Inhibitors of Protein Kinase C. 1.[1] 2,3-Bisarylmaleimides

Peter D. Davis,* Christopher H. Hill, Geoffrey Lawton, John S. Nixon, Sandra E. Wilkinson, Steven A. Hurst, Elizabeth Keech, and Susan E. Turner

Roche Products Limited, P.O. Box 8, Welwyn Garden City, Herts., AL7 3AY, U.K. *Received February 11, 1991*

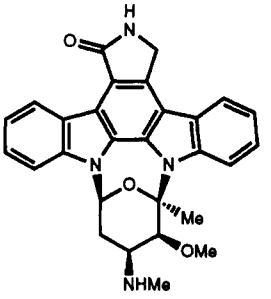
The design and synthesis of a series of novel inhibitors of protein kinase C (PKC) is described. These 2,3-bisarylmaleimides were derived from the structural lead provided by the indolocarbazoles, staurosporine and K252a. Optimum activity required the imide NH, both carbonyl groups, and the olefinic bond of the maleimide ring. 2,3-Bisindolylmaleimides were the most active, and the potency of these was improved by a chloro substituent at the 5-position of one indole ring (compound 28, IC_{50} 0.11 μM). In a series of (phenylindolyl)maleimides, nitro compound 74 was most active (IC_{50} 0.67 μM). Naphthalene 19 and benzothiophene 21 showed greater than 100-fold selectivity for inhibition of PKC over the closely related cAMP-dependent protein kinase (PKA).

Introduction

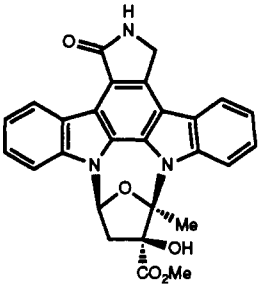
The enzyme family protein kinase C[2] (PKC) occupies a central role in the transduction of signals from a variety of mediators across the cell membrane.[3] Receptor occupation by a number of hormones, cytokines, neurotransmitters, and growth factors results in activation of PKC via activation of phospholipase C through either a G protein mechanism or a tyrosine kinase mechanism. PKC then propagates the signal by phosphorylation of proteins on serine or threonine, with ATP as cosubstrate, resulting in modification of the properties of these proteins. Thus PKC appears to regulate mechanisms of cell proliferation, secretion, and gene expression. Inhibition of PKC may provide therapy for diseases such as rheumatoid arthritis (through inhibition of T-cell proliferation[3]), cancer,[4] and AIDS (through inhibition of viral entry[5] and viral gene expression[6]).

The activation of PKC by its cofactors, calcium and diacylglycerol, most likely occurs as the enzyme transiently and weakly associates with membrane phospholipids. This membrane-bound form is susceptible to proteolysis, by calpain or other enzymes, to give a cytosolic form (PKM) which is fully active in the absence of diacylglycerol or phospholipid. The physiological role of PKM is unknown; however, if both active forms of the enzyme (PKC and PKM) are to be inhibited, either the protein substrate site or the ATP binding site should be blocked.

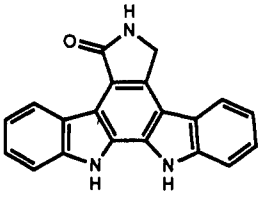
Our leads in the search for PKC inhibitors were the microbial products staurosporine[7] (1) and K252a[8] (2) which exert their effects, at least in part, by competing with ATP. In our assay 2 inhibited rat brain PKC with an IC_{50} of 470 nM and staurosporine was more potent (9 nM). These structures are related by a common aglycon (3) which itself has inhibitory activity.[9]



(1)



(2)



(3)

Although quite potent, these inhibitors show little selectivity for PKC over other protein kinases (Table I). Compound 2 also inhibits the closely related cAMP-dependent protein kinase (PKA) and is a far better inhibitor of phosphorylase kinase than of PKC.[10] Staurosporine is mildly selective for PKC over PKA but again inhibits phosphorylase kinase at much lower concentrations.[10]

Although aglycon 3 appeared to be a good lead compound for the design of potentially selective inhibitors, we were discouraged by the potential for DNA intercalation by such flat polyaromatic compounds. Indeed a compound containing an indolocarbazole, rebeccamycin, has been shown to induce single-strand breaks in DNA,[11] possibly through DNA binding. We therefore investigated whether activity would be retained in nonplanar analogues of the

(1) Part of this work has appeared in a preliminary communication: Davis, P. D.; Hill, C. H.; Keech, E.; Lawton, G.; Nixon, J. S.; Sedgwick, A. D.; Wadsworth, J.; Westmacott, D.; Wilkinson, S. E. Potent Selective Inhibitors of Protein Kinase C. *FEBS Letts.* **1989**, *259*, 61-63.

(2) Nishizuka, Y. The Role of Protein Kinase C in Cell Surface Signal Transduction and Tumour Promotion. *Nature* **1984**, *308*, 693-698.

(3) Farago, A.; Nishizuka, Y. Protein Kinase C in Transmembrane Signalling. *FEBS Lett.* **1990**, *268*, 350-354.

(4) Tritton, T. R.; Hickman, J. A. How To Kill Cancer Cells: Membranes And Cell Signalling As Targets in Cancer Chemotherapy. *Cancer Cells* **1990**, *2*, 95-105.

(5) Fields, P. A.; Bednarik, D. P.; Hess, A.; May, W. S. Human Immunodeficiency Virus Induces Phosphorylation of Its Cell Surface Receptor. *Nature* **1988**, *333*, 278-280.

(6) Jakobovits, A.; Rosenthal, A.; Capon, D. J. Trans-activation of HIV-1 LTR-directed Gene Expression By TAT Requires Protein Kinase C. *EMBO J.* **1990**, *9*, 1165-1170.

(7) Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, F. Staurosporine, A Potent Inhibitor of Phospholipid/Ca^{2+} Dependent Protein Kinase. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397-402.

(8) Kase, H.; Iwahishi, K.; Nakanishi, S.; Matsuda, Y.; Yamada, K.; Takahashi, M.; Murakata, C.; Sato, A.; Kaneko, M. K252 Compounds, Novel And Potent Inhibitors of Protein Kinase C And Cyclic Nucleotide-dependent Protein Kinases. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 436-440.

(9) Nakanishi, S.; Matsuda, Y.; Iwahashi, K.; Kase, H. K252b, c And d, Potent Inhibitors of Protein Kinase C of Microbial Origin. *J. Antibiot.* **1986**, *39*, 1066-1071.

(10) Elliott, L. H.; Wilkinson, S. E.; Sedgwick, A. D.; Hill, C. H.; Lawton, G.; Davis, P. D.; Nixon, J. S. K252a Is A Potent And Selective Inhibitor of Phosphorylase Kinase. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 148-154.

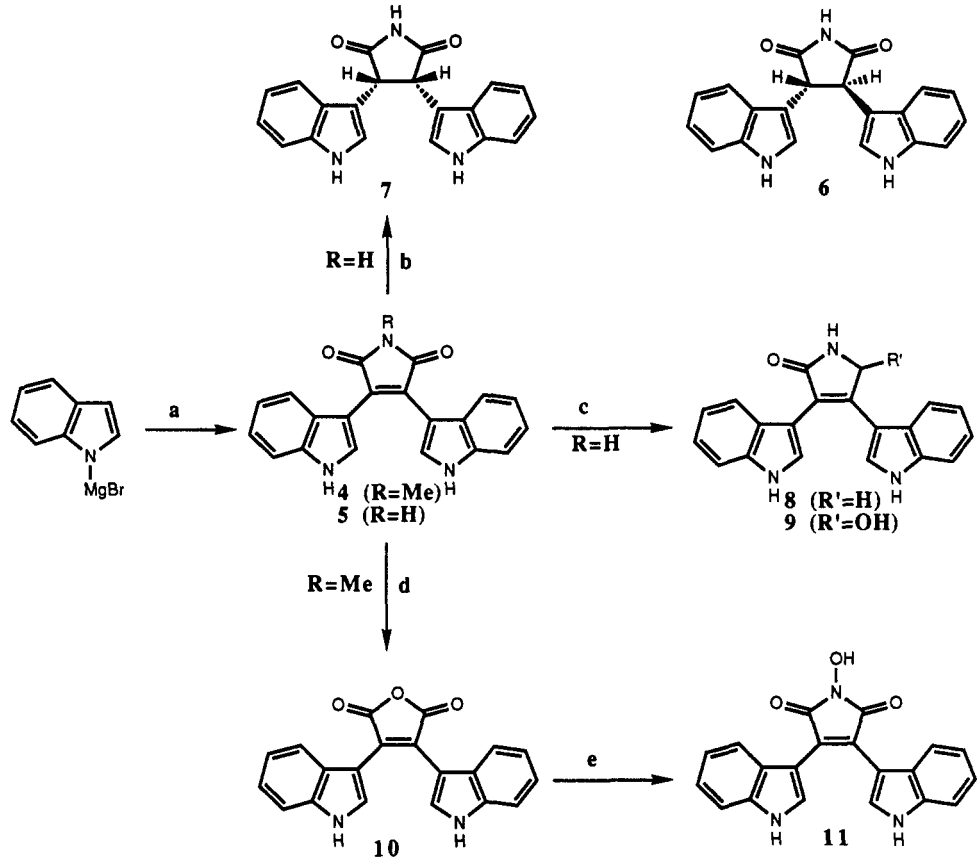
(11) Bush, J. A.; Long, B. H.; Catino, J. J.; Bradner, W. T. Production And Biological Activity of Rebeccamycin, A Novel Antitumour Agent. *J. Antibiot.* **1987**, *40*, 668-678.

0022-2623/92/1835-0177$03.00/0

Table I. Inhibition of Kinases by Indolocarbazoles[1,10]

	IC_{50} (nM)			
	PKC	PKA	Ca/CAM dep protein kinase	phosphorylase kinase
staurosporine	9 ± 1 (3)	121 ± 18 (2)	40 ± 7 (2)	0.5 ± 0.2 (2)
K252a	470 ± 11 (2)	140 ± 50 (3)	270 ± 40 (2)	1.7 ± 0.5 (2)

Scheme I[a]



[a] (a) (i) Methylmagnesium iodide, benzene, room temperature, (ii) 2,3-dibromo-*N*-methylmaleimide, 110 °C (R=Me) or 2,3-dibromomaleimide (R = H), 80 °C; (b) H_2 50 psi, Pd/C, DMF, 4 days; (c) $LiAlH_4$, THF, room temperature, 18 h; (d) KOH, ethanol, reflux, 1 h; (e) $NH_2OH{\cdot}HCl$, K_2CO_3, DMF/H_2O, 80 °C, 3 h.

aglycon. It was envisaged that compounds in which a bond had been formally removed and another carbonyl group added, e.g. bisindolylmaleimide 5[12] would not be able to attain a flat low-energy conformation due to steric repulsion both between the two indole rings and between each indole ring and its neighboring carbonyl group. Molecular mechanics calculations within the modeling program MOLOC[13] suggested a significant energy barrier to coplanarity of either indole ring with the maleimide ring.

This compound not only retained activity but displayed good selectivity for PKC over the related kinase PKA (vide infra). A systematic study of the structural features contributing to the activity of this compound was therefore performed.

Chemistry

Bisindolylmaleimide 4[12] was prepared by the addition of indolylmagnesium iodide to dibromo-*N*-methylmaleimide, and the analogous reaction with dibromomaleimide[14] gave bisindolylmaleimide 5. Crystallization of compound 5 from methanol gave crystals suitable for X-ray

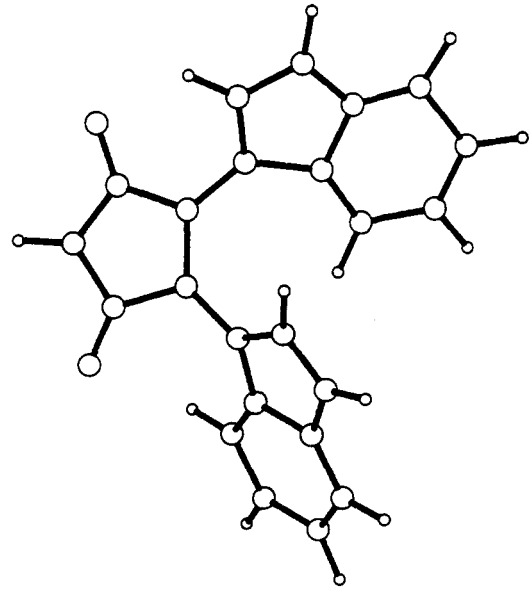


Figure 1. X-ray crystal structure of 5.

(12) Brenner, M.; Rexhausen, H.; Steffan, B.; Steglich, W. Synthesis of Arcyriarubin B And Related Bisindolylmaleimides. *Tetrahedron* **1988**, *44*, 2887–2892.

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

structure determination which showed, as expected, a conformation in which neither of the indoles were coplanar with the maleimide ring (Figure 1). Catalytic hydrogenation of maleimide **5** gave the *cis*-succinimide **7** (Scheme I) in which the coupling constant between the succinimide methine protons, measured by observation of the ^{13}C satellite peaks,[15] was 9 Hz. *trans*-2,3-Bisindolylsuccinimide **6** was prepared by the reaction of indolylmagnesium iodide with bromomaleimide. The trans stereochemistry was deduced from the lack of a measurable ^1H–^1H coupling constant between the succinimide methine protons. Reduction of imide **5** with lithium aluminum hydride gave a readily separated mixture of lactam **8** and hydroxy lactam **9**. Alkaline hydrolysis of *N*-methylimide **4** gave anhydride **10**,[12] which was converted into *N*-hydroxyimide **11** on treatment with hydroxylamine.

3-Aryl-4-indolylmaleimides **13–23, 25, 26, 59–61, 64–68,** and **70–80** were prepared by our published procedure[16] involving treatment of the corresponding arylacetic acid with 1-methylindole-3-glyoxylyl chloride and conversion of the resulting 3-aryl-4-indolylmaleic anhydrides into the imides. The latter conversion was effected by heating the anhydride with ammonia in aqueous DMF in a sealed vessel at 140 °C, except for the sensitive compounds **18** and **22** where our mild method,[17] involving treatment with hexamethyldisilazane and methanol, was used.

Bisindolylmaleimides **27–29, 32–34, 36, 37, 41, 43, 44, 48, 50, 51,** and **53–57** were synthesized by methylation of the substituted indole (sodium hydride, iodomethane, DMF), treatment with oxalyl chloride, and reaction of the resulting glyoxylyl chloride with 1-methylindole-3-acetic acid to give the bisindolylmaleic anhydrides. Conversion into the imide was achieved by heating with ammonia as before.

Pyrrole **24** was obtained by deprotection of the *N*-(benzenesulfonyl)pyrrole anhydride with sodium hydroxide in aqueous ethanol before conversion into the imide. Indole **12** was similarly prepared from *N*-(benzenesulfonyl)indole-3-acetic acid. Aromatic amines **30, 42, 49,** and **69** were prepared by catalytic hydrogenation of the corresponding (nitroaryl)maleic anhydrides (synthesis given above) followed by imide formation. Treatment of amines **30, 42,** and **49,** respectively, with acetic anhydride gave compounds **40, 47,** and **52.** Aniline **63** was prepared by deprotection of the *N-tert*-butoxycarbonyl-protected amine anhydride with trifluoroacetic acid and subsequent conversion into the imide.

Hydroxyindole **31** was prepared from the corresponding methoxyindole anhydride by heating with pyridinium hydrochloride in pyridine[18] to give the hydroxyindole anhydride, which was converted into the imide. Acid **38** and amide **39** were obtained as byproducts in the formation of imide methyl ester **37** as were acid **45** and amide **46** from the corresponding methyl ester. Sulfoxides **35** and **58** were prepared by *m*-chloroperbenzoic acid oxidation of sulfides **34** and **57,** respectively. Sulfone **62** was obtained from a similar oxidation of the sulfide anhydride before imide formation.

(14) Ciamician, G. L.; Silber, P. Ueber Einige Derivate Des Succinimids. *Chem. Ber.* **1884**, *17*, 553–559.

(15) Sheppard, N.; Turner, J. J. Internal Rotation In Substituted Ethanes And Cyclic Ethers. *Proc. R. Soc.* **1959**, *252*, 506–519.

(16) Davis, P. D.; Bit, R. A.; Hurst, S. A. A Convenient Synthesis Of Bisindolyl- and Indolylaryl-maleic Anhydrides. *Tetrahedron Lett.* **1990**, *31*, 2353–2356.

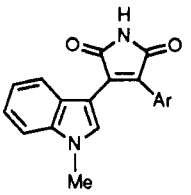
(17) Davis, P. D.; Bit, R. A. A Mild Conversion Of Maleic Anhydrides into Maleimides. *Tetrahedron Lett.* **1990**, *31*, 5201–5204.

(18) Filler, R.; Khan, B. T.; McMullen, C. W. Synthesis of 3-(Trifluoromethyl)tyrosine. *J. Org. Chem.* **1962**, *27*, 4660–4662.

Table II. PKC Inhibition by Pyrrole and Furan Derivatives

no.	formula	analysis	IC_{50} (μM)
4	$C_{21}H_{15}N_3O_2$	C, H, N	>100 (4)
5	$C_{20}H_{13}N_3O_2$	C, H, N	0.55 ± 0.17 (15)
6	$C_{20}H_{15}N_3O_2$	C, H, N	>100 (2)
7	$C_{20}H_{15}N_3O_2$	C, H, N	>100 (1)
8	$C_{20}H_{15}N_3O$	C, H, N	10.3 ± 1.3 (2)
9	$C_{20}H_{15}N_3O_2$	C, H, N	51 ± 8 (2)
10	$C_{20}H_{12}N_2O_3$	C, H, N	>100 (1)
11	$C_{20}H_{13}N_3O_3$	C, H, N	28.9 ± 11.4 (2)

Table III. Inhibition of PKC by (Arylindolyl)maleimides



no.	Ar	formula	analysis	IC_{50} (μM)
12	3-indolyl	$C_{21}H_{15}N_3O_2$[a]	C, H, N	0.22 ± 0.08 (3)
13	1-methyl-3-indolyl	$C_{22}H_{17}N_3O_2$	C, H, N	0.30 ± 0.06 (9)
14	1-benzyl-3-indolyl	$C_{28}H_{21}N_3O_2$	C, H, N	1.2 ± 0.5 (2)
15	1-propyl-3-indolyl	$C_{24}H_{21}N_3O_2$	C, H, N	0.48 ± 0.08 (2)
16	1-phenyl-3-indolyl	$C_{27}H_{19}N_3O_2$	b	0.36 ± 0.06 (2)
17	1-methyl-2-indolyl	$C_{22}H_{17}N_3O_2$	C, H, N	>100 (2)
18	1-indolyl	$C_{21}H_{15}N_3O_2$	C, H, N	3.0 ± 0.6 (2)
19	1-naphthyl	$C_{23}H_{16}N_2O_2$	C, H, N	0.81 ± 0.21 (2)
20	2-naphthyl	$C_{23}H_{16}N_2O_2$	C, H, N	2.8 ± 1.2 (3)
21	3-benzo[b]thienyl	$C_{21}H_{14}N_2O_2S$	C, H, N	0.90 ± 0.27 (2)
22	3-benzo[b]furanyl	$C_{21}H_{14}N_2O_3$	b	2.6 ± 0.7 (2)
23	phenyl	$C_{19}H_{14}N_2O_2$	C, H, N	7.7 ± 0.1 (2)
24	3-pyrrolyl	$C_{17}H_{13}N_3O_2$[c]	C, H, N	4.0 ± 2.0 (2)
25	3-thienyl	$C_{17}H_{12}N_2O_2S$	C, H, N	13.8 ± 3.9 (3)
26	2-thienyl	$C_{17}H_{12}N_2O_2S$	C, H, N	5.2 ± 2.2 (2)
27	3-(7-aza-1-methylindolyl)	$C_{21}H_{16}N_4O_2$	C, H, N	2.9 ± 0.6 (2)

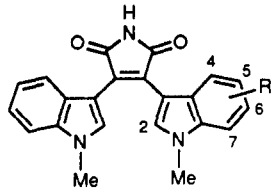
[a] Contained 0.075 equiv of diethyl ether. [b] Characterized by high-resolution mass spectrometry and ^1H NMR. Homogeneous by thin-layer chromatography. [c] Contained 0.1 equiv of ethyl acetate.

Results and Discussion

Compounds were assayed for inhibition of rat brain protein kinase C with ATP and histone as substrates (Tables II–V). The bisindolylmaleimide **5** showed good inhibitory activity and was systematically modified to ascertain the structural features contributing to the activity. The double bond of the maleimide ring was found to be essential; activity was lost in both the cis and trans isomers of the corresponding succinimides. A hydrogen-bond donor in the maleimide ring appeared important since neither anhydride **10** nor *N*-methylmaleimide **4** were active, although the *N*-hydroxymaleimide **11** retained some activity. Both carbonyl groups contributed to the potency since amide **8** was less active than the imide. Methylation of either one or both indole nitrogens in compound **5** increased activity (compounds **12** and **13**), although other larger groups such as benzyl were not tolerated so well (compound **14**).

Replacement of an indole group with a variety of other aryl groups reduced activity. The more active compounds contained groups of similar size, shape, and lipophilicity to indole while replacements such as *N*-methylindol-2-yl, occupying significantly different volumes to the indol-3-yl substituent, decreased activity most. This is consistent with fairly well defined hydrophobic binding pockets for the indole rings. There was also some dependence on electronic factors since pairs of heterocycles of similar lipophilicity and shape (e.g. 2- and 3-thienyl) had significantly different effects. These electronic factors may be

Table IV. PKC Inhibition by Substituted Bisindolylmaleimides



no.	substituent (R)	formula	analysis	IC_{50} (μM)
28	5-Cl	$C_{22}H_{16}ClN_3O_2$	C, H, N	0.11 ± 0.04 (2)
29	5-Me	$C_{23}H_{19}N_3O_2$	C, H, N	0.17 ± 0.02 (2)
30	5-NH_2	$C_{22}H_{18}N_4O_2$	C, H, N	0.49 ± 0.10 (2)
31	5-OH	$C_{22}H_{17}N_3O_3$[a]	C, H, N	0.92 ± 0.11 (2)
32	5-OMe	$C_{23}H_{19}N_3O_3$	C, H, N	0.60 ± 0.14 (3)
33	5-OCH_2Ph	$C_{29}H_{23}N_3O_3$	C, H, N	6.9 ± 1.8 (2)
34	5-SMe	$C_{23}H_{19}N_3O_3S$	C, H, N	0.38 ± 0.11 (2)
35	5-S(O)Me	$C_{23}H_{19}N_3O_3S$	C, H, N	3.0 ± 1.0 (2)
36	5-NO_2	$C_{22}H_{16}N_4O_4$	C, H, N	2.5 ± 0.4 (3)
37	5-CO_2Me	$C_{24}H_{19}N_3O_4$	C, H, N	1.4 ± 0.09 (2)
38	5-CO_2H	$C_{23}H_{17}N_3O_4$	[b]	>50 (2)
39	5-$CONH_2$	$C_{23}H_{18}N_4O_3$	[b]	16 ± 2.8 (2)
40	5-NHAc	$C_{24}H_{20}N_4O_3$[c]	C, H, N	15 ± 2.7 (2)
41	6-Cl	$C_{22}H_{16}ClN_3O_2$	C, H, N	1.6 ± 0.5 (2)
42	6-NH_2	$C_{22}H_{18}N_4O_2$[d]	C, H, N	0.31 ± 0.11 (2)
43	6-OMe	$C_{23}H_{19}N_3O_3$	C, H, N	1.1 ± 0.4 (2)
44	6-NO_2	$C_{22}H_{16}N_4O_4$[e]	C, H, N	1.1 ± 0.3 (2)
45	6-CO_2H	$C_{23}H_{17}N_3O_4$	[b]	27 ± 3.2 (2)
46	6-$CONH_2$	$C_{23}H_{18}N_4O_3$	[b]	9.5 ± 0.1 (2)
47	6-NHAc	$C_{24}H_{20}N_4O_3$	[b]	4.0 ± 1.3 (2)
48	7-Me	$C_{23}H_{19}N_3O_2$	C, H, N	0.28 ± 0.08 (2)
49	7-NH_2	$C_{22}H_{18}N_4O_2$	C, H, N	1.0 ± 0.2 (2)
50	7-OMe	$C_{23}H_{19}N_3O_3$	[b]	1.0 ± 0.4 (2)
51	7-NO_2	$C_{22}H_{16}N_4O_4$	C, H, N	1.5 ± 0.5 (2)
52	7-NHAc	$C_{24}H_{20}N_4O_3$[e]	C, H, N	2.4 ± 0.1 (2)
53	4-Me	$C_{23}H_{19}N_3O_2$	[b]	0.59 ± 0.21 (2)
54	4-OMe	$C_{23}H_{19}N_3O_3$	C, H, N	3.0 ± 1.1 (2)
55	4-NO_2	$C_{22}H_{16}N_4O_4$	C, H, N	2.0 ± 0.7 (2)
56	2-Me	$C_{23}H_{19}N_3O_2$[e]	C, H, N	0.47 ± 0.16 (2)
57	2-SMe	$C_{23}H_{19}N_3O_2S$	C, H, N	0.44 ± 0.14 (2)
58	2-S(O)Me	$C_{23}H_{19}N_3O_3S$	C, H, N	1.0 ± 0.4 (3)

[a] Contained 0.3 equiv of methanol. [b] Characterized by high-resolution mass spectroscopy and ^1H NMR. Homogeneous by thin-layer chromatography. [c] Contained 0.25 equiv of acetic anhydride. [d] Contained 0.25 equiv of DMF. [e] Contained 0.3 equiv of DMF.

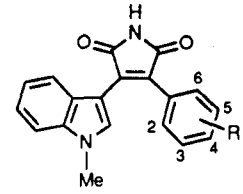
affecting the binding of the aryl groups themselves or changing the electronic properties of the maleimide system.

Quantitation of these effects, for example by Hansch analysis, is complicated by the existence of two arene binding sites, either of which might be occupied by each indole replacement. Furthermore the data set is too small to permit meaningful investigation of a correlation involving all the factors which appear to be involved—lipophilicity, electronics, and direction-dependent bulk. No correlation was found with single parameters, including π and group ionization potential.

In a series of substituted *N*-methylindoles (Table IV), only one substituent, the 5-chloro, improved activity. Electron-withdrawing or -releasing substituents generally failed to increase potency, probably because of steric interactions. Small, lipophilic substituents were tolerated at the 2-, 4-, 5-, and 7-positions and somewhat larger groups could be accommodated at the 1-position. At positions 4–7 larger substituents were detrimental to activity.

These SARs were used to define a minimum allowed volume of the arene binding sites. A common binding conformation was assumed and common structural elements superimposed. All substituents on indole rings were rotated to occupy a minimum volume in the plane of the indole which in each case also resulted in a low-energy

Table V. PKC Inhibition by Substituted (Phenylindolyl)maleimides



no.	substituent (R)	formula	analysis	IC_{50} (μM)
59	4-Cl	$C_{19}H_{13}ClN_2O_2$	C, H, N	>100 (2)
60	4-OMe	$C_{20}H_{16}N_2O_3$	C, H, N	40 ± 8 (2)
61	4-SMe	$C_{20}H_{16}N_2O_2S$	C, H, N	>50 (2)
62	4-SO_2Me	$C_{20}H_{16}N_2O_4S$	C, H, N	>100 (2)
63	4-NH_2	$C_{19}H_{15}N_3O_2$	C, H, N	7.5 ± 0.1 (2)
64	3-Me	$C_{20}H_{16}N_2O_2$	C, H, N	1.0 ± 0.3 (3)
65	3-Cl	$C_{19}H_{13}ClN_2O_2$	C, H, N	2.0 ± 0.6 (5)
66	3-Br	$C_{19}H_{13}BrN_2O_2$	C, H, N	2.2 ± 0.1 (2)
67	3-OMe	$C_{20}H_{16}N_2O_3$[a]	C, H, N	8.0 ± 2.7 (2)
68	3-OPh	$C_{25}H_{18}N_2O_3$	C, H, N	>100 (2)
69	3-NH_2	$C_{19}H_{15}N_3O_2$	C, H, N	3.2 ± 0.3 (2)
70	3-NO_2	$C_{19}H_{13}N_3O_4$	C, H, N	1.9 ± 0.9 (3)
71	3-CF_3	$C_{20}H_{13}F_3N_2O_2$	C, H, N	4.0 ± 1.3 (2)
72	2-Me	$C_{20}H_{16}N_2O_2$	C, H, N	2.2 ± 0.8 (2)
73	2-Cl	$C_{19}H_{13}ClN_2O_2$	C, H, N	0.90 ± 0.4 (3)
74	2-NO_2	$C_{19}H_{13}N_3O_4$	[b]	0.67 ± 0.19 (3)
75	2-CF_3	$C_{20}H_{13}F_3N_2O_2$	C, H, N	0.95 ± 0.35 (2)
76	2,3-Me_2	$C_{21}H_{18}N_2O_2$	C, H, N	2.4 ± 0 (2)
77	2,5-Me_2	$C_{21}H_{18}N_2O_2$	C, H, N	1.2 ± 0.3 (2)
78	2,6-Cl_2	$C_{19}H_{12}Cl_2N_2O_2$	[b]	2.4 ± 0.5 (2)
79	3,5-Cl_2	$C_{19}H_{12}Cl_2N_2O_2$	[b]	7.6 ± 2.8 (2)
80	2,3,6-Cl_3	$C_{19}H_{11}Cl_3N_2O_2$	C, H, N	1.0 ± 0.3 (2)

[a] Contains 0.1 equiv of DMF. [b] Characterized by high-resolution mass spectrometry and ^1H NMR. Homogeneous by thin-layer chromatography.

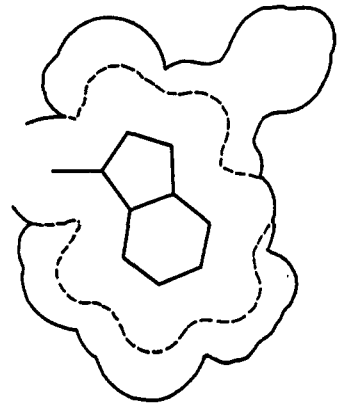


Figure 2. Van der Waals surfaces in the plane of the indole ring. Solid line: combined minimum allowed volume of the indole binding sites. Broken line: volume of indole for comparison.

conformation. A set of compounds (compounds **13**, **16**, **19**, **21**, **28**, **32**, **34**, **42**, **53**, **56**, **57**) with low IC_{50}s (≤1 μM), and therefore no apparent adverse steric interactions with the enzyme, was taken, and their 2-dimensional van der Waals surfaces were calculated. These surfaces were combined in a logical OR operation to give the allowed volume shown in Figure 2. This is the allowed volume of a combination of multiple arene binding sites and may not be a representation of a universal site. It may, however, be used to aid the design of further inhibitors.

The effect of substitution on the phenyl ring of compound **23** was investigated since, at least at the 2- and 3-positions, it was expected that substituents on this smaller ring would be better sterically accommodated than

Table VI. Selectivity of PKC Inhibitors

no.	inhibition of bovine PKA (IC_{50}, μM)[a]	selectivity IC_{50} PKA/ IC_{50} PKC	no.	inhibition of bovine PKA (IC_{50}, μM)[a]	selectivity IC_{50} PKA/ IC_{50} PKC
5	11.8 ± 3.6 (2)	22	**49**	23 ± 4.2 (2)	23
13	16.1 ± 1.7 (2)	54	**53**	13 ± 1.4 (2)	22
14	>100 (1)	>83	**55**	>100 (1)	>50
19	>100 (1)	>123	**56**	31 ± 1.4 (2)	66
21	>100 (1)	>111	**64**	>100 (1)	>100
23	>100 (1)	>13	**65**	>100 (1)	>50
28	8.4 ± 0 (2)	76	**69**	>100 (1)	>31
29	11.5 ± 2.1 (2)	68	**74**	21.5 ± 0.7 (2)	32
32	14.0 ± 2.8 (2)	23	**77**	82 ± 25.5 (2)	68
36	21.3 ± 3.8 (2)	9	**79**	>100 (1)	>13
42	14.5 ± 2.1 (2)	47			

[a] Concentrations above 100 μM were not studied because of potential solubility problems.

on indole (Table V). No 4-position substituent was better than hydrogen. This is probably a steric effect and was not unexpected since substituents at this position extend beyond the allowed volume previously determined by the aryl replacements. Most 3-position substituents marginally increased activity despite their markedly different electronic and hydrophobic properties. The large phenoxy group, however, was not tolerated. Substitution at the 2-position was also beneficial, giving up to an 8-fold increase in potency. The effects of substituents at the 2- and 3-positions were not additive and no further increase in potency was seen for di- and trisubstituted phenyl compounds. This may reflect binding of the substituted phenyl rings in different orientations or in different sites, for instance in either indole binding site.

The mode of PKC inhibition by staurosporine is the subject of debate; the inhibition kinetics have been described both as competitive with ATP[19] and "not competitive" with ATP.[7,20] The kinetics of inhibition by the maleimides described here is exemplified by compound **13**, which displays competitive inhibition with respect to ATP (Lineweaver–Burke plot; K_i = 0.16 μM).

Protein kinases possess extensive sequence homology in their ATP-binding domains,[21] and, consequently, lack of selectivity is a common drawback of ATP-competitive kinase inhibitors.[22] The inhibition of a cAMP-dependent protein kinase (PKA) by a representative sample of maleimides is shown in Table VI. Since the catalytic subunit of PKA is very closely related to the catalytic domain of PKC, the discrimination shown by the more selective inhibitors is remarkable. Naphthalene **19** and benzothiophene **21** were also assayed for inhibition of phosphorylase kinase and were again less active than against PKC (IC_{50}s for both were around 4 μM).

The compounds described here were assayed in 10% DMSO to ensure solubility of the inhibitors. The 10% DMSO produced a 10% increase in PKC activity, and an increase in IC_{50} values over those obtained in 1% DMSO was observed (Table VII). For example the potency of compound **5** increased 5-fold when assayed in 1% DMSO

Table VII. Effect of DMSO Concentration

	IC_{50} (μM)			
	PKC		PKA	
	1% DMSO	10% DMSO	1% DMSO	10% DMSO
1	0.003 ± 0.001 (4)	0.009 ± 0.001 (3)	0.018 ± 0.004 (2)	0.12 ± 0.02 (2)
5	0.1 ± 0.02 (16)	0.55 ± 0.17 (15)	3.1 ± 0.6 (2)	11.8 ± 3.6 (2)
13	0.12 ± 0.02 (3)	0.30 ± 0.06 (9)	*[a]	16.1 ± 1.7 (2)
19	0.40 ± 0.11 (2)	0.81 ± 0.21 (2)	*	>100 (1)
28	0.07 ± 0.01 (3)	0.11 ± 0.04 (2)	4.3 ± 0.3 (2)	8.4 ± 0 (2)
42	0.08 ± 0.01 (2)	0.31 ± 0.11 (2)	3.4 ± 1.1 (2)	14.0 ± 2.8 (2)
64	0.60 ± 0.05 (2)	1.04 ± 0.29 (3)	*	>100 (1)

[a] An asterisk (*) = insoluble.

(0.10 ± 0.02 μM, 16 determinations) and that of staurosporine increased 3-fold (0.003 ± 0.001 μM, 4 determinations). All other compounds compared in this manner exhibited smaller differences than compound **5**, and this might well reflect potential solubility problems in 1% DMSO. However, it is clear that the relative SARs obtained in 10% DMSO correlate closely with those obtained in 1% DMSO.

A similar effect was also seen with PKA. The increase in IC_{50} values for those compounds which could be assayed in 1% DMSO ranged from 2- to 3-fold. The exception to this was staurosporine which exhibited a 6-fold increase in activity in 1% DMSO. Again the difference could be explained by potential insolubility of the maleimides in 1% DMSO. Since the activities against both PKC and PKA are increased in 10% DMSO, the PKA/PKC ratios remain constant in both 1% and 10% DMSO.

In summary, a series of maleimides which inhibit PKC has been derived from the structural lead provided by staurosporine and **2**. Although not as potent as staurosporine, some of these compounds show much higher selectivity for PKC over PKA and phosphorylase kinase. The maleimides therefore represent new structural leads in the search for PKC inhibitors with the potency of staurosporine but with much improved selectivity.

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 a Bruker AC-250 or a Bruker WM-300 spectrometer, and chemical shifts are given in ppm from tetramethylsilane as internal standard. IR spectra were recorded on a Perkin-Elmer Model 782 spectrometer and UV spectra on a Kontron Model Uvikon 820 spectrophotometer. X-ray structure determination was performed on a Nicolet R3m/V instrument.

3,4-Bis(3-indolyl)-1*H*-pyrrole-2,5-dione (5). A solution of indole (23.4 g, 0.2 mol) in benzene (340 mL) was treated with methylmagnesium iodide (66 mL of a 3 M solution in diethyl ether, 0.2 mol), and the resulting solution was stirred at room temperature under nitrogen for 0.5 h. Dibromomaleimide (14.57 g, 57 mmol) was added, and the mixture was heated to reflux for 65 h, cooled, and evaporated to dryness. The residue was partitioned between dichloromethane (500 mL) and 2 M HCl (400 mL), and the insoluble material was filtered off. The dichloromethane extract was separated and dried (Na_2SO_4), and the solvent was removed by evaporation. Both the solid material and the extracted material were subjected to flash chromatography on silica gel using ethyl acetate/petroleum ether (1:1) as the eluant. The red product obtained was crystallized from acetone/dichloromethane and methanol/water to give 5.3 g (29%) of **5** as a red solid. An analytical sample was prepared by reprecipitation from methanol/water: mp 252–3 °C; UV (MeOH) λ_{max} 219 (ϵ

(19) Tamaoki, T.; Nakano, H. Potent And Specific Inhibitors of Protein Kinase C of Microbial Origin. *Biotechnology* **1990**, *8*, 732–735.

(20) Spacey, G. D.; Bonser, R. W.; Randall, R. W.; Garland, L. G. Selectivity of Protein Kinase Inhibitors In Human Intact Platelets. *Cellular Signalling* **1990**, *2*, 329–338.

(21) Hanks, S. K.; Quinn, A.; Hunter, T. The Protein Kinase C Family: Conserved Features And Deduced Phylogeny of The Catalytic Domain. *Science* **1988**, *241*, 42–52.

(22) Ruegg, U. T.; Burgess, G. M. Staurosporine, K252 and UCN-01: Potent But Nonspecific Inhibitors of Protein Kinases. *TiPS* **1989**, *10*, 218–220.

40 400), 277 (10 200), 373 (5000), 457 nm (7400); IR (Nujol) ν_{max} 1750, 1690 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.62 (2 H, t, J = 8 Hz, indole, 5-H), 6.80 (2 H, d, J = 8 Hz, indole, 4-H), 6.97 (2 H, t, J = 8 Hz, indole, 6-H), 7.36 (2 H, d, J = 8 Hz, indole, 7-H), 7.73 (2 H, s, indole, 2-H), 10.90 (1 H, bs, imide, N-H), and 11.66 (2 H, bs, indole, NH); MS m/z 327 (M^+). Anal. ($C_{20}H_{13}N_3O_2$) C, H, N.

***trans*-3,4-Bis(3-indolyl)-2,5-pyrrolidinedione (6).** A solution of indole (5.8 g, 49.6 mmol) in toluene (100 mL) was added dropwise to a stirred solution of 3 M methylmagnesium chloride in THF (16.5 mL, 49.5 mmol). The mixture obtained was heated to 45 °C for 1 h and was then treated with a solution of bromomaleimide[23] (1.34 g, 8.27 mmol) in toluene (50 mL). The mixture obtained was heated to reflux for 6 h, cooled, and acidified with 2 M HCl (200 mL). The mixture was extracted with EtOAc (2 × 250 mL), and the combined extracts were washed with water (250 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed twice on silica gel (ether/petroleum ether, 2:1, and CH_2Cl_2/EtOAc, 9:1) to give **6** (150 mg, 6%) as a white solid: mp 264–5 °C; UV (MeOH) λ_{max} 221 (ϵ 65 000), 281 (13 100), 289 nm (11 900); IR (Nujol) ν_{max} 1760, 1710, 1700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.52 (2 H, s, imide, 3-H and 4-H), 6.87 (2 H, t, J = 8 Hz, indole-H), 7.04 (2 H, t, J = 8 Hz, indole-H), 7.19 (2 H, d, J = 2.5 Hz, indole 2-H), 7.31 (2 H, d, J = 8 Hz, indole-H), 7.37 (2 H, d, J = 8 Hz, indole-H), 11.03 (2 H, bs, indole 1-H), 11.45 (1 H, bs, imide 1-H); MS m/z 329 (M^+). Anal. ($C_{20}H_{15}N_3O_2$) C, H, N.

***cis*-3,4-Bis(3-indolyl)-2,5-pyrrolidinedione (7).** 10% Pd/C (40 mg, 0.04 mmol) was added to a solution of 3,4-bis(3-indolyl)-1*H*-pyrrole-2,5-dione (**5**) (200 mg, 0.6 mmol) in DMF (10 mL). The black suspension was shaken under a hydrogen atmosphere (50 psi) for 4 days, the catalyst was filtered off, and the filtrate was concentrated in vacuo to give a cream-colored solid. Crystallization from ethyl acetate/petroleum ether gave 140 mg (70%) of pure **7** as a white solid: mp 235–8 °C; UV (MeOH) λ_{max} 219 (ϵ 55 000), 274 (10 700), 280 (10 900), 290 nm (9200); IR (Nujol) ν_{max} 1750, 1715, 1695 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.91 (2 H, s, imide 3- and 4-H), 6.75–7.00 (6 H, m, indole-H), 7.13 (2 H, d, J = 8 Hz, indole-H), 7.40 (2 H, d, J = 8 Hz, indole-H), 10.63 (2 H, bs, indole 1-H) and 11.58 (1 H, bs, imide 1-H); MS m/z 329 (M^+). Anal. ($C_{20}H_{15}N_3O_2$) C, H, N.

3,4-Bis(3-indolyl)-3-pyrrolin-2-one (8) and 5-Hydroxy-3,4-bis(3-indolyl)-3-pyrrolin-2-one (9). Lithium aluminum hydride (20 mL of a 1 M solution in diethyl ether, 20 mmol) was added to a stirred solution of 3,4-bis(3-indolyl)-1*H*-pyrrole-2,5-dione (**5**) (1.0 g, 3.05 mmol) in THF (140 mL). The mixture was stirred at room temperature for 18 h under a nitrogen atmosphere. The mixture was cooled to 0 °C, quenched carefully with water (50 mL), and then acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (3 × 75 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution (30 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography on silica gel using 5–10% methanol in dichloromethane for the elution. The first product eluted was triturated with ethyl acetate/hexane to give 175 mg (18%) of the lactam **8** as a cream-colored solid: mp 290–3 °C; UV (MeOH) λ_{max} 224 (ϵ 41 600), 278 (14 400), 339 nm (12 900); IR (Nujol) ν_{max} 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.54 (2 H, s, lactam 5-H), 6.73 (1 H, t, J = 8 Hz, indole-H), 6.86 (1 H, t, J = 8 Hz, indole-H), 6.90–7.10 (3 H, m, indole-H), 7.25–7.44 (4 H, m, indole-H), 7.49 (1 H, d, J = 2.5 Hz, indole 2-H), 8.22 (1 H, s, lactam 1-H), 11.26 (1 H, t, indole 1-H), and 11.38 (1 H, bs, indole 1-H); MS m/z 313 (M^+). Anal. ($C_{20}H_{15}N_3O$) C, H, N.

The second product eluted was crystallized from ethyl acetate/chloroform to give 490 mg (49%) of **9** as a cream-colored solid: mp >250 °C; UV (MeOH) λ_{max} 209 (ϵ 38 100), 224 (40 900), 278 (12 100), 353 nm (11 600); IR (Nujol) ν_{max} 3600–2850, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.93–6.06 (2 H, m, lactam 5-H and OH), 6.63–6.76 (2 H, m, indole-H), 6.90–7.03 (3 H, m, indole-H), 7.15 (1 H, d, J = 8 Hz, indole-H), 7.28–7.38 (2 H, m, indole-H), 7.43 (1 H, d, J = 2.5 Hz, indole 2-H), 7.47 (1 H, d, J = 2.5 Hz, indole 2-H), 8.47 (1 H, bs, lactam 1-H), 11.25 (1 H, bd, J = 2.5 Hz, indole 1-H) and 11.38 (1 H, bd, J = 2.5 Hz, indole 1-H); MS m/z 329 (M^+). Anal. ($C_{20}H_{15}N_3O_2$) C, H, N.

1-Hydroxy-3,4-bis(3-indolyl)-2,5-pyrroledione (11). A solution of hydroxylamine hydrochloride (300 mg, 0.92 mmol) and potassium carbonate (650 mg, 4.71 mmol) in water (50 mL) was added to a stirred solution of 3,4-bis(3-indolyl)-2,5-furandione in DMF (50 mL). The mixture obtained was heated to 80 °C for 3 h, cooled, and concentrated under reduced pressure. The residue was partitioned between ethyl acetate (250 mL) and water (150 mL), and the organic extract was washed with saturated $NaHCO_3$ (50 mL) and water (50 mL), dried ($MgSO_4$), and evaporated to dryness. Purification was effected by flash chromatography (EtOAc/petroleum ether, 1:1) to give **11** as a red solid (205 mg, 65%): mp 232 °C; IR (Nujol) ν_{max} 1695, 1760, and 3000–3450 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.65 (2 H, t, J = 7 Hz, indole), 6.80 (2 H, d, J = 7 Hz, indole), 6.97 (2 H, t, J = 7 Hz, indole), 7.39 (2 H, d, J = 7 Hz, indole), 7.79 (2 H, s, indole 2-H), 10.45 (1 H, bs, NOH) and 11.74 (2 H, bs, NH); MS m/z 343 (M^+). Anal. ($C_{20}H_{13}N_3O_3$) C, H, N.

General Method. 3-(5-Methoxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione (32). A solution of 5-methoxyindole (5.0 g, 34 mmol) in DMF (50 mL) at 0 °C was treated with sodium hydride (80% in oil, 1.2 g, 40 mmol). After 30 min iodomethane (5.68 g, 40 mmol) was added and the mixture was allowed to warm to room temperature. After a further 16 h, the mixture was poured into water (250 mL), acidified with 2 N hydrochloric acid, and allowed to stand for 4 h at 4 °C. The solid was filtered and dried to give 5.31 g (97%) of 5-methoxy-1-methylindole:[24] ^1H NMR ($CDCl_3$) δ 3.79 (3 H, s, methyl), 3.89 (3 H, s, methyl), 6.43 (1 H, d, J = 2.5 Hz, indole-H), 6.92 (1 H, dd, J = 8, 2.5 Hz, indole 6-H), 7.05 (1 H, d, J = 2.5 Hz, indole-H), 7.13 (1 H, d, J = 2.5 Hz, indole-H), 7.25 (1 H, d, J = 8 Hz, indole-H); MS m/z 161 (M^+). A solution of 5-methoxy-1-methylindole (5.3 g, 33 mmol) in dichloromethane (50 mL) at 0 °C was treated with oxalyl chloride (4.6 g, 36 mmol). The mixture was allowed to warm to room temperature and stir for 3 h before the solvent was removed under reduced pressure. 5-Methoxy-1-methylindole-3-glyoxylyl chloride was obtained as a purple solid (7.64 g, 95%) and used without purification: ^1H NMR ($CDCl_3$) δ 3.89 (3 H, s, methyl), 3.93 (3 H, s, methyl), 7.02 (1 H, dd, J = 8, 2 Hz, indole 6-H), 7.30 (1 H, d, J = 8 Hz, indole 7-H), 7.87 (1 H, d, J = 2 Hz, indole 4-H), 8.09 (1 H, s, indole 2-H); MS m/z 251 (M^+).

A solution of 5-methoxy-1-methylindole-3-glyoxylyl chloride (7.85 g, 31 mmol) in dichloromethane (100 mL) was treated with triethylamine (7.85 g, 78 mmol) followed by a solution of 1-methylindole-3-acetic acid (5.86 g, 31 mmol) in dichloromethane (50 mL). The mixture was allowed to stir for 16 h before the solvent was removed under reduced pressure and the residue was chromatographed on silica gel with 1% methanol in dichloromethane as eluant.

3-(5-Methoxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)-furan-2,5-dione was obtained as a red solid: mp 234–237 °C; ^1H NMR ($CDCl_3$) δ 3.11 (3 H, s, OMe), 3.89 (6 H, s, indole *N*-methyls), 6.24 (1 H, s, methoxyindole 4-H), 6.79 (1 H, t, J = 8 Hz, indole-H), 6.86 (1 H, d, J = 8 Hz, indole-H), 7.03 (1 H, d, J = 8 Hz, indole-H), 7.13–7.33 (2 H, m, indole-Hs), 7.34 (1 H, d, J = 8 Hz, indole-H), 7.70 (1 H, s, indole 2-H), 7.84 (1 H, s, indole 2-H). Accurate mass (M^+) 386.1269 ($C_{23}H_{18}N_2O_4$ requires 386.1266): yield 1.94 g, 16%. The anhydride (100 mg, 0.26 mmol) was dissolved in DMF (3 mL) and treated with 33% aqueous ammonia (10 mL). The mixture was heated at 140 °C in a sealed steel bomb for 3 h before being allowed to cool. The red solid was collected by filtration and dried to give 3-(5-methoxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione: mp 240–245 °C; ^1H NMR (DMSO-d_6) δ 2.95 (3 H, s, OMe), 3.83 (3 H, s, indole *N*-methyl), 3.84 (3 H, s, indole *N*-methyl), 6.03 (1 H, d, J = 2.5 Hz, methoxyindole 4-H), 6.58 (1 H, dd, J = 8, 2.5 Hz, methoxyindole 6-H), 6.70 (1 H, t, J = 8 Hz, indole-H), 6.90 (1 H, d, J = 8 Hz, indole-H), 7.07 (1 H, t, J = 8 Hz, indole-H), 7.28 (1 H, d, J = 8 Hz, indole-H), 7.43

(23) Nicolaus, R. A.; Nicoletti, R. New Synthesis Of Methoxymaleimide. *Rend. Acad. Sci. Fis e Mat (Soc. Nazl. Sci., Napoli) Ser. 4* **1959**, *26*, 149–53; *Chem. Abstr.* **1961**; *55*, 09373B.

(24) Taborsky, R. G.; Delvigs, P.; Page, I.; Crawford, N. Synthesis and Preliminary Pharmacology of Some 1-Methylindoles. *J. Med. Chem.* **1965**, *8*, 460–466.

(1 H, d, J = 8 Hz, indole-H), 7.72 (1 H, s, indole 2-H), 7.91 (1 H, s, indole 2-H), 10.88 (1 H, s, NH); MS m/z 385 (M^+). Anal. ($C_{23}H_{19}N_3O_3$) C, H, N. Yield: 72 mg, 72%.

3-(1-Methyl-3-indolyl)-4-(1-methyl-7-nitro-3-indolyl)-pyrrole-2,5-dione (51). A solution of 7-nitroindole (4.8 g, 30 mmol) in DMF (50 mL) at 0 °C was treated with sodium hydride (80% in oil, 1.06 g, 35 mmol). After 30 min iodomethane (4.97 g, 35 mmol) was added and the mixture was allowed to warm to room temperature. After a further 3 h the mixture was poured into water (250 mL), acidified with 2 N hydrochloric acid, and allowed to stand for 16 h at 4 °C. The yellow solid was filtered and dried to give 5.25 g (99%) of 1-methyl-7-nitroindole:[25] mp 48–49 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (3 H, s, methyl), 6.65 (1 H, d, J = 3.5 Hz, indole 3-H), 7.14 (1 H, d, J = 3.5 Hz, indole 2-H), 7.15 (1 H, t, J = 7.5 Hz, indole 5-H), 7.83 (1 H, dd, J = 7.5, 1.2 Hz, indole-H), 7.88 (1 H, dd, J = 7.5, 1.2 Hz, indole-H); MS m/z 176 (M^+). A solution of 1-methyl-7-nitroindole (5.2 g, 30 mmol) in dichloromethane (50 mL) at 0 °C was treated with oxalyl chloride (4.5 g, 35 mmol). The mixture was allowed to warm to room temperature and stir for 3 h before the solvent was removed under reduced pressure.

1-Methyl-7-nitroindole-3-glyoxylyl chloride was obtained as a brown solid (7.64 g, 95%) and used without purification: ^{1}H NMR ($CDCl_3$) δ 7.48 (1 H, t, J = 7.5 Hz, indole 5-H), 7.96 (1 H, dd, J = 7.5, 1.2 Hz, indole-H), 8.30 (1 H, s, indole 2-H), 8.75 (dd, J = 7.5, 1.2 Hz, indole-H); MS m/z 266 (M^+). A solution of 1-methyl-7-nitroindole-3-glyoxylyl chloride (7.6 g, 29 mmol) in dichloromethane (100 mL) was treated with triethylamine (7.2 g, 71 mmol) followed by a solution of 1-methylindole-3-acetic acid (5.68 g, 30 mmol) in dichloromethane (50 mL). The mixture was allowed to stir for 3 h before the solvent was removed under reduced pressure and the residue was chromatographed on silica gel with 2% methanol in dichloromethane as eluant.

3-(1-Methyl-3-indolyl)-4-(1-methyl-7-nitro-3-indolyl)furan-2,5-dione was obtained as a red solid: mp 252–255 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (3 H, s, methyl), 3.93 (3 H, s, methyl), 6.66 (1 H, d, J = 7.5 Hz, indole-H), 6.77 (1 H, dt, J = 7.5, 1.2 Hz, indole-H), 6.84 (1 H, d, J = 7.5 Hz, indole-H), 7.17 (1 H, dt, J = 7.5, 1.2 Hz, indole-H), 7.30 (1 H, t, J = 7.5 Hz, nitroindole 5-H), 7.35 (1 H, dd, J = 7.5, 1.2 Hz, indole-H), 7.70 (1 H, dd, J = 7.5, 1.2 Hz, indole-H), 7.74 (1 H, s, indole 2-H), 7.99 (1 H, s, indole 2-H); IR (Nujol) ν_{max} 1820, 1740 cm^{-1}; accurate mass 401.1017 ($C_{22}H_{15}N_3O_5$ requires 401.1012). Anal. ($C_{22}H_{15}N_3O_5$) C, H, N. Yield: 3.27 g, 28%.

3-(1-Methyl-3-indolyl)-4-(1-methyl-7-nitro-3-indolyl)furan-2,5-dione (150 mg, 0.37 mmol) was dissolved in DMF (1 mL) and treated with 33% aqueous ammonia (10 mL). The mixture was heated at 140 °C in a sealed steel bomb for 5 h before being allowed to cool. The red solid was collected by filtration and dried to give 3-(1-methyl-3-indolyl)-4-(1-methyl-7-nitro-3-indolyl)-pyrrole-2,5-dione: mp 264–266 °C; ^{1}H NMR (DMSO-d_6) δ 3.84 (3 H, s, methyl), 3.88 (3 H, s, methyl), 6.59 (1 H, d, J = 7.5 Hz, indole-H), 6.66 (1 H, d, J = 7.5 Hz, indole-H), 6.83 (1 H, t, J = 7.5 Hz, indole-H), 7.05 (1 H, t, J = 7.5 Hz, indole-H), 7.27 (1 H, d, J = 7.5 Hz, indole-H), 7.44 (1 H, t, J = 7.5 Hz, indole-H), 7.69 (1 H, d, J = 7.5 Hz, indole-H), 7.89 (1 H, s, indole 2-H), 7.96 (1 H, s, indole 2-H), 11.08 (1 H, bs, imide NH); IR (Nujol) ν_{max} 3400, 1755, 1710 cm^{-1}; MS m/z 400 (M^+). Anal. ($C_{22}H_{16}N_4O_4$) C, H, N. Yield: 109 mg, 73%.

Desulfonylation. 3-(1-Methyl-3-indolyl)-4-(3-pyrrolyl)-pyrrole-2,5-dione (24). A solution of 3-(1-(benzenesulfonyl)-3-pyrrolyl)-4-(1-methyl-3-indolyl)furan-2,5-dione (255 mg, 0.59 mmol) in ethanol (10 mL) was treated with 2.5 M sodium hydroxide solution (2.5 mL) for 16 h. Water was added, and the solution was washed with two portions of ether. The aqueous solution was acidified and extracted with ethyl acetate. This extract was dried, and the solvent was removed under reduced pressure.

3-(1-Methyl-3-indolyl)-4-(3-pyrrolyl)furan-2,5-dione was isolated as an orange solid (140 mg, 81%) and used without purification: ^{1}H NMR ($CDCl_3$) 3.92 (3 H, s, NMe), 6.40 (1 H, m, aromatic), 6.71 (1 H, m, aromatic), 7.05 (2 H, m, aromatic), 7.30 (1 H, m, aromatic), 7.42 (1 H, d, J = 7.5 Hz, aromatic), 7.52 (1 H, m, aromatic), 7.63 (1 H, s, indole 2-H), 8.55 (1 H, bs, pyrrole NH). A solution of this anhydride (135 mg, 0.46 mmol) in DMF (5 mL) was treated with 30% aqueous ammonia (5 mL) and heated at 140 °C in a sealed steel bomb for 4 h. The cooled mixture was diluted with water and extracted with dichloromethane. The dried (Na_2SO_4) solution was concentrated, and the residue was chromatographed on silica gel with 50% ethyl acetate in hexane. Imide **24** was obtained as a red solid: mp 240–241 °C; ^{1}H NMR (DMSO-d_6) δ 3.91 (3 H, s, NMe), 6.08 (1 H, m, aromatic), 6.67 (1 H, m, aromatic), 6.95 (2 H, m, aromatic), 7.20 (1 H, m, aromatic), 7.35 (1 H, m, aromatic), 7.52 (1 H, d, J = 7.5 Hz, aromatic), 7.69 (1 H, s, indole 2-H), 10.76 (1 H, s, pyrrole NH), 11.19 (1 H, bs, imide NH); MS m/z 291 (M^+). Anal. ($C_{17}H_{13}N_3O_2 \cdot 0.1EtOAc$) C, H, N.

Oxidation of Sulfides. 3-(1-Methyl-3-indolyl)-4-(1-methyl-5-(methylsulfinyl)-3-indolyl)pyrrole-2,5-dione (35). A solution of sulfide **34** (70 mg, 0.175 mmol) in dichloromethane (50 mL) at 0 °C was treated with m-chloroperbenzoic acid (80%, 38 mg, 0.176 mmol), and the resulting solution was stirred for 8 h. The mixture was washed successively with saturated aqueous sodium bicarbonate solution (20 mL) and water (20 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from ethyl acetate to give sulfoxide **35** as an orange solid: mp 292 °C; ^{1}H NMR ($CDCl_3$) δ 1.95 (3 H, s, SMe), 3.94 (3 H, s, NMe), 3.96 (3 H, s, NMe), 6.52–6.68 (2 H, m, indole-Hs), 6.97 (1 H, s, (methylsulfinyl)indole 4-H), 7.02 (1 H, t, J = 7.5 Hz, indole-H), 7.22–7.30 (2 H, m, indole-Hs), 7.32–7.44 (2 H, m, indole-Hs), 7.64 (1 H, bs, NH), 7.89 (1 H, s, indole 2-H), 7.93 (1 H, s, indole 2-H); MS m/z 417 (M^+). Anal. ($C_{23}H_{19}N_3O_3S$) C, H, N.

Demethylation. 3-(5-Hydroxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione (31). A solution of 1-(5-methoxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)furan-2,5-dione (100 mg, 0.26 mmol) and pyridine hydrochloride (400 mg, 3.1 mmol) in pyridine (3 mL) was heated at 220 °C in a sealed glass tube for 2 h. The cooled mixture was partitioned between dichloromethane and water, and the organic phase was washed with two portions of water and one portion of 0.5 N hydrochloric acid. The dried ($MgSO_4$) and concentrated solution was chromatographed on silica gel with 1% methanol in dichloromethane to give 3-(5-hydroxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)-furan-2,5-dione as a red solid: mp 128–132 °C; ^{1}H NMR ($CDCl_3$) δ 3.75 (3 H, s, N-methyl), 3.76 (3 H, s, N-methyl), 6.25 (1 H, d, J = 2.5 Hz, hydroxyindole 4-H), 6.70 (1 H, dd, J = 8, 2.5 Hz, hydroxyindole 6-H), 6.82 (1 H, t, J = 8 Hz, indole-H), 6.96 (1 H, d, J = 8 Hz, indole-H), 7.14 (1 H, d, J = 8 Hz, indole-H), 7.15 (1 H, t, J = 8 Hz, indole-H), 7.28 (1 H, d, J = 8 Hz, indole-H), 7.59 (1 H, s, indole 2-H), 7.65 (1 H, s, indole 2-H); accurate mass (M^+) 372.1119 ($C_{22}H_{16}N_2O_4$ requires 372.1110); yield 58 mg, 60%.

3-(5-Hydroxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)-furan-2,5-dione (58 mg, 0.16 mmol) was dissolved in DMF (1 mL) and treated with 33% aqueous ammonia (20 mL). The mixture was heated at 140 °C in a sealed steel bomb for 5 h before being allowed to cool. The solvent was removed under reduced pressure, and the residue was triturated with water to give a red solid, 3-(5-hydroxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione, which was filtered and dried: mp 284–287 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (3 H, s, indole N-methyl), 3.85 (3 H, s, indole N-methyl), 6.21 (1 H, d, J = 2.5 Hz, hydroxyindole 4-H), 6.55 (1 H, dd, J = 8, 2.5 Hz, hydroxyindole 6-H), 6.67 (1 H, t, J = 8 Hz, indole-H), 6.79 (1 H, d, J = 8 Hz, indole-H), 7.05 (1 H, t, J = 8 Hz, indole-H), 7.20 (1 H, d, J = 8 Hz, indole-H), 7.42 (1 H, d, J = 8 Hz, indole-H), 7.63 (1 H, s, indole 2-H), 7.79 (1 H, s, indole 2-H), 8.51 (1 H, s, OH), 10.84 (1 H, s, NH); MS m/z 385 (M^+). Anal. ($C_{23}H_{19}N_3O_3$) C, H, N. Yield: 18 mg, 30%.

Reduction of Nitro Groups. 3-(7-Amino-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione (49). 3-(1-Methyl-3-indolyl)-4-(1-methyl-7-nitro-3-indolyl)furan-2,5-dione (2 g, 5 mmol) was dissolved in warm THF (80 mL). The solution was shaken with 10% Pd/C (200 mg) in an atmosphere of hydrogen at ambient pressure and temperature for 16 h. Solvent was removed under reduced pressure from the filtered solution. Chromatography on silica gel with ethyl acetate provided 3-(7-amino-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)furan-2,5-dione

(25) Vance, W. A.; Okamoto, H. S.; Wang, Y. Y. Structure–activity Relationships Of Nitro And Methylnitro Derivatives Of Indoline, Indole, Indazole And Benzimidazole In Salmonella Typhimurium. *Mutat. Res.* **1986**, *173*, 169–176.

as a red solid: mp 286–288 °C; ^{1}H NMR ($CDCl_3$) δ 3.87 (3 H, s, methyl), 4.22 (3 H, s, methyl), 6.38 (1 H, t, J = 7.5 Hz, indole-H), 6.54 (1 H, t, J = 7.5 Hz, indole-H), 6.83 (1 H, t, J = 7.5 Hz, indole-H), 7.00 (1 H, d, J = 7.5 Hz, indole-H), 7.16 (1 H, t, J = 7.5 Hz, indole-H), 7.32 (1 H, t, J = 7.5 Hz, indole-H), 7.62 (1 H, s, indole 2-H), 7.75 (1 H, s, indole 2-H); accurate mass 371.1265 ($C_{22}H_{17}N_3O_3$ requires 371.1270); yield 410 mg, 22%.

3-(7-Amino-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)furan-2,5-dione (265 mg, 0.71 mmol) was dissolved in DMF (10 mL) and treated with 33% aqueous ammonia (10 mL). The mixture was heated at 140 °C in a sealed steel bomb for 5 h before being allowed to cool. The red solid was collected by filtration and dried.

3-(7-Amino-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)-pyrrole-2,5-dione was obtained as a red solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (3 H, s, methyl), 4.12 (3 H, s, methyl), 4.90 (2 H, s, amine H), 6.04 (1 H, d, J = 7.5 Hz, indole-H), 6.27 (1 H, d, J = 7.5 Hz, indole-H), 6.33 (1 H, t, J = 7.5 Hz, indole-H), 6.71 (1 H, t, J = 7.5 Hz, indole-H), 6.39 (1 H, d, J = 7.5 Hz, indole-H), 7.04 (1 H, t, J = 7.5 Hz, indole-H), 7.41 (1 H, d, J = 7.5 Hz, indole-H), 7.58 (1 H, s, indole 2-H), 7.72 (1 H, s, indole 2-H), 10.80 (1 H, bs, imide NH); IR (Nujol) ν_{max} 3415, 3345, 1752, 1708 cm^{-1}; MS m/z 370 (M^+); yield 159 mg, 60%.

Acetylation of Aromatic Amines. 3-(7-(Acetylamino)-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione (52). A solution of the amine **49** (50 mg, 0.13 mmol) in acetic anhydride (2 mL) was stirred for 2 h. The solvent was removed under reduced pressure, and the residue was crystallized from DMF/water to give, after drying, 3-(7-(acetylamino)-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione as a red solid containing 0.35 equiv of DMF: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 2.08 (3 H, s, amide methyl), 3.87 (3 H, s, methyl), 3.94 (3 H, s, methyl), 6.59–6.80 (5 H, complex signal, indole-Hs) 7.07 (1 H, t, J = 7.5 Hz, indole-H), 7.43 (1 H, d, J = 7.5 Hz, indole-H), 7.69 (1 H, s, indole 2-H), 7.82 (1 H, s, indole 2-H), 9.74 (1 H, s, amide NH), 10.94 (1 H, bs, imide NH); MS m/z 412 (M^+). Anal. ($C_{24}H_{20}N_4O_3$·0.35DMF) C, H, N. Yield: 32 mg, 50%.

Crystal Data for 5. $C_{20}H_{15}N_3O_2$·0.5H_2O·0.5MeOH; MW 352.37; orthorhombic; *Fdd*2; a = 37.606 (4) Å, b = 16.2585 Å, and c = 11.5221 Å; V = 7044.75 (12) Å^3; Z = 16; density (calcd) 1.31 mg/m^3; λ 0.710 69 Å; F(000) = 2896; observed reflections 1396; solved by direct methods and refined by full-matrix least-squares methods; final R 0.069.

Molecular Modeling. Structures were built from fragments obtained from the Cambridge Structural Database[26] and from the X-ray crystal structure of **5**. Molecular mechanics calculations and geometry optimizations were performed within MOLOC.[13] The map in Figure 2 was calculated within ChemX[27] from structures built and optimized within MOLOC; 100 data points and a contour level of 1 were used.

Inhibition of Rat Brain PKC. Compounds were assayed for PKC inhibitory activity as described previously.[1] 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 compound **5** gave an IC_{50} value of 0.55 ± 0.17 μM (15 determinations) and for compound **13** gave 0.30 ± 0.06 μM (9 determinations). Stock solutions of inhibitors were prepared in DMSO and assays were performed in 10% aqueous DMSO to ensure solubility of inhibitors.

Inhibition of Bovine Heart PKA. Compounds were assayed for PKA inhibitory activity as described previously.[1] 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 compound **5** gave an IC_{50} value of 11.8 ± 3.6 μM and for compound **13** gave 16.1 ± 1.7 μM. The assay mixture contained 10% DMSO as before.

Inhibition of Phosphorylase Kinase. Assays were performed as described previously.[10]

Acknowledgment. We thank Dr. J. Daly and Dr. P. Schoenholzer for the X-ray determination of compound **5**, Messrs. R. A. Bit, J. J. Long, P. Malsher, and I. Smith for synthetic contributions, Mr. I. Whitcombe for NMR spectra and members of the Department of Physical Methods, Roche Products Ltd., for other physical measurements.

Registry No. **4**, 113963-68-1; **5**, 119139-23-0; **6**, 137467-07-3; **7**, 137467-08-4; **8**, 85753-78-2; **9**, 125313-53-3; **10**, 115684-57-6; **11**, 137108-08-8; **12**, 133052-89-8; **13**, 125313-72-6; **14**, 125313-88-4; **15**, 137467-09-5; **16**, 125314-42-3; **17**, 125314-19-4; **18**, 125314-23-0; **19**, 125313-42-0; **20**, 137467-10-8; **21**, 125313-44-2; **22**, 125314-24-1; **23**, 125313-97-5; **24**, 125314-63-8; **25**, 125313-45-3; **26**, 125314-10-5; **27**, 137467-11-9; **28**, 125313-77-1; **29**, 125313-93-1; **30**, 125313-46-4; **31**, 125313-48-6; **32**, 125313-89-5; **33**, 125313-90-8; **34**, 125313-74-8; **35**, 125313-78-2; **36**, 125313-41-9; **37**, 131305-28-7; **38**, 137467-12-0; **39**, 137494-44-1; **40**, 125313-47-5; **41**, 125313-95-3; **42**, 125314-12-7; **43**, 125313-75-9; **44**, 125313-92-0; **45**, 137467-13-1; **46**, 137467-14-2; **47**, 125314-15-0; **48**, 125313-94-2; **49**, 125314-11-6; **50**, 125334-44-3; **51**, 125313-91-9; **52**, 125314-14-9; **53**, 125334-47-6; **54**, 125313-73-7; **55**, 125313-96-4; **56**, 125314-18-3; **57**, 125313-56-6; **58**, 125334-43-2; **59**, 125313-99-7; **60**, 125313-98-6; **61**, 125314-00-3; **62**, 125314-16-1; **63**, 125314-02-5; **64**, 137467-15-3; **65**, 125314-04-7; **66**, 125314-05-8; **67**, 137467-16-4; **68**, 137467-17-5; **69**, 125314-13-8; **70**, 125314-03-6; **71**, 125314-09-2; **72**, 125334-48-7; **73**, 125314-07-0; **74**, 125314-01-4; **75**, 125314-08-1; **76**, 125314-20-7; **77**, 125314-06-9; **78**, 125334-49-8; **79**, 125314-21-8; **80**, 125314-22-9; indole, 120-72-9; dibromomaleimide, 1122-10-7; bromomaleimide, 98026-79-0; 3,4-bis(3-indolyl)-2,5-furandione, 115684-57-6; 5-methoxyindole, 1006-94-6; 5-methoxy-1-methylindole, 2521-13-3; oxalyl chloride, 79-37-8; 5-methoxy-1-methylindole-3-glyoxylyl chloride, 16382-43-7; 1-methylindole-3-acetic acid, 1912-48-7; 3-(5-methoxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)furan-2,5-dione, 125314-90-1; 7-nitroindole, 6960-42-5; 1-methyl-7-nitroindole, 101489-23-0; 1-methyl-7-nitroindole-3-glyoxylyl chloride, 137467-18-6; 3-(1-methyl-3-indolyl)-4-(1-methyl-7-nitro-3-indolyl)furan-2,5-dione, 137467-19-7; 3-(1-benzenesulfonyl)-3-pyrrolyl)-4-(1-methyl-3-indolyl)furan-2,5-dione, 125334-54-5; 3-(1-methyl-3-indolyl)-4-(3-pyridyl)furan-2,5-dione, 125315-18-6; 3-(5-hydroxy-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)furan-2,5-dione, 125314-89-8; 3-(7-amino-1-methyl-3-indolyl)-4-(1-methyl-3-indolyl)furan-2,5-dione, 137467-20-0; protein kinase, 9026-43-1.

Supplementary Material Available: Further details of structure determination for **5**, including atomic coordinates, bond lengths, and bond angles, and Lineweaver–Burk and Dixon plots for compound **13** (9 pages). Ordering information is given on any current masthead page.

(26) The Cambridge Structural Database is distributed by Cambridge Crystallographic Data Centre, University Chemical Laboratories, Lensfield Rd., Cambridge, U.K.

(27) ChemX is distributed by Chemical Design Ltd. Oxford, U.K.