H, m, CH_2NHCH_3), 3.84 (3 H, s, NCH_3), 4.32 (2 H, t, J = 7 Hz, NCH_2), 6.55–6.72 (3 H, m, indole-H), 6.85 (1 H, d, J = 8 Hz, indole-H), 6.93–7.06 (2 H, m, indole-H), 7.39 (1 H, d, J = 8 Hz, indole-H), 7.51 (1 H, d, J = 8 Hz, indole-H), 7.76 (1 H, s, indole 2-H), 7.83 (1 H, s, indole 2-H), 8.83–9.05 (2 H, bs, NH_2Me^+), 10.92 (1 H, bs, imide); IR ϑ_{max} (nujol) 1705, 1750, and 2400–3300 cm^{-1}; MS m/z 412 (M^+). Analysis ($C_{25}H_{24}N_4O_2$·HCl) C, H, N.

3-[1-[3-(Dimethylamino)propyl]-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Hydrochloride (8). A suspension of 3-[1-(3-hydroxypropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (**5b**) (250 mg, 0.63 mmol) and 2,6-lutidine (134 mg, 1.25 mmol) in dichloromethane (50 mL) was added to a stirred solution of trifluoromethanesulfonic anhydride (263 mg, 0.93 mmol) in dichloromethane (40 mL) at 0 °C under nitrogen. After 0.5 h a 33% solution of dimethylamine in ethanol (1 mL, 5.6 mmol) was added and the mixture was allowed to warm to room temperature. After 1.5 h the solvent was removed in vacuo and the residue was purified by flash chromatography (dichloromethane/methanol/acetic acid/water, 90:18:3:2). The product was partitioned between ethyl acetate (200 mL) and 10% sodium bicarbonate (150 mL), and the organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in ethyl acetate (25 mL) and treated with a saturated solution of HCl in ethyl acetate (3 mL). The precipitate was filtered off and dried to give 155 mg (54%) of **8** as a red solid: mp 186–190 °C; ^{1}H NMR (DMSO-d_6) 2.04–2.30 (2 H, m, $CH_2CH_2CH_2N(CH_3)_2$), 2.75–2.85 (6 H, m, $N(CH_3)_2$), 3.04–3.19 (2 H, m, $CH_2N(CH_3)_2$), 3.98 (3 H, s, NCH_3), 4.43 (2 H, t, J = 7 Hz, NCH_2), 6.70–6.88 (3 H, m, indole-H), 7.05 (1 H, d, J = 8 Hz, indole-H), 7.10–7.22 (2 H, m, indole-H), 7.54 (1 H, d, J = 8 Hz, indole-H), 7.64 (1 H, d, J = 8 Hz, indole-H), 7.87 (1 H, s, indole 2-H), 7.83 (1 H, s, indole 2-H), 10.55 (1 H, bs, $NH(Me)_2^+$), 11.06 (1 H, bs, imide); IR ϑ_{max} (nujol) 1700, 1750, and 2300–3600 cm^{-1}; MS m/z 426 (M^+). Analysis ($C_{26}H_{26}N_4O_2$·HCl) C, H, N.

Trimethyl[3-[3-[3-(1-methyl-3-indolyl)-2,5-dioxo-3-pyrrolin-4-yl]-1-indolyl]propyl]ammonium Trifluoromethanesulfonate (9). A suspension of 3-[1-(3-hydroxypropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (**5b**) (250 mg, 0.63 mmol) and 2,6-lutidine (134 mg, 1.25 mmol) in

dichloromethane (50 mL) was added to a stirred solution of trifluoromethanesulfonic anhydride (263 mg, 0.93 mmol) in dichloromethane (40 mL) at 0 °C under nitrogen. After 0.5 h a 33% solution of trimethylamine in ethanol (1 mL, 4.2 mmol) was added and the mixture was allowed to warm to room temperature. After 1.5 h the solvent was removed in vacuo, and the residue was purified by flash chromatography (dichloromethane/methanol, 9:1) to give 197 mg (59%) of a red gum. A sample was crystallized from water to give **9** as a red solid: mp 186–189 °C; ^{1}H NMR (DMSO-d_6) 2.09–2.26 (2 H, m, $CH_2CH_2CH_2N(CH_3)_3$), 3.04 (9 H, s, $N(CH_3)_3$), 3.28–3.40 (2 H, m, $CH_2N(CH_3)_3$), 3.88 (3 H, s, NCH_3), 4.27 (2 H, t, J = 7 Hz, NCH_2), 6.60–6.82 (3 H, m, indole-H), 6.94–7.05 (3 H, m, indole-H), 7.45 (1 H, d, J = 8 Hz, indole-H), 7.54 (1 H, d, J = 8 Hz, indole-H), 7.76 (1 H, s, indole 2-H), 7.79 (1 H, s, indole 2-H), 10.96 (1 H, bs, imide); IR ϑ_{max} (nujol) 1705, 1750, 2500–3700 cm^{-1}; MS m/z 440 (M^+); exact mass 441.2267 ($C_{27}H_{29}N_4O_2$ requires 441.2288).

3-(1-Methyl-3-indolyl)-4-[1-[3-(methylsulfonyloxy)propyl]-3-indolyl]-1*H*-pyrrole-2,5-dione (10b). Methanesulfonic anhydride (190 mg, 1.1 mmol) was added to a stirred solution of 3-[1-(3-hydroxypropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (**5b**) (400 mg, 1 mmol) in dichloromethane (40 mL) and pyridine (1 mL). After 2 h more pyridine (1 mL) and methanesulfonic anhydride (40 mg) were added and the solution obtained was stirred at room temperature for a further 18 h. The reaction mixture was washed with water (40 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from ethyl acetate/petrol to give (350 mg, 73%) of **10b** as a red solid: mp 202–4 °C; ^{1}H NMR (DMSO-d_6) 2.13 (2 H, quintet, J = 6.5 Hz, $CH_2CH_2CH_2OMs$), 3.16 (3 H, s, OSO_2CH_3), 3.87 (3 H, s, NCH_3), 4.15 (2 H, t, J = 6.5 Hz, CH_2OMs), 4.34 (2 H, t, J = 6.5 Hz, CH_2N), 6.49–6.78 (3 H, m, indole-H), 6.92 (1 H, d, J = 8 Hz, indole-H), 6.99–7.12 (2 H, m, indole-H), 7.43 (1 H, d, J = 8 Hz, indole-H), 7.50 (1 H, d, J = 8 Hz, indole-H), 7.73 (1 H, s, indole 2-H), 7.86 (1 H, s, indole 2-H), 10.95 (1 H, bs, NH); IR ϑ_{max} (nujol) 1695 and 1755 cm^{-1}; MS m/z 477 (M^+). Analysis ($C_{25}H_{23}N_3O_5S$) C, H, N.

3-[1-[3-(Amidinothio)propyl]-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Methanesulfonate (11b). A mixture of 3-(1-methyl-3-indolyl)-4-[1-[3-(methylsulfonyloxy)-propyl]-3-indolyl]-1*H*-pyrrole-2,5-dione (**10b**) (1.46 g, 3.05 mmol) and thiourea (364 mg, 4.78 mmol) in ethanol (45 mL) was heated to reflux under nitrogen for 18 h and then stirred at room temperature for 3 days. The solid formed was filtered off, washed with ethanol (50 mL), ether (50 mL), and dried in vacuo to give **11b** (1.33 g, 79%) as a red solid: mp 236–238 °C; ^{1}H NMR (DMSO-d_6) 1.95–2.10 (2 H, m, $CH_2CH_2CH_2S$), 2.39 (3 H, s, OMs), 2.97–3.07 (2 H, m, SCH_2), 3.87 (3 H, s, NCH_3), 4.23–4.38 (2 H, m, $N\text{-}CH_2$), 6.55–6.65 (2 H, m, indole-H), 6.76 (1 H, t, J = 8 Hz, indole-H), 6.97–7.13 (3 H, m, indole-H), 7.44 (1 H, d, J = 8 Hz, indole-H), 7.50 (1 H, d, J = 8 Hz, indole-H), 7.67 (1 H, s, indole 2-H), 7.88 (1 H, s, indole 2-H), 9.10 (4 H, bs, $SC(=NH_2^+)NH_2$), 10.95 (1 H, bs, imide NH); IR ϑ_{max} (nujol) 1695, 1755, and 2500–3500 cm^{-1}; MS m/z (DCI) 458 ([M + H]$^+$). Analysis ($C_{25}H_{23}N_5O_2S\cdot CH_4O_3S$) C, H, N.

3-(1-Methyl-3-indolyl)-4-[1-[3-(2-nitroguanidino)propyl]-3-indolyl]-1*H*-pyrrole-2,5-dione (12). A mixture of 3-[1-(3-aminopropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione acetate (**6b**) (500 mg, 1.1 mmol), sodium bicarbonate (110 mg, 1.32 mmol) and 3,5-dimethyl-N^2-nitro-1-pyrazole-1-carboxamidine (242 mg, 1.32 mmol) was heated in refluxing ethanol for 4 h. The solvent was removed, and the residue chromatographed on silica gel with a gradient of 1% to 5% methanol in dichloromethane to afford **12** as a red solid: mp 268–270 °C dec; yield 500 mg, 94%; ^{1}H NMR (DMSO-d_6) 1.9 (2 H, m, $CH_2CH_2CH_2$), 3.11 (2 H, m, CH_2CH_2NH), 3.87 (3 H, s, Me), 4.25 (2 H, t, CH_2-indole N), 6.6–6.7 (2 H, complex signal, indole-H), 6.72 (1 H, t, J = 7.5 Hz, indole-H), 6.94 (1 H, d, J = 7.5 Hz, indole-H), 7.0–7.1 (2 H, complex signal, indole-H), 7.49 (1 H, d, J = 7.5 Hz, indole-H), 7.75 (1 H, s, indole 2-H), 7.86 (1 H, s, indole 2-H), 10.93 (1 H, s, imide); IR ϑ_{max} (nujol) 1710, 1750, and 3000–3500 cm^{-1}; MS m/z 486 ([M + H]$^+$). Analysis ($C_{25}H_{23}N_7O_4\cdot 0.3MeOH$) C, H, N.

3-[1-(3-Guanidinopropyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Nitrate (13). A mixture of 3-[1-(3-aminopropyl)indolyl]-4-(1-methylindolyl)-1*H*-pyrrole-2,5-dione monoacetate (**6b**) (1.5 g, 3.0 mmol) and sodium bicarbonate (250 mg, 3 mmol) in refluxing ethanol (50 mL) was treated with 3,5-dimethylpyrazole-1-carboxamidine nitrate (900 mg, 4.47 mmol) for 16 h. The solvent was removed from the cooled solution, and the residue was chromatographed on silica gel with a gradient elution (1% to 10% methanol in dichloromethane) to afford **13** as a red solid: mp 179–181 °C; yield 844 mg, 56%; ^{1}H NMR (DMSO-d_6) 1.93 (2 H, m, $CH_2CH_2CH_2$), 3.07 (2 H, m, CH_2CH_2NH), 3.88 (3 H, s, Me), 4.25 (2 H, t, J = 7.5 Hz, CH_2-indole N), 6.60 (1 H, t, J = 7.5 Hz, indole-H), 6.63 (1 H, t, J = 7.5 Hz, indole-H), 6.22 (1 H, t, J = 7.5 Hz, indole-H), 6.8–7.3 (7 H, complex signal, indole-H and guanidinium), 7.43 (1 H, d, J = 7.5 Hz, indole-H), 7.48 (1 H, d, J = 7.5 Hz, indole-H), 7.59 (1 H, bt, J = 5 Hz, guanidinium), 7.72 (1 H, s, indole 2-H), 7.88 (1 H, s, indole 2-H), 10.94 (1 H, bs, imide); IR ϑ_{max} (nujol) 1650, 1690, 1755, and 3100–3400 cm^{-1}; exact mass 441.2000 (M + H)$^+$ ($C_{25}H_{25}N_6O_2$ (M + H)$^+$ requires 441.2037). Analysis ($C_{25}H_{24}N_6O_2\cdot HNO_3$) C, H, N.

3-[1-(4-Cyanobutyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (14). A solution of 4-(1-methyl-3-indolyl)-3-[1-[4-(methylsulfonyloxy)butyl]-3-indolyl]-1*H*-pyrrole-2,5-dione (**10c**) (387 mg, 0.79 mmol) in dry dimethyl sulfoxide (20 mL) was treated with sodium cyanide (116 mg, 2.36 mmol) and heated at 50 °C under a nitrogen atmosphere for 8 h. The cooled mixture was diluted with water (100 mL) and extracted with ethyl acetate (3 × 20 mL), and the combined extracts were dried ($MgSO_4$) and evaporated. The residue was subjected to flash chromatography on silica gel with toluene/acetic acid (9:1) as eluent. The red product was triturated with ether to give **14** (37 mg, 11%): mp 195–198 °C; ^{1}H NMR (DMSO-d_6) 1.45 (2 H, m, CH_2), 1.8 (2 H, m, CH_2), 2.5 (2 H, t, J = 7 Hz, CH_2CN), 3.85 (3 H, s, $N\text{-}CH_3$), 4.25 (2 H, t, J = 7 Hz, NCH_2), 6.6 (2 H, m, indole-H), 6.7 (1 H, t, J = 8 Hz, indole-H), 6.9 (1 H, d, J = 8 Hz, indole-H), 7.0 (2 H, m, indole-H), 7.4 (1 H, d, J = 8 Hz, indole-H), 7.5 (1 H, d, J = 8 Hz, indole-H), 7.7 (1 H, s, indole 2-H), 7.9 (1 H, s, indole 2-H), 10.9 (1 H, bs, imide); IR ϑ_{max} (nujol) 1690 and 1755 cm^{-1}; MS m/z 422 (M^+). Analysis ($C_{25}H_{25}N_6O_2\cdot 0.3C_2H_4O_2$) C, H, N.

3-[1-(4-Amidinobutyl)-3-indolyl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (15). An ice-cooled solution of **14** (150 mg, 0.35 mmol) in dry ethanol (20 mL) was saturated with hydrogen chloride and left at ambient temperature for 18 h. The solvent was evaporated and the residue redissolved in dry ethanol (20 mL). The solution was cooled in ice, saturated with ammonia, and left at ambient temperature for 18 h. The solvent was evaporated and the residue subjected to flash chromatography with CH_2Cl_2/MeOH/AcOH/H_2O (120:14:3:2) as eluent. The red product was suspended in ethyl acetate (1 mL), treated with saturated HCl ethyl acetate (1 mL), and stirred 30 min. The suspension was filtered and dried to give 123 mg (73%) **15** as its hydrochloride: mp 133–136 °C dec; ^{1}H NMR (DMSO-d_6) 1.55 (2 H, m, CH_2), 1.75 (2 H, m, CH_2), 2.35 (2 H, t, J = 7 Hz, CH_2-amidine), 3.85 (3 H, s, $N\text{-}CH_3$), 4.25 (2 H, t, J = 7 Hz, $N\text{-}CH_2$), 6.6 (3 H, m, indole-H), 6.9 (1 H, d, J = 8 Hz, indole-H), 7.0 (2 H, t, J = 8 Hz, indole-H), 7.4 (1 H, d, J = 8 Hz, indole-H), 7.5 (1 H, d, J = 8 Hz, indole-H), 7.75 (1 H, s, indole 2-H), 7.85 (1 H, s, indole 2-H), 8.8 (2 H, bs, NH_2), 9.5 (2 H, bs, NH_2^+), 10.9 (1 H, bs, imide); IR ϑ_{max} (nujol) 1690, 1705, 1750, and 3000–3400 cm^{-1}; MS m/z (FAB + ve) 440 ([M + H]$^+$). Analysis ($C_{26}H_{25}N_5O_2\cdot 1.2HCl$) C, H, N, Cl.

Molecular Modeling. Template forcing studies were performed within MOLOC.[27] Structures were displayed on a Silicon Graphics IRIS workstation with RASTER3D as modified by Ethan A. Merritt, Dept. of Biological Structure, University of Washington SM-20, Seattle, Washington 98195 (available electronically from anonymous ftp site: xrays.bchem.washington.edu (128.208.112.3)). MOPACv5 is available from the Quantum Chemistry Program Exchange (QCPE 455).

Inhibition of Rat Brain PKC. Compounds were assayed for PKC inhibitory activity as described previously.[23] In each assay, data points were determined in triplicate and the quoted IC_{50} values are the mean of at least two independent assay results. Replicate independent determinations performed for **6b** gave an

(27) Gerber, P. R.; Gubernator, K.; Mueller, K. Generic Shapes for the Conformation Analysis of Macrocyclic Structures. *Helv. Chim. Acta* **1988**, *71*, 1429–1441.

IC_{50} value of 0.075 ± 0.019 μM (17 determinations).

Inhibition of Bovine Heart PKA. Compounds were assayed for PKA inhibitory activity as described previously.[23] In each assay, data points were determined in triplicate and the quoted IC_{50} values are the mean of at least two independent assay results. Replicate independent determinations performed for **6b** gave an IC_{50} value of 5.2 ± 1.2 μM (12 determinations).

Mixed Lymphocyte Reaction. MLR assays were performed by the method of Fitzharris and Knight.[28] In each assay, data points were determined in triplicate and the quoted IC_{50} values are the mean of at least four independent assay results. Replicate independent determinations performed for **6b** gave an IC_{50} value of 2.0 ± 0.7 μM (five determinations).

Plasma Level Determinations. Starved, female AP1 rats were dosed orally with compound at 100 mg/kg, wet-milled in 10% succinylated gelatin or intravenously with compound at 20 mg/kg dissolved in propylene glycol. Blood samples were taken by cardiac puncture at appropriate time intervals and centrifuged. Samples of the supernatant plasma (1.5 mL) were extracted with three portions of ethyl acetate (1.5 mL each). The combined extracts were evaporated to dryness (water bath at 60 °C), and the residue was dissolved in DMSO (0.2 mL). Levels of inhibitor were measured in the standard PKC assay by reference to a standard curve prepared from plasma samples spiked with known amounts of inhibitor. Graph points are the mean of triplicate determinations.

(28) Fitzharris, P.; Knight, R. A. Generation of Suppressor Cells in the Autologous Mixed Lymphocyte Reaction. *Clin. Exp. Immunol.* **1981**, *46*, 185–195.

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Registry No. **3a**, 125315-16-4; **3b**, 125314-98-9; **3c**, 138489-06-2; **3d**, 138489-07-3; **4a**, 125315-15-3; **4b**, 125314-97-8; **4c**, 138489-08-4; **4d**, 138489-09-5; **5a**, 125314-59-2; **5b**, 125313-60-2; **5c**, 125313-79-3; **5d**, 125313-81-7; **6a**, 138516-30-0; **6b**, 138516-31-1; **6c**, 138489-11-9; **6d**, 138516-32-2; **7**, 138489-12-0; **8**, 138489-13-1; **9**, 125811-23-6; **10a**, 138489-14-2; **10b**, 125315-06-2; **10c**, 138489-15-3; **10d**, 138489-16-4; **11a**, 138489-17-5; **11b**, 138489-18-6; **11c**, 138489-19-7; **11d**, 138489-20-0; **12**, 125314-46-7; **13**, 138489-21-1; **14**, 125314-73-0; **15**, 138489-22-2; $Br(CH_2)_3OAc$, 592-33-6; $Br(CH_2)_4OAc$, 4753-59-7; $Br(CH_2)_5OAc$, 15848-22-3; indole, 120-72-9; ethylene oxide, 75-21-8; 1-methylindole-3-acetic acid, 1912-48-7; thiourea, 627-56-5; 3,5-dimethyl-N^2-nitro-1-pyrazole-1-carboxamidine, 2946-89-6; 3,5-dimethylpyrazole-1-carboxamidine nitrate, 38184-47-3; 1-(2-hydroxyethyl)indole, 121459-15-2.

Supplementary Material Available: A listing of the coordinates of models shown in Figure 1 (structure factors) (5 pages). Ordering information is given on any current masthead page.