Inhibitors of Protein Kinase C. 3. Potent and Highly Selective Bisindolylmaleimides by Conformational Restriction

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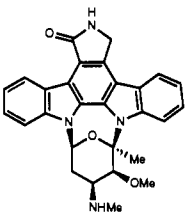
The protein kinase inhibitor staurosporine has been used to design a series of selective bisindolylmaleimide inhibitors of protein kinase C (PKC). Guided by molecular graphics, conformational restriction of the cationic side chain has led to ATP competitive inhibitors of improved potency and selectivity. Two compounds have been further evaluated and were shown to inhibit PKC of human origin and prevent T-cell activation in a human allogeneic mixed lymphocyte reaction. One of these compounds was orally absorbed in mice and antagonized a phorbol ester induced paw edema in a dose-dependent manner. This compound also selectively inhibited the secondary T-cell mediated response in a developing adjuvant arthritis model in rats and provides evidence for the potential use of PKC inhibitors as therapeutic immunomodulators.

Introduction

The protein kinase C (PKC) isoenzyme family[1,2] plays a pivotal role in the signal transduction pathways of a variety of hormones, cytokines, neurotransmitters, and growth factors.[3] Activation of PKC following receptor occupation leads to catalytic transfer of the γ-phosphate of ATP to serine or threonine in acceptor proteins. By altering the properties of these protein substrates PKC appears to modulate mechanisms of cell proliferation and gene expression[4] and is implicated in the pathogenesis of a variety of diseases.[5-9] In light of the proposed involvement of PKC in T-cell activation[10] and proliferation[3] we are particularly interested in the therapeutic potential of PKC inhibitors for the treatment of autoimmune diseases, such as rheumatoid arthritis.

We have described[11] a series of bisindolylmaleimide PKC inhibitors (e.g. **2a**) which are more selective than the protein kinase inhibitor staurosporine (1). These earlier inhibitors are less potent than staurosporine and only modestly selective for PKC over phosphorylase kinase. Subsequently we have shown that the introduction of a cationic side chain into these compounds resulted in inhibitors of greater potency and selectivity.[12] The most potent of these (**2c**) is an isothiourea, which is as potent as staurosporine against PKC but diplays a considerably better selectivity profile. However, this compound is not readily absorbed on oral dosing to rats. In contrast, the amine **2b** was found to be orally absorbed in rats and was therefore chosen as a promising lead, despite its lower in vitro potency.



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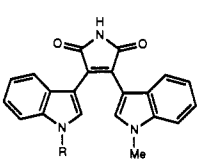

2a R = CH_3
2b R = $(CH_2)_3NH_2$
2c R = $(CH_2)_3S(C{=}NH)NH_2$

To improve upon the potency of this amine a conformational restriction approach was adopted. In our previous study we have demonstrated that small lipophilic substituents are tolerated at the 2-, 4-, 5-, and 7-positions around one indole ring.[11] Modeling studies suggested that formation of a ring between the indole nitrogen and 2-position would result in an ideal template for attaching cationic substituents which should be able to access the putative amine binding site. To test this theory the parent tetrahydropyrido[1,2-*a*]indole **3b** was constructed[13] and converted into the desired bisindolylmaleimide **5b** (Scheme I). The activity of this compound against PKC (IC_{50} 155 nM) demonstrated that the six-membered ring was well tolerated and could be used for the design of further inhibitors. Both five- and seven-membered ring analogues **5a** and **5c** were also well accommodated.

In our earlier work we postulated that the bisindolylmaleimides and staurosporine adopted a common mode

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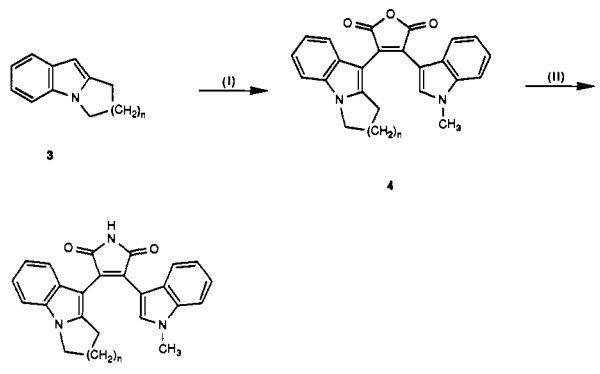
Scheme I [a]



		Yield %	
	n	(i)	(ii)
a	1	8	77
b	2	10	70
c	3	23	86

[a] Conditions: (i) 1. $(COCl)_2$; 2. 1-methylindole-3-acetic acid, triethylamine; (ii) 33% aqueous ammonia, DMF, 140 °C.

of binding. On the basis of common structure–activity relationships,[12,14] we have again chosen to model the bisindolylmaleimides in a low-energy conformation which best approximates the staurosporine aglycon. The spatial position of the methylamino nitrogen in the staurosporine pyranose ring was then used as a guide for inhibitor design.

Both conformations of the staurosporine sugar moiety[15] were initially considered when undertaking the modeling studies, and compounds were designed with amino substituents attached to the "template" ring. An example of this is shown in Figure 1a where an aminomethyl group is attached to the 7-position of the tetrahydropyridoindole in the preferred pseudoequatorial orientation. The amino group has then been modeled in low-energy conformations that place the nitrogen proximal to the position of the amino group in either staurosporine boat or chair conformer. The best fit for this compound was found to be for the boat amine, where the amino group could be brought to within 0.5 Å in a conformation of 0.8 kcal mol^{-1} above the global minimum, compared to 2.1 Å with 6.4 kcal mol^{-1} enthalpy penalty for the chair amine. This process was repeated for the enantiomer (Figure 1b), which also fitted better to the boat amine (0.5 Å, 1.0 kcal mol^{-1}) than to the chair (2.3 Å, 6.4 kcal mol^{-1}). Figures 1c and 1d show the corresponding 8-aminomethyl-substituted compounds **23a** and **23b**, which both fit better to the boat amine position than to the chair. Compounds based on smaller (**9a**) and larger (**9f–h**) rings were also modeled, together with compounds possessing shorter (**13**) and longer (**9c** and **9e**) substituents.

Conformationally restricted analogues of amine **2b** were thus designed which should be able to interact with the putative amine binding site.

Chemistry

The required bisindolylmaleimides bearing a conformationally restricted side chain were prepared from the corresponding 3-unsubstituted indoles.[13] These were coupled with 1-methylindole-3-acetic acid[16] to give the desired maleic anhydrides **4**, **7**, **11**, **16** and **22**, which were converted into the required imides with either ammonia or HMDS/methanol.[17] Those compounds substituted by an acetoxyalkyl group (Scheme II) were converted into the required primary amines **9** via alcohols **8**. The alcohol **8b** was also used as an intermediate for the preparation of the alkylated amines **18** (Scheme V).

The protected amines **10** and **15** were coupled by the same procedure as used for the acetate **6**. The maleic anhydrides **11** were then converted into imides **12**, followed by deprotection to the desired amines **13** (Scheme III). The maleic anhydride product from coupling **15** was first deprotected to give amine **16** and then converted into the imide **17** (Scheme IV).

Both enantiomers **23** of the most active compound **9b** were prepared via chromatographic separation followed by crystallization of the menthyl esters **20** (Scheme VI). The homochiral acetates were then converted into the final products **23** by a similar procedure to that described above.

Results and Discussion

Amines **9a–f** all showed improved inhibitory potency (Table I) over the parent bisindolylmaleimides **5** (IC_{50}s 155–450 nM) and therefore confirm our previous conclusion that the amino groups are able to interact favorably with the enzyme.[12] In addition these conformationally restricted amines all demonstrate increased activity against the isolated enzyme when compared to the more flexible analogue **2b** (IC_{50} 75 nM).[12] This is consistent with the beneficial entropy gain associated with a conformational restriction approach.[18]

Both enantiomers (**23a** and **23b**) of the most active inhibitor **9b** (IC_{50} 7.6 nM) were synthesized and both were shown to be more potent than the parent unsubstituted bisindolylmaleimides **5**. This was predicted by modeling studies (Figures 1c and 1d) of the enantiomers which showed that both compounds are able to access the proposed common amine binding site recognized by staurosporine. This is to be expected since it is likely that the tetrahydropyridoindole moiety of each enantiomer will adopt a half chair conformation, with the aminoalkyl substituent adopting the thermodynamically preferred pseudoequatorial position. The amine group for each enantiomer can therefore occupy a similar spatial position; however, any effects due to the steric differences between the two half chairs cannot be predicted.

The absolute stereochemistry of the more active enantiomer (**23a**) was unambiguously established as *S* by X-ray crystallography of the intermediate menthyl ester (**20a**, Figure 2). The higher potency of this homochiral compound (**23a**) over its enantiomer is consistent with the better fit for the *S* enantiomer (1.3 Å with a 2.3 kcal mol^{-1} enthalpy penalty) over the *R* enantiomer (1.3 Å, 4.7 kcal mol^{-1}) in the graphics model (Figures 1c and 1d).

Both enantiomers of the aminoalkyl compound **9d** also fit well when modeled onto the amine of the boat form of

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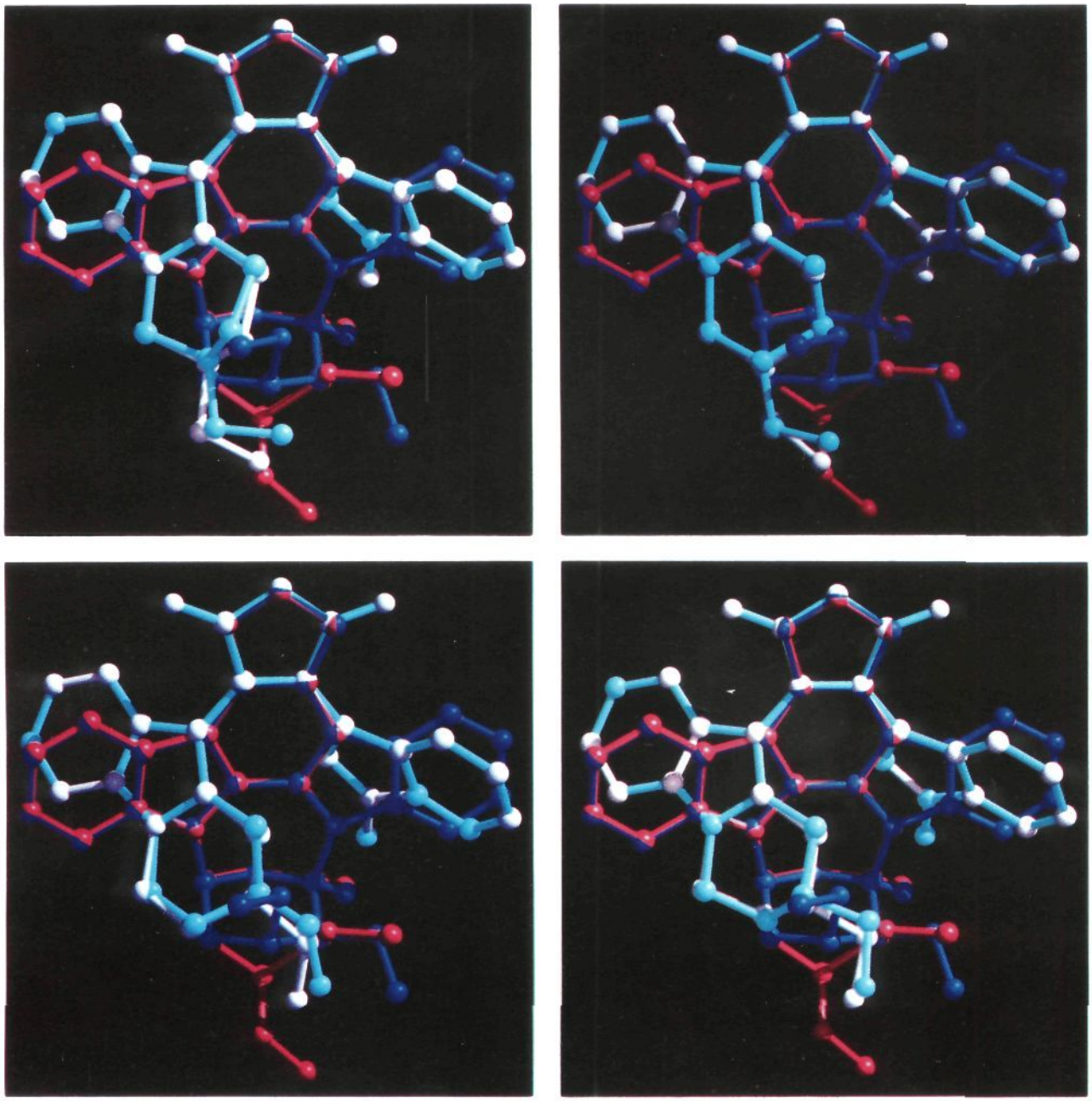
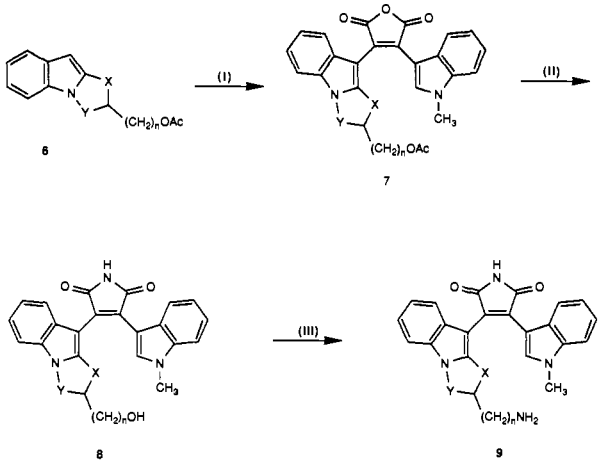


Figure 1. Both conformers of staurosporine (blue, chair and orange, boat) overlayed with modeled conformations of bisindolylmaleimides by matching lactam and imide rings. (a, top left) *S* enantiomer of compound **9d** in low-energy conformations (white and cyan) modeled with an amino group near to the corresponding position in space of the staurosporine boat and chair amines, respectively. (b, top right) *R* enantiomer of compound **9d** in low-energy conformations (white and cyan) modeled with an amino group near to the corresponding position in space of the staurosporine boat and chair amines, respectively. (c, bottom left) *S* enantiomer (**23a**) of compound **9b** in low-energy conformations (white and cyan) modeled with an amino group near to the corresponding position in space of the staurosporine boat and chair amines, respectively. (d, bottom right) *R* enantiomer (**23b**) of compound **9b** in low-energy conformations (white and cyan) modeled with an amino group near to the corresponding position in space of the staurosporine boat and chair amines, respectively.

staurosporine, but neither show a good fit onto the "chair" amine. Since the racemic mixture **9d** has been shown to retain good inhibitory activity, it would appear that the boat form of staurosporine is the best model for bisindolylmaleimides. This confirms our earlier findings and suggests that the protonated boat form of staurosporine is the bioactive conformation. However, the "goodness of fit" for the enantiomers of **9d** are better than those for **9b**, whereas **9b** is a more potent inhibitor of PKC. Clearly the spatial position of the staurosporine boat nitrogen is not optimal for our inhibitors and an amino pharmacophore model, based upon our most potent compounds, will need to be established for the design of future inhibitors.

As expected, compounds with longer aminoalkyl side chains (**9c** and **9e**) also retain good inhibitory activity, as do some of those with different sized template rings (**9a** and **9f**). However, those compounds with an amino group directly attached to the ring (**13**) lose inhibitory activity associated with the amine binding and are only as active as the parent systems **5**. These results are consistent with the modeling studies as exemplified by compound **13a**, which does not overlay well onto the amine of the

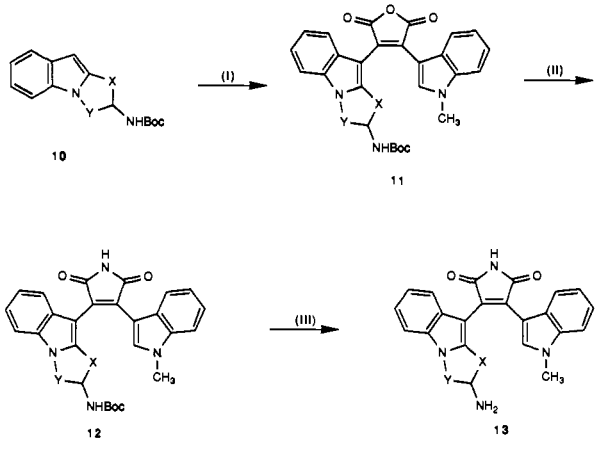
Scheme II[a]



	X	Y	n	yield % (I)	(II)	(III)
a	CH_2	CH_2	1	19	75	23
b	CH_2	CH_2CH_2	1	32	94	52
c	CH_2	CH_2CH_2	2	15	61	8
d	CH_2CH_2	CH_2	1	32	64	57
e	CH_2CH_2	CH_2	2	21	74	60
f	CH_2CH_2	CH_2CH_2	1	40	33	55
g	$CH_2CH_2CH_2$	CH_2	1	32	61	46
h	CH_2	$CH_2CH_2CH_2$	1	44	44	33

[a] Conditions: (i) 1. $(COCl)_2$; 2. 1-methylindole-3-acetic acid, triethylamine; (ii) 33% aqueous ammonia, DMF, 140 °C; (iii) 1. collidine, triflic anhydride; 2. 33% aqueous ammonia.

Scheme III[a]



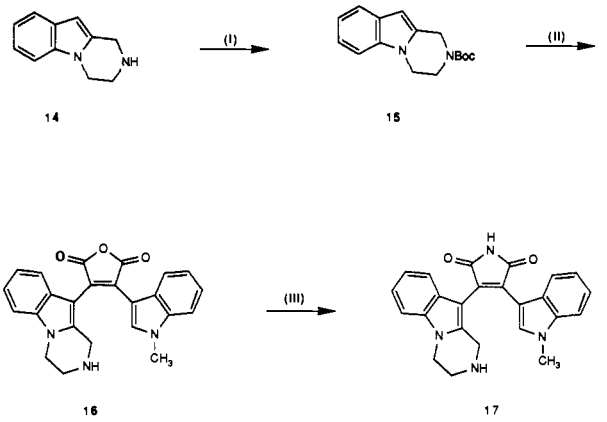
	X	Y	yield % (I)	(II)	(III)
a	CH_2	CH_2CH_2	20	85	70
b	CH_2CH_2	CH_2	24	82	67
c	CH_2CH_2	CH_2CH_2	24	80	77

[a] Conditions: (i) 1. $(COCl)_2$; 2. 1-methylindole-3-acetic acid, triethylamine; (ii) HMDS, MeOH; (iii) HCl/ethyl acetate.

staurosporine boat conformer (*S* enantiomer, 2.3 Å with a 3.6 kcal mol^{-1} enthalpy penalty; *R* enantiomer, 2.4 Å, 5.4 kcal mol^{-1}). Indeed, the same is true for **17**, where the nitrogen is incorporated into the ring, which does not match well onto staurosporine boat amine (3.2 Å, 1.6 kcal mol^{-1}), and is unable to access the putative amine binding site. Staurosporine has therefore proved to be a useful tool for localizing the amine binding site for our bisindolylmaleimides, but it is likely that future extensions of this work will require a more sophisticated model.

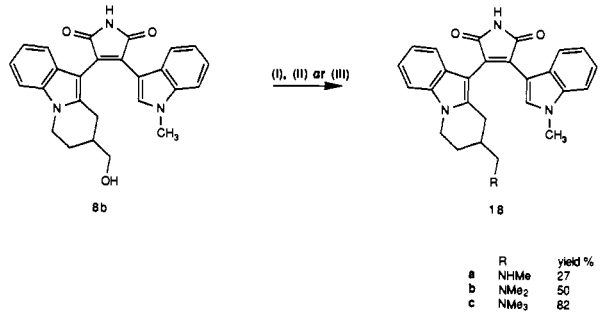
The most potent compound, primary amine **9b**, was found to be competitive with respect to ATP (K_i = 4.3 nM) in common with earlier bisindolylmaleimides[19] and

Scheme IV[a]



[a] Conditions: (i) di-*tert*-butyl dicarbonate, triethylamine, 82%; (ii) 1. $(COCl)_2$; 2. 1-methylindole-3-acetic acid, triethylamine; 3. HCl/ethyl acetate, 29%; (iii) 33% aqueous ammonia, DMF, 80 °C, 78%.

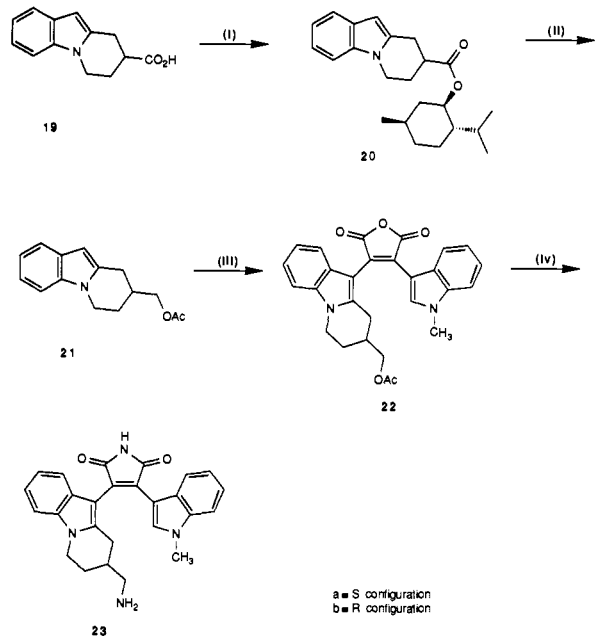
Scheme V [a]



	R	yield %
a	NHMe	27
b	NMe_2	50
c	NMe_3	82

[a] Conditions: (i) 1. collidine, triflic anhydride; 2. methylamine; (ii) 1. collidine, triflic anhydride; 2. dimethylamine; (iii) 1. collidine, triflic anhydride; 2. trimethylamine.

Scheme VI [a]



[a] Conditions: (i) DCC, DMAP, *l*-menthol, separate; (ii) 1. LAH; 2. Ac_2O, NEt_3; (iii) 1. $(COCl)_2$; 2. 1-methylindole-3-acetic acid, triethylamine; (iv) 1. NH_4OH/DMF, 140 °C; 2. collidine, triflic anhydride; 3. 33% aqueous ammonia.

is also a potent inhibitor of human derived PKC (Table II). Despite the strong sequence homology in the ATP binding regions,[20] both **9b** and **18b** are also highly selective

Table I. Inhibition of PKC

no.	formula	anal.	IC_{50} vs PKC (nM)	IC_{50} vs PKA (μM)
	staurosporine		9 ± 1 (3)	0.121 ± 0.018 (2)
2b	$C_{24}H_{22}N_4O_2 \cdot C_2H_4O_2$	C,H,N	75 ± 19 (17)	5.2 ± 1.2 (12)
5a	$C_{24}H_{19}N_3O_2 \cdot 0.2H_2O$	C,H,N	210 ± 0 (2)	68 ± 50[b] (2)
5b	$C_{25}H_{21}N_3O_2$	C,H,N	155 ± 7 (2)	23 ± 9.9 (2)
5c	$C_{26}H_{23}N_3O_2$	C,H,N	450 ± 200[b] (3)	>100[b] (2)
9a	$C_{25}H_{22}N_4O_2 \cdot 0.65H_2O$	C,H,N	14 ± 8 (2)	3.9 ± 0.5 (2)
9b	$C_{26}H_{24}N_4O_2 \cdot HCl$	C,H,N	7.6 ± 0.2 (10)	2.8 ± 1.2 (6)
9c	$C_{27}H_{26}N_4O_2$	a	47 ± 0.7 (2)	24 ± 8.5 (2)
9d	$C_{26}H_{24}N_4O_2$	a	15 ± 0 (2)	1.1 ± 0.4 (2)
9e	$C_{27}H_{26}N_4O_2 \cdot HCl$	C,H,N	23 ± 1 (2)	4.5 ± 0.7 (2)
9f	$C_{27}H_{26}N_4O_2 \cdot 2.15C_2H_4O_2$	C,H,N	16.5 ± 1.5 (2)	2.6 ± 1.1 (3)
9g	$C_{27}H_{26}N_4O_2$	a	400 ± 113 (2)	12.8 ± 1.1 (2)
9h	$C_{27}H_{26}N_4O_2 \cdot 2CH_3CO_2H$	C,H,N	272 ± 3 (2)	34 ± 8.5 (2)
13a	$C_{25}H_{22}N_4O_2$	a	110 ± 14 (2)	3.3 ± 0.8 (2)
13b	$C_{25}H_{22}N_4O_2$	a	135 ± 21 (2)	4.1 ± 2.1 (2)
13c	$C_{26}H_{24}N_4O_2$	C,H,N	100 ± 28 (2)	2.4 ± 0.5 (2)
17	$C_{24}H_{20}N_4O_2$	C,H,N	540 ± 60 (2)	3.2 ± 0.2 (2)
18a	$C_{27}H_{26}N_4O_2 \cdot 1.3HCl$	C,H,N	9.8 ± 0.2 (2)	4.8 ± 1.1 (2)
18b	$C_{28}H_{28}N_4O_2 \cdot HCl$	C,H,N	42 ± 8 (5)	8.6 ± 3.9 (4)
18c	$C_{30}H_{31}N_4O_5F_3S \cdot 0.2H_2O$	C,H,N	49 ± 2 (2)	92.5 ± 3.5 (2)
23a	$C_{26}H_{24}N_4O_2 \cdot C_2H_4O_2$	C,H,N	4.1 ± 1 (2)	12.1 ± 2.8 (2)
23b	$C_{26}H_{24}N_4O_2 \cdot C_2H_4O_2$	C,H,N	10 ± 3 (2)	0.9 ± 0.4 (2)

[a] Characterized by high-resolution mass spectrometry. Homogeneous by thin-layer chromatography. [b] Poorly soluble at inhibitory concentrations.

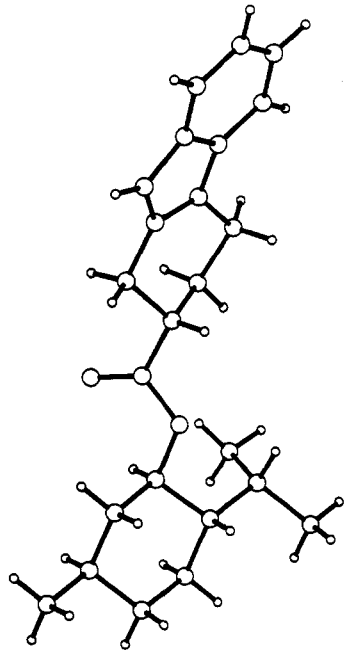


Figure 2. X-ray crystal structure of **20a**.

for PKC over other closely related serine/threonine protein kinases and tyrosine specific protein kinases (Table II). Monomethylation of the primary amine group has little effect on the activity against PKC (e.g **18a** IC_{50} = 9.8 nM versus **9b** IC_{50} = 7.6 nM), but the corresponding tertiary and quaternary amines **18b** and **18c** are about 5-fold less active, possibly due to steric effects. The quaternary amine **18c** is particularly noteworthy in view of its 2000-fold selectivity for PKC over PKA (Table I); however this compound is unable to penetrate cells efficiently.

Amines **9b** and **18b** were further evaluated in cellular systems and found to dose dependently antagonize a TPA-stimulated 47-kDa protein phosphorylation in platelets,[21] TPA-stimulated CD3 down regulation in T-cells,[22] and an allogeneic mixed lymphocyte reaction[23] (Table III). The reduced potency of these ATP-competitive inhibitors observed in the cellular assays can be explained by the higher concentration of ATP present in cells (1–10 mM) compared to the isolated enzyme assay (10 μM). Both compounds were also evaluated in vivo by oral administration to mice at 100 mg kg^{-1}. The PKC-inhibitory activity in plasma following oral dosing of primary amine **9b** was found to be relatively low, and peak inhibitory activity was little more than a cellular IC_{50} value.[24] In contrast the peak inhibitory activity of tertiary amine **18b** following oral administration was equivalent to 30 μM **18b**, at least 15-fold higher than the cellular IC_{50} value. The reason for this difference is not clear in the absence of a detailed pharmacokinetic study; however, it is possible that the tertiary amine is better absorbed. Indeed, the measured apparent partition coefficient for the primary amine ($\log D = 0.04$) is sufficiently different from that of the tertiary amine ($\log D = 0.61$) for there to be a difference in absorption kinetics. In addition, a common route for the metabolism of tertiary amines is by N-dealkylation[25] which could result in the production of more potent metabolites for this compound. Compound **18b** was thus selected as the more promising lead for in vivo evaluation and was found to produce a dose-dependent inhibition of a phorbol ester-induced paw edema in mice with an MED of 15 mg kg^{-1}.[26] This tertiary amine also selectively inhibited the immune mediated secondary inflammation in a developing adjuvant arthritis model in the rat (MED = 12.5 mg kg^{-1}).[27]

In conclusion, a series of potent and selective PKC inhibitors has been designed from the parent bisindolylmaleimides using staurosporine as a guide. Conformational restriction of an aminoalkyl side chain has localized the amine binding site and should allow an amino pharmacophore model to be identified for our inhibitors. This will permit the design of more potent inhibitors of PKC, possibly by further conformational restriction of the amine side chain.

These compounds are able to antagonize PKC in an intracellular environment and amine **18b** has been shown to be orally active in in vivo models of inflammation. The results suggest that these inhibitors could prove to be of therapeutic benefit in the treatment of autoimmune diseases such as rheumatoid arthritis.

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Table II. Selectivity Profile for Amines 9b and 18b

	IC50 (μM)		
	9b	18b	staurosporine
rat brain PKC	0.0076 ± 0.0002 (10)	0.042 ± 0.008 (5)	0.009 ± 0.001 (3)
human platelet PKC	0.012 ± 0.005 (4)	0.045 ± 0.004 (2)	not determined
human neutrophil PKC	0.0048 ± 0.0015 (6)	0.032 (1)	0.0025 ± 0.0004 (2)
human neutrophil PKA	1.2 ± 0.5 (5)	5.9 ± 0.8 (3)	0.019 (1)
phosphorylase kinase (PhK)	1.3 ± 0.2 (3)	1.1 ± 0.4 (5)	0.0005 ± 0.0002 (2)
myosin light chain kinase (MLCK)	3.7 ± 1.0 (3)	17.8 ± 0.4 (2)	0.040 ± 0.013 (3)
casein Kinase II	>100 (2)	>100 (2)	7 ± 5.6 (2)
$p56^{lck}$	>100 (2)	>100 (2)	2.1 (1)
$p60^{src}$	>100 (2)	>100 (2)	3 (1)

Table III. Cellular Activities for Amines 9b and 18b

	p47 phosphorylation IC50 (μM)	CD3 down-regulation IC50 (μM)	mixed lymphocyte reaction IC50 (μM)
9b	0.46 ± 0.26 (3)	0.6 ± 0.3 (3)	0.47 ± 0.43 (4)
18b	0.95 ± 0.17 (3)	2.0 ± 0 (2)	0.54 ± 0.22 (3)

Experimental Section

General. Melting points were determined on a Büchi 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. ^{1}H NMR spectra were recorded on a Bruker AC-250, a Bruker WM-300, or a Bruker AM-400 spectrometer and chemical shifts are given in ppm (δ) from tetramethylsilane as internal standard. IR spectra were recorded on either a Perkin-Elmer Model 782 spectrometer or a Nicolet Model 520 FT-IR spectrometer. Optical rotations were measured in a Perkin-Elmer 241 polarimeter.

3-[6,7,8,9-Tetrahydropyrido[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)furan-2,5-dione (4b). A stirred, ice-cooled solution of 6,7,8,9-tetrahydropyrido[1,2-*a*]indole (450 mg, 2.6 mmol) in anhydrous dichloromethane (13 mL) was treated dropwise with oxalyl chloride (350 mg, 2.8 mmol). After 2 h solvent was evaporated and the residue was dissolved in dichloromethane (26 mL). The solution was added dropwise to a stirred solution of 1-methylindole-3-acetic acid (497 mg, 2.63 mmol) and triethylamine (0.73 mL) in dichloromethane (26 mL) and the mixture was stirred at room temperature for 60 h. The solvent was removed by evaporation and the residue purified by flash chromatography (dichloromethane). Trituration with ethyl acetate gave 100 mg (10%) of **4b** as a red solid: mp 276–278 °C; ^{1}H NMR (DMSO-d_6, 353 K) δ 1.43–1.53 (2 H, m), 1.76–1.86 (2 H, m), 2.24–2.39 (2 H, bm), 3.91 (3 H, s, NCH_3), 4.05 (2 H, t, J = 5 Hz, NCH_2), 6.42 (1 H, d, J = 8 Hz, indole-H), 6.68 (1 H, t, J = 8 Hz, indole-H), 7.01 (1 H, t, J = 8 Hz, indole-H), 7.08–7.19 (2 H, m, indole-H), 7.36–7.45 (3 H, m, indole-H), 8.09 (1 H, s, indole-2H); IR ν_{max} (Nujol) 1760, 1820 cm^{-1}; MS m/z 396 (M^+). Anal. ($C_{25}H_{20}N_2O_3$) C, H, N.

3-[6,7,8,9-Tetrahydropyrido[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (5b). A solution of **4b** (72 mg, 0.18 mmol) in a mixture of *N*,*N*-dimethylformamide (5 mL) and 0.880 aqueous ammonia (5 mL) was heated at 140 °C in a sealed vessel for 4 h. The cooled suspension was filtered and the solid dried in vacuo to give 50 mg (70%) **5b** as a red solid: mp 286–289 °C; ^{1}H NMR (DMSO-d_6, 343 K) δ 1.35–1.55 (2 H, bm), 1.70–1.85 (2 H, bm), 2.10–2.45 (2 H, bm), 3.87 (3 H, s, NCH_3), 4.02 (2 H, t, J = 5 Hz, NCH_2), 6.38 (1 H, d, J = 8 Hz, indole-H), 6.60 (1 H, t, J = 8 Hz, indole-H), 6.98 (1 H, t, J = 8 Hz, indole-H), 7.02–7.15 (2 H, m, indole-H), 7.30–7.45 (3 H, m, indole-H), 7.95 (1 H, s, indole-2H), 10.58 (1 H, bs, imide-H); IR ν_{max} (Nujol) 1690, 1745 cm^{-1}; MS m/z 395 (M^+). Anal. ($C_{25}H_{21}N_3O_2$) C, H, N.

3-[8-(Acetoxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)furan-2,5-dione (7b). A stirred, ice-cooled solution of 8-(acetoxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indole (60 g, 0.247 mol) in anhydrous diethyl ether (0.9 L) was treated dropwise with oxalyl chloride (23.7 mL, 0.271 mol) during 10 min. After 0.5 h solvent was evaporated and the residue reevaporated with toluene (400 mL) and dissolved in dichloromethane (0.5 L). The solution was added dropwise to a stirred, ice-cooled solution of 1-methylindole-3-acetic acid (46.7 g, 0.247 mol) and triethylamine (69 mL, 0.497 mol) in dichloromethane (0.5 L). Cooling was removed, and the solution was stirred 65 h and then washed with 5% aqueous sodium bicarbonate (2 × 0.5 L) and saturated aqueous sodium chloride (0.5 L). The solution was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (ethyl acetate/hexane, 1:19). Crystallization from ethyl acetate/hexane gave 36.65 g (32%) **7b** as a red solid: mp 192–195 °C; ^{1}H NMR (DMSO-d_6, 343 K) δ 1.45–1.6 (1 H, bm), 1.7–1.85 (1 H, bm), 1.9–2.05 (2 H, bm), 1.9 (3 H, s, OAc), 2.5–2.6 (1 H, bm), 3.65–3.8 (2 H, m, CH_2OAc), 3.8–3.9 (1 H, m, CHN), 3.86 (3 H, s, NCH_3), 4.25–4.35 (1 H, m, CHN), 6.4 (1 H, d, J = 8 Hz, indole-H), 6.65 (1 H, t, J = 8 Hz, indole-H), 7.0 (1 H, t, J = 8 Hz, indole-H), 7.05–7.15 (2 H, m, indole-H), 7.45–7.5 (3 H, m, indole-H), 8.07 (1 H, s, indole-2H); IR ν_{max} (Nujol) 1745, 1820 cm^{-1}; MS m/z 468 (M^+). Anal. ($C_{28}H_{24}N_2O_5 \cdot 0.2C_4H_8O_2$) C, H, N.

3-[8-(Hydroxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (8b). A solution of **7b** (28.6 g, 0.061 mol) in a mixture of *N*,*N*-dimethylformamide (150 mL) and 0.880 aqueous ammonia (200 mL) was heated at 140 °C in a sealed vessel for 9 h. The cooled suspension was filtered, and the solid was washed with water and dried in vacuo at 100 °C to give 24.46 g (94%) of **8b** as a red solid: mp 256–257 °C; ^{1}H NMR (DMSO-d_6, 343 K) δ 1.4–1.7 (2 H, bm), 1.95–2.1 (2 H, bm), 2.5–2.65 (1 H, bm), 3.1–3.15 (2 H, bm), 3.75–3.9 (1 H, bm), 3.84 (3 H, s, NCH_3), 4.2–4.3 (2 H, m, CH_2N), 6.4 (1 H, d, J = 8 Hz, indole-H), 6.58 (1 H, t, J = 8 Hz, indole-H), 6.9 (1 H, t, J = 8 Hz, indole-H), 7.0 (2 H, m, indole-H), 7.25 (1 H, d, J = 8 Hz, indole-H), 7.36 (2 H, t, J = 8 Hz, indole-H), 7.92 (1 H, s, indole-2H); IR ν_{max} (Nujol) 1720, 1755, 3400–3550 cm^{-1}; MS m/z 425 (M^+). Anal. ($C_{26}H_{23}N_3O_3$) C, H, N.

3-[8-(Aminomethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Hydrochloride (9b). A suspension of **8b** (3.9 g, 9.2 mmol) in dichloromethane (0.4 L) containing 2,4,6-collidine (2.43 mL, 18.4 mmol) was added dropwise under nitrogen atmosphere to a stirred, ice-cooled solution of trifluoromethanesulfonic anhydride (3.03 mL, 18.4 mmol) in dichloromethane (0.5 L). After 2 h the solution was treated with 0.880 aqueous ammonia (60 mL) and the mixture stirred vigorously for 17 h. The layers were separated, and the organic phase was washed with water (250 mL), 5% aqueous sodium bicarbonate (100 mL), and water (3 × 350 mL). The solution was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (dichloromethane/methanol/acetic acid/water, 90:18:2:3). The product was suspended in ethyl acetate (85 mL) and treated with saturated hydrogen chloride in ethyl acetate (15 mL). After stirring for 2 h the solid was filtered and dried in vacuo at 140 °C to give 2.3 g (52%) of **9b** as an orange powder: mp >300 °C dec; ^{1}H NMR (DMSO-d_6, 343 K) δ 1.5–1.8 (1 H, bm), 2.0–2.3 (3 H, m), 2.55–2.7 (2 H, bm), 2.7–2.9 (1 H, bm), 3.85 (3 H, s, NCH_3), 3.85–4.0 (1 H, m, NCH), 4.2–4.35 (1 H, m, NCH), 6.48 (1 H, d, J = 8 Hz, indole-H), 6.58 (1 H, t, J = 8 Hz, indole-H), 6.86 (1 H, t, J = 8 Hz, indole-H), 7.05 (2 H, m, indole-H), 7.16 (1 H, bd, indole-H), 7.4 (2 H, m, indole-H), 7.92 (1 H, s, indole-2H), 8.0–8.5 (3 H, bm, NH_3^+),

10.5–11.0 (1 H, bs, imide-NH); IR ν_{max} (Nujol) 1690, 1740, 3000–3200, 3300, 3640 cm^{-1}; MS m/z 424 (M$^+$). Anal. ($C_{26}H_{25}N_4O_2Cl$) C, H, N, Cl.

3-[8-(*tert*-Butoxyformamido)-7,8,9,10-tetrahydro-6*H*-azepino[1,2-*a*]indol-11-yl]-4-(1-methyl-3-indolyl)furan-2,5-dione (11c). A solution of 8-(*tert*-butoxyformamido)-7,8,9,10-tetrahydro-6*H*-azepino[1,2-*a*]indole (500 mg, 1.67 mmol) in dry dichloromethane (20 mL) was cooled to 0 °C and treated with oxalyl chloride (220 mg, 1.8 mmol). The solution obtained was stirred for 5 min and the solvent removed under reduced pressure. The residue was dissolved in dichloromethane (30 mL), and the solution was treated successively with 1-methylindole-3-acetic acid (338 mg, 1.8 mmol) and triethylamine (760 μL, 5.3 mmol). The reaction mixture was stirred for 70 h at room temperature and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:1) and crystallized from ethyl acetate/hexane to give 210 mg (24%) of **11c** as a red solid: mp 223–5 °C; ^{1}H NMR (DMSO-d_6, 353 K) δ 1.20–1.60 (2 H, bm), 1.46 (9 H, s, $(CH_3)_3$), 1.70–1.82 (1 H, m), 2.05–2.15 (1 H, bm), 2.90–3.00 (1 H, bm), 3.05–3.18 (1 H, bm), 3.58–3.72 (1 H, bm), 3.90 (3 H, s, NCH_3), 3.95–4.05 (1 H, m, NCH), 4.54–4.64 (1 H, m, NCH), 6.53–6.68 (2 H, m, indole-H, NH), 6.71 (1 H, t, J = 8 Hz, indole-H), 6.89 (1 H, t, J = 8 Hz, indole-H), 7.08–7.22 (3 H, m, indole-H), 7.47 (1 H, d, J = 8 Hz, indole-H), 7.53 (1 H, d, J = 8 Hz, indole-H), 8.09 (1 H, s, indole-2H); IR ν_{max} (Nujol) 1680, 1760, 3346 cm^{-1}; MS m/z 525 (M$^+$). Anal. ($C_{31}H_{31}N_3O_5$) C, H, N.

3-[8-(*tert*-Butoxyformamido)-7,8,9,10-tetrahydro-6*H*-azepino[1,2-*a*]indol-11-yl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (12c). A solution of **11c** (200 mg, 0.38 mmol), hexamethyldisilazane (1 mL), and methanol (100 μL) in dimethylformamide (10 mL) was heated at 50 °C for 4 h and left to stand for 48 h. The solvent was removed under reduced pressure, and the residue was partitioned between ethyl acetate (50 mL) and water (50 mL), dried ($MgSO_4$), and evaporated. Crystallization from ether gave 160 mg (80%) of **12c** as a red solid: mp 176–8 °C; ^{1}H NMR (DMSO-d_6, 353 K) δ 1.35–1.60 (2 H, bm), 1.46 (9 H, s, $(CH_3)_3$), 1.55–1.70 (1 H, m), 2.00–2.10 (1 H, bm), 2.85–2.95 (1 H, bm), 3.02–3.15 (1 H, bm), 3.55–3.75 (1 H, bm), 3.88 (3 H, s, NCH_3), 3.95–4.05 (1 H, m, NCH), 4.52–4.62 (1 H, m, NCH), 6.48–6.68 (3 H, bm, indole-H, NH), 6.80–6.90 (1 H, bm, indole-H), 7.00–7.23 (3 H, m, indole-H), 7.42 (1 H, d, J = 8 Hz, indole-H), 7.48 (1 H, d, J = 8 Hz, indole-H), 7.95 (1 H, s, indole-2H), 10.65 (1 H, bs, imide-NH); IR ν_{max} (Nujol) 1716, 1755, 3200 cm^{-1}; MS m/z 524 (M$^+$). Anal. ($C_{31}H_{32}N_4O_4 \cdot 0.5H_2O$) C, H, N.

3-[8-Amino-7,8,9,10-tetrahydro-6*H*-azepino[1,2-*a*]indol-11-yl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Hydrochloride (13c). A suspension of **12c** (120 mg, 0.23 mmol) in ethyl acetate (2 mL) was treated with saturated hydrogen chloride in ethyl acetate (4 mL). The mixture was stirred 3 h, the suspension filtered, and the solid washed with ethyl acetate. The solid was dried in vacuo to give 75 mg (77%) of **13c** as a red solid: mp >330 °C; ^{1}H NMR (DMSO-d_6, 353 K) δ 1.58–1.75 (2 H, bm), 2.05–2.17 (1 H, m), 2.30–2.45 (1 H, bm), 2.95–3.18 (3 H, bm), 3.40–3.55 (1 H, bm), 3.89 (3 H, s, NCH_3), 3.90–4.10 (1 H, bs, NCH), 4.67–4.77 (1 H, m, NCH), 6.48–6.90 (3 H, bm, indole-H), 7.00–7.20 (3 H, bm, indole-H), 7.42 (1 H, d, J = 8 Hz, indole-H), 7.52 (1 H, d, J = 8 Hz, indole-H), 7.95 (1 H, s, indole-2H), 8.28 (3 H, bs, NH), 10.70 (1 H, bs, imide-NH); IR ν_{max} (Nujol) 1700, 1750, 2600–3600 cm^{-1}; MS m/z 424 (M$^+$). Anal. ($C_{26}H_{25}N_4O_2Cl \cdot 0.4H_2O$) C, H, N.

***tert*-Butyl 1,2,3,4-Tetrahydropyrazino[1,2-*a*]indole-2-carboxylate (15).** A solution of 1,2,3,4-tetrahydropyrazino[1,2-*a*]-indole (450 mg, 2.62 mmol) in dichloromethane (30 mL) under nitrogen atmosphere was cooled in ice and treated with triethylamine (303 mg, 3 mmol) and di-*tert*-butyl dicarbonate (615 mg, 2.8 mmol). The solution was stirred for 4 h and then washed with 5% aqueous sodium bicarbonate (20 mL) and dried (Na_2SO_4). Evaporation of the solvent and crystallization from methanol gave 580 mg (82%) of **15** as a white solid: mp 103–105 °C; ^{1}H NMR (DMSO-d_6, 353 K) δ 1.45 (9 H, s, ^{t}Bu), 3.84 (2 H, t, J = 6 Hz, $CH_2CH_2NCO_2{}^t$Bu), 4.08 (2 H, t, J = 6 Hz, $CH_2CH_2NCO_2{}^t$Bu), 4.75 (2 H, s, $InCH_2NCO_2{}^t$Bu), 6.28 (1 H, s, indole-3H), 7.0–7.15 (2 H, m, indole-H), 7.37 (1 H, d, J = 8 Hz, indole-H), 7.5 (1 H, d, J = 8 Hz, indole-H); IR ν_{max} (Nujol) 1690 cm^{-1}; MS m/z 272 (M$^+$). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

3-(1,2,3,4-Tetrahydropyrazino[1,2-*a*]indol-10-yl)-4-(1-methyl-3-indolyl)-2,5-furandione Hydrochloride (16). An ice-cooled solution of **15** (7.9 g, 29 mmol) in anhydrous diethyl ether (200 mL) under nitrogen atmosphere was treated with oxalyl chloride (2.9 mL, 33 mmol). After 30 min solvent was evaporated and the residue redissolved in sieve-dried dichloromethane (200 mL). The solution was added dropwise to a stirred mixture of 1-methylindole-3-acetic acid (6.2 g, 33 mmol) and triethylamine (9.2 mL, 66 mmol) in sieve-dried dichloromethane (200 mL) under nitrogen atmosphere. The mixture was stirred for 17 h and solvent evaporated. Flash chromatography (ethyl acetate/dichloromethane, 2:98) gave 4.8 g of 3-[2-(*tert*-butoxycarbonyl)-1,2,3,4-tetrahydropyrazino[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)-2,5-furandione as an orange solid. The product was dissolved in ethyl acetate (100 mL) and treated with saturated hydrogen chloride in ethyl acetate (30 mL). The mixture was stirred 18 h, the suspension filtered, and the solid washed with diethyl ether (2 × 50 mL). The solid was dried in vacuo to give 3.7 g (29%) of **16** as a red solid: mp 292–295 °C; ^{1}H NMR (DMSO-d_6, 353 K) δ 3.6 (2 H, t, J = 6 Hz, $CH_2CH_2NH_2{}^+$), 3.93 (3 H, s, NCH_3), 4.27 (2 H, bs, $InCH_2NH_2{}^+$), 4.48 (2 H, t, J = 6 Hz, $CH_2CH_2NH_2{}^+$), 6.7–6.8 (2 H, m, indole-H), 6.95 (1 H, t, J = 8 Hz, indole-H), 7.1–7.25 (3 H, m, indole-H), 7.48 (1 H, d, J = 8 Hz, indole-H), 7.53 (1 H, d, J = 8 Hz, indole-H), 8.1 (1 H, s, indole-2H), 10.0–10.5 (2 H, bs, $NH_2{}^+$); IR ν_{max} (Nujol) 1760, 1830 cm^{-1}; MS m/z 397 (M$^+$). Anal. ($C_{24}H_{19}N_3O_3 \cdot 1.1HCl$) C, H, N, Cl.

3-(1,2,3,4-Tetrahydropyrazino[1,2-*a*]indol-10-yl)-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione (17). A suspension of **16** (1 g, 2.3 mmol) in dimethylformamide (8 mL) was treated with 0.880 aqueous ammonia (50 mL) and the mixture heated at 80 °C in a sealed vessel for 9 h. The mixture was diluted with water (50 mL), stored at 0 °C 1 h, and filtered, and the solid was washed with water (2 × 25 mL). The product was dried in vacuo to give 710 mg (78%) of **17** as a dark red crystalline solid: mp 285–287 °C; ^{1}H NMR (DMSO-d_6, 353 K) δ 3.0 (2 H, t, CH_2CH_2NH), 3.5 (2 H, bs, $InCH_2NH$), 3.9 (3 H, s, NCH_3), 4.0 (2 H, t, J = 6 Hz, CH_2CH_2NH), 6.5 (1 H, d, J = 8 Hz, indole-H), 6.65 (1 H, t, J = 8 Hz, indole-H), 6.97 (1 H, t, J = 8 Hz, indole-H), 7.06–7.16 (2 H, m, indole-H), 7.35–7.5 (3 H, m, indole-H), 7.95 (1 H, s, indole-2H); IR ν_{max} (Nujol) 1700, 1750 cm^{-1}; MS m/z 369 (M$^+$). Anal. ($C_{24}H_{20}N_4O_2$) C, H, N.

3-[6,7,8,9-Tetrahydro-8-[(methylamino)methyl]pyrido-[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Hydrochloride (18a). A suspension of **8b** (200 mg, 0.47 mmol) in dichloromethane (30 mL) containing 2,4,6-collidine (100 mg) was added dropwise under nitrogen atmosphere to a stirred, ice-cooled solution of trifluoromethanesulfonic anhydride (265 mg, 0.94 mmol) in dichloromethane (40 mL). After 5 h the solution was treated with a 33% solution of methylamine in methylated spirit (1.25 mL) and the mixture obtained was stirred vigorously for 22 h. The solvent was concentrated by evaporation and the precipitated solid was filtered off and purified by flash chromatography (dichloromethane/methanol/acetic acid/water, 90:18:2:3). The product was suspended in ethyl acetate saturated with hydrogen chloride for 2 h and the resulting solid was filtered and dried in vacuo to give 55 mg (27%) of **18a** as an orange powder: mp 337–340 °C dec; ^{1}H NMR (DMSO-d_6, 343 K) δ 1.55–1.85 (1 H, bm), 1.95–2.30 (2 H, m), 2.45 (3 H, s, $NHCH_3$), 2.40–2.90 (4 H, bm), 3.86 (3 H, s, NCH_3), 3.85–4.00 (1 H, m, NCH), 4.20–4.35 (1 H, m, NCH), 6.43 (1 H, d, J = 8 Hz, indole-H), 6.60 (1 H, t, J = 8 Hz, indole-H), 6.92 (1 H, t, J = 8 Hz, indole-H), 7.00–7.15 (2 H, m, indole-H), 7.20 (1 H, bd, indole-H), 7.35–7.45 (2 H, m, indole-H), 7.95 (1 H, s, indole-2H), 10.65 (1 H, bs, imide-NH); IR ν_{max} (Nujol) 1690, 1740, 3000–3600 cm^{-1}; MS m/z 438 (M$^+$). Anal. ($C_{27}H_{26}N_4O_2 \cdot 1.3HCl$) C, H, N.

3-[8-[(Dimethylamino)methyl]-6,7,8,9-tetrahydropyrido-[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Hydrochloride (18b). A suspension of **8b** (140 mg, 0.33 mmol) in dichloromethane (25 mL) containing 2,4,6-collidine (70 mg, 0.66 mmol) was added dropwise under nitrogen atmosphere to a stirred, ice-cooled solution of trifluoromethanesulfonic anhydride (185 mg, 0.66 mmol) in dichloromethane (30 mL). After 1.25 h the solution was treated with a 33% solution of dimethylamine in ethanol (0.8 mL, 3.3 mmol) and the mixtured stirred vigorously for 2.5 h. The solvent was removed under reduced pressure and the residue was triturated with methanol

and filtered off. The product was suspended in ethyl acetate saturated with hydrogen chloride for 2 h, filtered, and dried in vacuo to give 70 mg (50%) of **18b** as an orange powder: mp 335–336 °C dec; ^{1}H NMR (DMSO-d_6, 343 K) δ 1.55–1.80 (1 H, bm), 2.10–2.30 (2 H, m), 2.45–2.90 (4 H, bm), 2.55 (6 H, bs, $N(CH_3)_2$), 3.80–4.0 (1 H, m, NCH), 3.88 (3 H, s, NCH_3), 4.2–4.35 (1 H, m, NCH), 6.48 (1 H, d, J = 8 Hz, indole-H), 6.61 (1 H, t, J = 8 Hz, indole-H), 6.95 (1 H, t, J = 8 Hz, indole-H), 7.05–7.18 (2 H, m, indole-H), 7.30 (1 H, d, J = 8 Hz, indole-H), 7.38–7.48 (2 H, m, indole-H), 7.98 (1 H, s, indole-2H), 10.30 (1 H, bs, NH^+), 10.65 (1 H, bs, imide-NH); IR ν_{max} (Nujol) 1700, 1745, 2800–3600 cm^{-1}; MS m/z 452 (M^+). Anal. ($C_{28}H_{29}N_4O_2Cl$) C, H, N.

3-[6,7,8,9-Tetrahydro-8-[(trimethylamino)methyl]pyrido-[1,2-*a*]indol-10-yl]-4-(1-methyl-3-indolyl)-1*H*-pyrrole-2,5-dione Trifluoromethanesulfonate (18c). A suspension of **8b** (200 mg, 0.47 mmol) in dichloromethane (30 mL) containing 2,4,6-collidine (100 mg, 0.94 mmol) was added dropwise under nitrogen atmosphere to a stirred, ice-cooled solution of trifluoromethanesulfonic anhydride (265 mg, 0.94 mmol) in dichloromethane (30 mL). After 5 h the solution was treated with a 33% solution of trimethylamine in ethanol (0.8 mL, 3.3 mmol) and the mixture stirred vigorously for 18 h. The resulting precipitate was filtered off and dried in vacuo to give 237 mg (82%) of **18c** as an orange powder: mp 320–324 °C; ^{1}H NMR (DMSO-d_6, 343 K) δ 1.60–1.85 (1 H, bm), 2.00–2.30 (2 H, m), 2.55–3.15 (3 H, bm), 2.94 (9 H, s, $N(CH_3)_3$), 3.25–3.40 (1 H, bm), 3.88 (3 H, s, NCH_3), 3.90–4.05 (1 H, m, NCH), 4.25–4.40 (1 H, m, NCH), 6.48 (1 H, d, J = 8 Hz, indole-H), 6.53 (1 H, t, J = 8 Hz, indole-H), 6.96 (1 H, t, J = 8 Hz, indole-H), 7.05–7.20 (2 H, m, indole-H), 7.32 (1 H, d, J = 8 Hz, indole-H), 7.38–7.48 (2 H, m, indole-H), 8.00 (1 H, s, indole-2H), 10.65 (1 H, bs, imide-NH); IR ν_{max} (Nujol) 1710, 1760, 3100–3650 cm^{-1}; MS m/z 467 (M^+). Anal. ($C_{30}H_{31}N_4O_5F_3S\cdot 0.2H_2O$) C, H, N.

6,7,8,9-Tetrahydropyrido[1,2-*a*]indole-8(*S*)- and 8(*R*)-carboxylic Acid *l*-Menthyl Ester (20a and 20b). A mixture of 6,7,8,9-tetrahydropyrido[1,2-*a*]indole-8-carboxylic acid (4.47 g, 20.8 mmol) and *l*-menthol (3.9 g, 25 mmol) in dichloromethane (100 mL) was treated with 4-(dimethylamino)pyridine (0.25 g, 2.05 mmol) and cooled in ice. Dicyclohexylcarbodiimide (6.08 g, 22.9 mmol) in dichloromethane (20 mL) was added dropwise during 10 min. After 30 min the suspension was filtered through a pad of Hyflo and the filtrate evaporated. Flash chromatography (diethyl ether/hexane, 1:5) gave 6.09 g (83%) of mixed diastereoisomers as an oil. The isomers were separated either by flash chromatography (diethyl ether/hexane, 1:19) or fractional crystallization from 2-propanol.

20a: mp 117–118 °C; [α], 20 °C, 589 nm = −76.2°, c = 1% in chloroform; ^{1}H NMR ($CDCl_3$) δ 0.77 (3 H, d, J = 6 Hz), 0.85–1.15 (3 H, m), 0.88 (3 H, d, J = 6 Hz), 0.9 (3 H, d, J = 6 Hz), 1.35–1.55 (2 H, m), 1.65–1.75 (2 H, m), 1.8–1.9 (1 H, m), 1.95–2.05 (1 H, m), 2.1–2.25 (1 H, m), 2.4–2.5 (1 H, m), 2.75–2.9 (1 H, m), 3.05–3.15 (1 H, m), 3.25–3.35 (1 H, m), 3.85–3.95 (1 H, m), 4.3–4.4 (1 H, m), 4.7–4.8 (1 H, m), 6.24 (1 H, s, indole-3H), 7.05–7.2 (2 H, m, indole-H), 7.26 (1 H, d, J = 8 Hz, indole-H), 7.53 (1 H, d, J = 8 Hz, indole-H); IR ν_{max} (Nujol) 1720 cm^{-1}; MS m/z 353 (M^+). Anal. ($C_{23}H_{31}NO_2$) C, H, N.

20b: mp 87–89 °C; [α], 20 °C, 589 nm = −22.8°, c = 1% in chloroform; ^{1}H NMR ($CDCl_3$) δ 0.74 (3 H, d, J = 6 Hz), 0.85–1.15 (3 H, m), 0.85 (3 H, d, J = 6 Hz), 0.92 (3 H, d, J = 6 Hz), 1.3–1.55 (2 H, m), 1.64–1.8 (3 H, m), 1.95–2.05 (1 H, m), 2.15–2.3 (1 H, m), 2.37–2.5 (1 H, m), 2.77–2.9 (1 H, m), 3.06–3.18 (1 H, m), 3.23–3.32 (1 H, m), 3.85–4.0 (1 H, m), 4.22–4.32 (1 H, m), 4.66–4.8 (1 H, m), 6.24 (1 H, s, indole-3H), 7.05–7.2 (2 H, m, indole-H), 7.25 (1 H, d, J = 8 Hz, indole-H), 7.52 (1 H, d, J = 8 Hz, indole-H); IR ν_{max} (Nujol) 1710 cm^{-1}; MS m/z 353 (M^+). Anal. ($C_{23}H_{31}NO_2$) C, H, N.

8(*S*)-(Acetoxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indole (21a). A solution of **20a** (0.8 g, 2.27 mmol) in dry tetrahydrofuran (15 mL) under nitrogen atmosphere was treated dropwise with 1 M lithium aluminum hydride (2 mL, 2 mmol). After 10 min the mixture was cooled in ice and treated successively with ethyl acetate (5 mL) and water (30 mL) and acidified with 1 M hydrochloric acid. The mixture was extracted with diethyl ether (3 × 20 mL), and the combined extracts were dried (Na_2SO_4) and evaporated. Flash chromatography (ethyl acetate/hexane, 1:1) gave the intermediate alcohol as a white solid which was dissolved in dichloromethane (5 mL). Acetic anhydride (0.43 g, 4.21 mmol) and triethylamine (0.9 mL, 6.5 mmol) were added, and the solution was left for 17 h. Solvent was evaporated and the residue partitioned between 5% aqueous sodium bicarbonate (20 mL) and diethyl ether (2 × 20 mL). The extract was dried (Na_2SO_4) and evaporated, and the residue was recrystallized from aqueous ethanol to give 0.518 g (93%) of **21a** as a white solid: mp 63–64 °C; [α], 20 °C, 589 nm = −43.7°, c = 1% in chloroform; ^{1}H NMR ($CDCl_3$) δ 1.75–1.9 (1 H, m), 2.1 (3 H, s, OAc), 2.15–2.3 (2 H, m), 2.6–2.75 (1 H, m), 3.1–3.2 (1 H, m), 3.8–3.94 (1 H, m), 4.12 (2 H, d, J = 7 Hz, CH_2OAc), 4.25–4.35 (1 H, m), 6.23 (1 H, s, indole-3H), 7.05–7.2 (2 H, m, indole-H), 7.25 (1 H, d, J = 8 Hz, indole-H), 7.52 (1 H, d, J = 8 Hz, indole-H); IR ν_{max} (Nujol) 1730 cm^{-1}; MS m/z 243 (M^+). Anal. ($C_{15}H_{17}NO_2$) C, H, N.

Similarly **21b** was prepared in 94% yield from **20b**: mp 63–64 °C; [α], 20 °C, 589 nm = 44.8°, c = 1% in chloroform. Anal. ($C_{15}H_{17}NO_2$) C, H, N.

Molecular Modeling. Template forcing studies were performed within MOLOC.[28] Structures were displayed on a Silicon Graphics IRIS workstation with RASTER3D as modified by Ethan A. Merritt, Department of Biological Structure, University of Washington SM-20, Seattle, WA 98195 (available electronically from anonymous ftp site: xrays.bchem.washington.edu (128.208.112.3)).

Inhibition of Rat Brain PKC. Compounds were assayed for PKC inhibitory activity as described previously.[19] 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 **9b** gave an IC_{50} value of 7.6 ± 0.2 nM (10 determinations).

Inhibition of Human Neutrophil PKC. Compounds were assayed for PKC inhibitory activity as described previously.[30]

Inhibition of Human Platelet PKC. Platelet PKC was isolated by the method of Mamiya et al.[31] Compounds were assayed for inhibitory activity as described for rat brain PKC.

Inhibition of Bovine Heart PKA. Compounds were assayed for PKA inhibitory activity as described previously.[19] 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 **9b** gave an IC_{50} value of 2.8 ± 1.2 μM (six determinations).

Inhibition of Human Neutrophil PKA. Compounds were assayed for PKA inhibitory activity as described previously.[30]

Inhibition of Rabbit Muscle Phosphorylase Kinase (PhK). Compounds were assayed for PhK inhibitory activity as described previously.[29] 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.

Inhibition of Turkey Gizzard Myosin Light Chain Kinase (MLCK). Myosin light chain kinase (15 μL), isolated from turkey gizzards,[32] was added to 0.1 mM $CaCl_2$, 100 ng of calmodulin, 10 μM [γ-^{32}P]ATP, 0.1 mg of turkey gizzard myosin light chains,[33] and inhibitor or solvent-only controls (final concentration of DMSO was 10%) in 85 μL of 25 mM Tris·HCl, 10 mM $MgCl_2$ at pH 7.4. After 10 min at 30 °C, the reaction was stopped by the addition of 1 mL of 10% trichloroacetic acid. Acid-precipitable protein was then collected as described previously.[19]

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Inhibition of Casein Kinase II. Casein kinase II (5 μL), isolated from rat liver, was added to 10 μM [γ-^{32}P]ATP, 125 μg of phosphorylated β-casein, and inhibitor or solvent-only controls (final DMSO concentration was 10%) in 45 μL of 50 mM MOPS, 50 mM NaCl, 10 mM $MgCl_2$ at pH 7.0. After incubation for 50 min at 37 °C, the reaction was stopped by the addition of 1 mL of 10% trichloroacetic acid. Acid-precipitable protein was then collected as described previously.[19]

Inhibition of p56^{lck}. Human p56^{lck} (2 ng) was added to 75 μg of angiotensin II, 10 μM [γ-^{32}P]ATP, and inhibitor or solvent-only controls (final concentration of DMSO was 10%) in 50 mM HEPES, 25 mM $MnCl_2$ at pH 6.8 (25 μL). After 90 min at 30 °C the assay was stopped by the addition of 25 μL of 40 mM EDTA at pH 8. Twenty-five microliter sample aliquots were spotted onto phosphocellulose binding paper, dried, and washed twice with 75 mM orthophosphoric acid. The papers were then washed with ethanol and dried, and incorporated radioactivity was determined by liquid scintillation spectrometry.

Inhibition of p60^{src}. Twenty units of human p60$^{c\text{-}src}$ (Oncogen Science Inc.) was added to 60 μg of angiotensin II, 10 μM [γ^{32}P]-ATP, 10 mM $MgCl_2$, and inhibitor or solvent-only controls (final concentration of DMSO was 10%) in 50 mM HEPES, 0.1 mM EDTA, 0.015% Brij 35 at pH 7.5 (50 μL). After 60 min at 30 °C the assay was stopped by the addition of 50 μL of 40 mM EDTA. Fifty microliter sample aliquots were then processed for liquid scintillation spectrometry as described above.

Inhibition of p47 Phosphorylation in Platelets. Compounds were assayed as described previously.[19] In each assay, data points were determined in triplicate and the quoted IC_{50} values are the mean of at least three independent assay results.

Inhibition of CD3 Down-Regulation. Compounds were assayed as described previously.[19] 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.

Mixed Lymphocyte Reaction. MLR assays were performed by the method of Fitzharris and Knight.[23] In each assay, data points were determined in triplicate and the quoted IC_{50} values are the mean of at least three independent assay results.

Supplementary Material Available: Coordinates of models shown in Figure 1 and X-ray coordinates (17 pages). Ordering information is given on any current masthead page.

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