Recent discovery and development of selective protein kinase C inhibitors

Hong Hu

Evidence for inappropriate activation of protein kinase C (PKC) in a variety of human disease processes suggests that PKC represents a novel cellular target for development of therapeutic agents. A number of potent and selective PKC inhibitors identified recently have demonstrated potential therapeutic applications in both *in vitro* and *in vivo* studies. This article reviews the recent development of some of these PKC inhibitors based on their chemical class.

Protein kinase C (PKC), a key element in the signal transduction pathway, is a family of serine/threonine protein kinases discovered by Nishizuka and coworkers in the 1970s (Refs 1,2). To date, 12 isozymes of the PKC family have been identified and cloned, namely α, β_I, β_{II}, γ, δ, ε, η, θ, μ, ζ, λ and ι (Refs 3–6). PKC isozymes are similar in structure and contain a single polypeptide chain, with a catalytic domain in the C-terminus and a regulatory domain in the N-terminus. The catalytic domain has binding sites for protein substrates and ATP; the regulatory domain has binding sites for Ca^{2+}, phospholipid and activators such as diacylglycerol (DAG) and phorbol esters. On the basis of molecular heterogeneity, activation requirements and cofactor specificity, PKC isozymes are classified into three groups: classical or conventional PKCs (cPKC: α, β_I, β_{II} and γ); novel PKCs (nPKC: δ, ε, η, θ and μ); and atypical PKCs (aPKC: ζ, λ and ι)[7,8]. cPKCs are calcium- and phospholipid-dependent, and are activated by DAG. nPKCs are also activated by DAG, but their activation does not require calcium. aPKCs are independent of calcium and DAG, and their activity is regulated by fatty acids[9]. cPKCs and nPKCs serve as major cellular receptors for phorbol esters, which are naturally occurring tumor promoters and have been shown to be specific activators of PKC (Ref. 10).

In addition to the different activation requirements and cofactor specificities, PKC isozymes are expressed differentially in cells and tissues[11,12]. It is believed that each member of the PKC family plays a unique role in physiological processes. Therefore, isozyme-specific PKC inhibitors should be of great value in investigating the distinct function of each isozyme.

The role of PKC in various pathological processes strongly indicates that selective PKC inhibitors may have a wide range of therapeutic applications in the control of PKC-mediated disorders. A number of studies have suggested that inappropriate activation of PKC is involved in diseases such as cancer, inflammation, viral infection, immune and CNS disorders, and cardiovascular malfunction[13]. The potential of PKC as a target for cancer treatment and the role of PKC in cancer biology and in multidrug resistance have been studied extensively[3,12,14–18]. Recently, PKC has also been found to be involved in vascular complications of diabetes[19] and in insulin resistance[20,21], suggesting the potential utilization of PKC inhibitors in the treatment of diabetes and its associated complications.

Over the past decade, intensive efforts have been made in developing potent and selective PKC inhibitors; this has led to the discovery of a number of chemical classes of PKC

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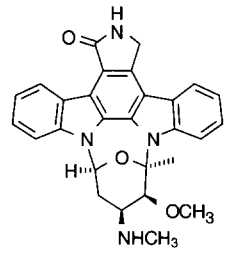

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Figure 1. *Chemical structure of staurosporine.*

inhibitors[22–27]. This review focuses on the recent development of selected groups of PKC inhibitors, with emphasis on their selectivity profiles.

Staurosporine-related inhibitors

Staurosporine (**1**, Figure 1), a microbial alkaloid isolated from *Streptomyces staurosporeus*, was identified as a potent PKC inhibitor in 1986 (Ref. 28). It remained the most potent lead compound until the recent discovery of (–)-balanol (see below). Staurosporine is not a selective kinase inhibitor because it also inhibits cAMP-dependent protein kinases (PKA), cGMP-dependent protein kinases (PKG) and tyrosine protein kinases at similar concentrations[29,30]. This has led to substantial efforts in search of staurosporine analogs with improved kinase selectivity. A number of staurosporine-related inhibitors with a high degree of selectivity for PKC compared with other closely related kinases have recently been identified[31–36]. Furthermore, some of these inhibitors have been demonstrated to have excellent selectivity for a specific PKC isozyme or a group of isozymes, and their therapeutic applications have been indicated in *in vitro* and *in vivo* studies[32–37].

Indolocarbazoles

A series of indolocarbazoles, in which the glycosidic moiety of staurosporine was omitted, has been developed as potent and selective PKC inhibitors by Gödecke Preclinical Research in Germany. Of these, compound Gö 6976 (**2**) has proved to have the highest specificity for PKC (Box 1)[31]. It inhibited PKC at 20 nM with at least 5000-fold selectivity for PKC compared with PKA. Inhibition of other kinases was three orders of magnitude less effective than inhibition of PKC. In addition to high specificity for PKC, Gö 6976 displayed excellent isozyme selectivity for Ca^{2+}-dependent PKC-α and -β_I (IC_{50} = 2–6 nM). It had no effect on the Ca^{2+}-independent δ, ε and ζ isozymes at concentrations up to 3 μM (Ref. 37). Unfortunately, low solubility and poor bioavailability of this compound make it an unlikely candidate for further development.

Gö 7612 (**3**) and Gö 7874 (**4**), two imide derivatives from the newer generation of indolocarbazoles, were found to be two- to fourfold more potent and tenfold more selective than staurosporine for inhibition of PKC with respect to other kinases (Box 1)[38]. The same study showed that this class of molecules, as exemplified by Gö 6976 and Gö 7612, inhibits PKC by interaction with the catalytic domain of PKC, in the same way as staurosporine.

Several indolocarbazoles (**5–7**, Figure 2) that have a three-atom sulfone-containing bridge over the two nitrogens of the indole rings have been discovered in the Schering-Plough research laboratories[39]. In studies using partially purified rat-brain PKC, these compounds exhibited a 50% inhibition

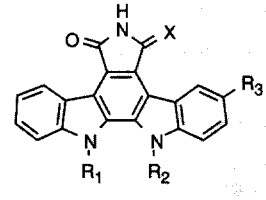
Box 1. Kinase inhibition by indolocarbazoles

Lactam series

X = H, H	**R_1**	**R_2**	**R_3**
Gö 6976 (**2**)	CH_3	$(CH_2)_2CN$	H

Imide series

X = O	**R_1**	**R_2**	**R_3**
Gö 7612 (**3**)	CH_3	$(CH_2)_2CN$	OCH_3
Gö 7874 (**4**)	CH_3	$CH_2CHOHCH_2NMe_2$	OCH_3



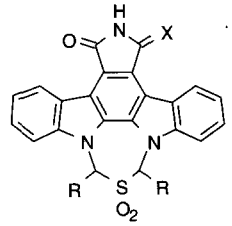
	IC_{50} (μM)				
	PKC[a]	**PKA[b]**	**PKG[c]**	**MLCK[d]**	**TSK[e]**
Gö 6976 (**2**)	0.020	>100	6.2	5.8	>10
Gö 7612 (**3**)	0.002	0.4	>3	0.2	>10
Gö 7874 (**4**)	0.004	0.5	4.8	0.12	NA
Staurosporine (**1**)	0.009	0.04	0.018	0.01	0.4

[a]Mixtures of isozymes α, β, γ
[b]cAMP-dependent protein kinase
[c]cGMP-dependent protein kinase
[d]Myosin light chain kinase
[e]Tyrosine-specific protein kinase from murine B cells; NA, not available

(IC_{50}) of PKC at 2, 5 and 2.4 nM, respectively. Staurosporine had an IC_{50} of 6 nM in the same assay.

Bisindolylmaleimides

It has been suggested that there is a cationic binding site in the PKC active site to which staurosporine binds via its amino-substituted glycosidic moiety. Using staurosporine as a guide, a number of bisindolylmaleimides bearing an amino alkyl group, such as GF 109203X (**8**, Box 2), have been generated. This class of molecules are believed to share a common mode of binding to PKC with staurosporine[4]. GF 109203X was discovered in 1991 as a potent PKC inhibitor with high selectivity for PKC over tyrosine kinases[40]. Recently, the extended studies on the selectivity for PKC and anti-inflammatory properties of GF 109203X (also known as Gö 6850) have been reported[32]. GF 109203X was found to have a higher specificity for PKC over a wide range of other kinases compared with staurosporine (Box 2). The efficacy of GF 109203X was observed in various experimental animal models of inflammation, including phorbol myristate acetate (PMA) and arachidonic acid induced edema in murine epidermis, and in a T-cell-dependent model of delayed-type hyper-sensitivity. Cellular studies demonstrated that GF 109203X effectively inhibited release of PGE_2 and superoxide from PMA-stimulated macrophages and neutrophils, respectively. These studies suggested a prominent role for PKC in the pathogenesis of acute inflammation, and demonstrated the therapeutic potential of selective PKC inhibitors for control of inflammation.

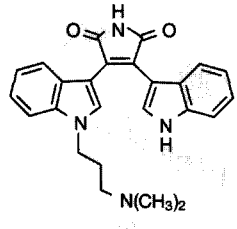


5 X = O, R = H
6 X = H_2, R = H
7 X = O, R = CH_3

Figure 2. *Chemical structures of three-atom bridged indolocarbazoles.*

Molecular modeling studies on GF 109203X and staurosporine suggested that the formation of a ring between the nitrogen and the vicinal C-2 of the indole ring would result in an ideal template for attaching cationic substituents, which should be able to access the putative amine binding site. Ro 32-0432 (**9**, Box 3) is an earlier successful example of this concept,

Box 2. Kinase inhibition by GF 109203X

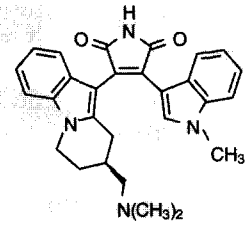


GF 109203X (Gö 6850, **8**)

Kinases	IC_{50} (μM)	
	GF 109203X (ratio)[a]	**Staurosporine (ratio)[a]**
PKC	0.032 (–)	0.009 (–)
PKA	33 (1030)	0.04 (4.4)
PKG	4.6 (144)	0.018 (2.0)
Myosin light chain kinase	0.6 (19)	0.01 (1.1)
Tyrosine kinase 1	94 (2937)	0.4 (44)
Tyrosine kinase 2	>100 (>3125)	>1 (>111)

[a]Relative to PKC

Box 3. Kinase inhibition by bisindolylmaleimides



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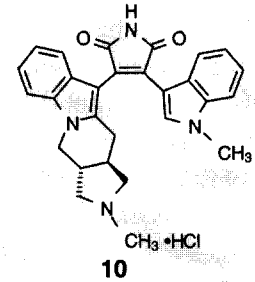
Ro 32-0432 (**9**) (single enantiomer, as drawn)



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11

Ro 32-0556 (**10**) (single enantiomer)
Ro 32-0557 (**11**) (single enantiomer, as drawn)

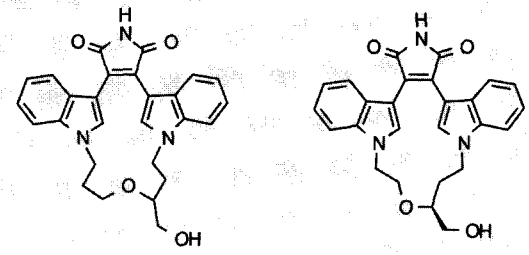
	IC_{50} (nM)		
	PKC[a]	**PKA**	**PhK[b]**
Ro 32-0432 (**9**)	17.2	22575	15625
Ro 32-0556 (**10**)	8	5500	3800
Ro 32-0557 (**11**)	5	2800	1688

[a]Rat brain protein kinase C
[b]Phosphorylase kinase

having high potency and selectivity in kinase assays as well as oral activity in *in vivo* studies[41]. It modulated T-cell function both *in vitro* and *in vivo*, providing evidence for the potential use of potent and selective PKC inhibitors as therapeutic immunomodulators[4].

The conformationally more restricted diastereomers Ro 32-0556 (**10**) and Ro 32-0557 (**11**) were recently shown to be more potent than **9**, while retaining 300- to 500-fold selectivity for PKC over PKA and phosphorylase kinase (Box 3). In addition, **11** exhibited greater selectivity for the Ca^{2+}-dependent PKC isoenzymes (α, β_I, β_{II}, γ) than the Ca^{2+}-independent ε isozyme. Despite the improved *in vitro* potency, **11** was not significantly more active than **9** in the developing adjuvant arthritis model[33].

Box 4. Kinase inhibition by bisindolylmaleimide macrocycles

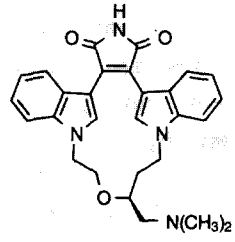


12 **13**

	PKC[a] (IC_{50}, μM)	PKC-β_{II}[b] (IC_{50}, μM)	PKC-α/PKC-β_{II}	PKA/PKC
LY 317644 (**12**)	6.40	0.27	25	>10,000
LY 326449 (**13**)	0.55	0.032	20	>10,000

[a]Rat brain protein kinase C
[b]Human PKC isozyme

Box 5. Kinase inhibition by LY 333531



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Kinases	IC_{50} (μM)	
	LY 333531 (14)	**Staurosporine**
PKC-α	0.36	0.0045
PKC-β_I	0.0047	0.023
PKC-β_{II}	0.0059	0.019
PKC-γ	0.3	0.11
PKC-δ	0.25	0.028
PKC-ε	0.6	0.018
PKC-ζ	>100	>1.5
PKC-η	0.052	0.005
PKA	>100	0.1
Ca^{2+}-calmodulin kinase	8	0.004
Casein kinase	>100	14
Src tyrosine kinase	>100	0.001

Bisindolylmaleimide macrocycles

N,N'-Bridged bisindolylmaleimide macrocycles incorporating a 14- or 15-membered ring were developed recently in the Lilly Research Laboratories as a new structural type of PKC inhibitor in the staurosporine family. Compounds LY 317644 (**12**) and LY 326449 (**13**) were shown to have a >10,000-fold selectivity for PKC over PKA (Box 4)[34]. Further SAR studies led to identification of LY 333531 (**14**) as a specific PKC-β isozyme inhibitor (Box 5)[35]. It inhibited PKC-β_I/β_{II} with an IC_{50} of 5 nM, which is significantly more effective than the inhibition of other PKC isozymes (50-fold) and non-PKC kinases (1,000-fold). When administered orally, **14** normalized the glomerular filtration rate, albumin excretion rate and retinal circulation in diabetic rats in a dose-responsive manner in parallel with its PKC inhibitory activity. It has been reported that PKC-β_{II} is preferentially activated in retina, heart and aorta in diabetic rats[42] and that the activation of PKC-β_{II} may cause some of the abnormalities observed in vascular complications of diabetes such as hemodynamic

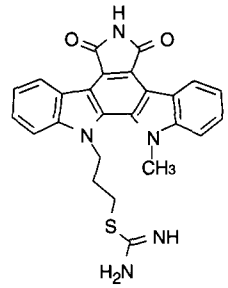
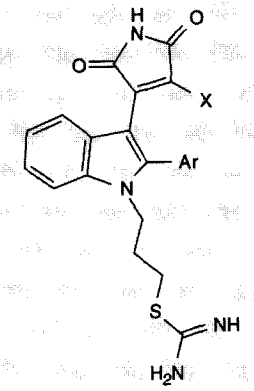
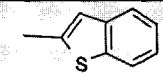
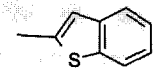
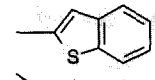
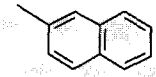


Figure 3. Chemical structure of Ro 31-8220.

Box 6. Protein kinase C inhibition by 2-arylindolylmaleimides



	Ar	X	PKC-β (IC_{50}, nM)
16		H	3
17		CN	2
18		NMe_2	20
19		H	10

changes[19]. Studies with compound **14** provided strong evidence that activation of PKC, especially the β isozyme, is involved in some of the vascular complications of diabetes, and suggested that selective inhibition of PKC-β may be potentially useful for preventing such complications. Kinetic analysis showed that LY 333531 is a competitive inhibitor of ATP, with a K_i of 2 nM for PKC-β_1 (Ref. 35).

Monoindolylmaleimides

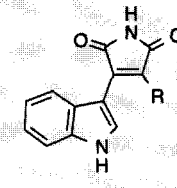
A novel series of 2-arylindolylmaleimides (**16–19**, Box 6), derived from the bisindolylmaleimide Ro 31-8220 (**15**, Figure 3) by modifying and transferring one indole group from the maleimide moiety to the C-2 position of the other indole ring, was found to be among the most potent inhibitors of PKC (Ref. 36). Compound **15**, although an equally potent and better kinase-selective inhibitor than staurosporine, is not as promising as other Roche compounds, such as **9**, due to a lack of oral activity[43]. Monoindolylmaleimides **16–19** were shown to have excellent PKC-β potency (Box 6). Moreover, compound **16** demonstrated selectivity for PKC-β (IC_{50} = 3 nM) over PKA (IC_{50} = 500 nM) and tyrosine kinase $p60^{src}$ (inactive). It inhibited PKC-ε with an IC_{50} of 10 nM. Significantly, compound **16** showed anticancer activity in several *in vitro* and *in vivo* models in which PKC-β was overexpressed. It is hoped that compounds such as **16** will prove useful in the management of carcinogenesis.

A bisubstrate approach, which allows for the design of PKC inhibitors that interact simultaneously with the ATP and the protein substrate-binding domains of PKC, has resulted in a series of monoindolylmaleimide derivatives with an amine side-chain on the maleimide ring[44]. The most active compounds (**20** and **21**, Box 7) inhibited PKC with an IC_{50} of 8 μM, and showed a >26-fold selectivity for PKC over PKA (Ref. 45). This study further indicated that compound **20** inhibits PKC by interaction at the ATP binding site. The arginine in compound **21** is involved in the recognition of protein substrates by PKC.

Balanoids

(–)-Balanol (**22**, Box 8), a fungal metabolite of *Verticillium balanoides*, was first isolated and identified in our laboratories as one of the most potent naturally occurring PKC inhibitors[46]. Later, it was also found to be a constituent of *Fusarium merismoides* by Japanese scientists[47]. Because of its potency against PKC, its structural novelty and uniqueness, and its low availability from natural sources, balanol has attracted considerable attention both as a synthetic target and as a new lead compound for the development of PKC inhibitors with therapeutic applications. The total

Box 7. Protein kinase C inhibition by monoindolylmaleimides

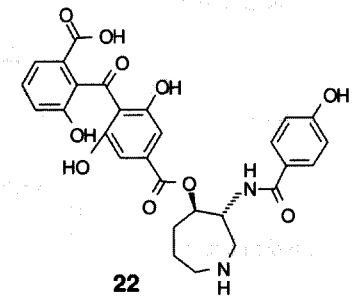


R	IC_{50} (μM) PKC	PKA
20 $NH-(CH_2)_5-NH_2$	8	300
21 $NH-(CH_2)_5-NH-(CH_2)_2-NH-Arg$	8	210

synthesis of (–)-balanol was completed shortly after its isolation and structure elucidation in our laboratories[48–52], and this was closely followed by other successful attempts[53–56]. The accomplishment of the total synthesis of balanol has allowed ready access to its synthetic analogs and thus an understanding of thestructure–activity relationships of this class of molecule.

(–)-Balanol has shown nanomolar inhibition for most of the PKC isozymes, but it lacks isozyme/kinase selectivity (Box 8) and sufficient cellular activity (Tables 1 and 2). Systematic structural modifications, including change of substitution pattern on the benzophenone and the benzamido moieties, replacement of the central perhydroazepine ring, and substitution of the amide bond, have been pursued in the search for analogs with improved properties. Selected balanol analogs are shown in Figure 4, and their PKC inhibition profiles are described in Table 1. A tetra-*ortho*-substitution pattern and the presence of an acidic functionality are critical features of the benzophenone moiety. Any deviations, such as removing or replacing the carboxyl group, or re-locating the hydroxyl or carboxyl group on the benzophenone ring, resulted in dramatic decreases in activity[57]. Replacement of the azepine ring with a smaller ring or a bicyclic nucleus, on the other hand, has provided a number of promising analogs (compounds **23–27**). For example, the pyrrolidine analog (**23**) was found to be more

Box 8. Kinase inhibition by balanol



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Kinases	IC_{50} (μM)		
	(–)-Balanol (22)	**(+)-Balanol**	**(±)-Balanol**
PKC-α	0.03	3.0	0.067
PKC-β_I	0.01	0.5	0.03
PKC-β_{II}	0.01	0.88	0.03
PKC-γ	0.01	0.40	0.03
PKC-δ	0.004	0.42	0.23
PKC-ε	0.01	<1.0	0.38
PKC-η	0.003	0.26	0.02
PKC-ζ	5.9	>150	3.5
PKA	0.08	5.2	NA[a]
Ca^{2+}-calmodulin kinase	0.03	NA	NA
Casein kinase	0.015	NA	NA
Src tyrosine kinase	>10	NA	>100

[a]NA, not available

Table 1. Protein kinase C inhibition by balanol analogs IC_{50}, μm

	PKC[a]								PKA
	α	β_I	β_{II}	γ	δ	ε	η	ζ	
(–)-Balanol	0.03	0.01	0.01	0.01	0.004	0.01	0.003	5.9	0.08
(±)-Balanol	0.067	0.03	0.03	0.03	0.23	0.38	0.02	3.5	NA[b]
23	0.022	0.01	0.033	0.012	0.005	0.01	0.004	>0.15	0.07
24	0.04	0.04	0.05	0.01	0.0009	0.05	0.0006	22	0.03
25	0.02	0.004	0.002	0.005	0.005	0.01	0.002	1.2	0.05
26	0.05	0.045	0.03	0.035	0.02	0.03	0.02	37	1.9
(–)-**27**	0.24	0.06	0.05	0.05	0.05	1.90	<0.05	NA	50.0
(+)-**28**	0.21	0.03	0.007	0.48	0.003	0.46	0.003	>50	>50
29	0.06	0.004	0.003	0.04	0.005	0.15	0.003	>50	>50

[a]Human protein kinase C (PKC) isozymes; IC_{50} values were calculated from four point curves of tenfold dilutions
[b]NA, not available

Table 2. Cellular activities of balanol analogs[a]

	IC_{50} (μM)			
	Balanol	**30**	**31**	**32**
Neutrophil[b]	>10	0.15[c]	0.09	0.26

[a]All racemic

[b]Measure of phorbol myristate acetate induced superoxide release in human neutrophils

[c]30 min preincubation; others with 1 h preincubation

potent than balanol. The cyclopentane analog (**24**) proved to be as potent as balanol against most PKC isozymes, and more potent than balanol against PKC-δ and -η (Ref. 58). Compound **25**, a modification of **24**, displayed increased inhibition of PKC-β and -γ, but was otherwise as potent as balanol[59]. These ring analogs, like balanol, were equally active against PKA, while some of the bicyclic analogs, such as norbornane (**26**)[60] and indane (–)-(**27**)[61], not only retained a level of activity comparable to that of balanol against most PKC isozymes, but also displayed a 35–1,000-fold selectivity for most PKC isozymes relative to PKA. Potent activity and increased selectivity were also demonstrated by balanol analogs **28** and **29**, in which the carboxamide functionality is replaced (J.M. Defauw *et al.*, unpublished; see also Ref. 62).

Although required for potency in the enzyme assays, the high density of polar groups on the benzophenone seemed to prevent balanol analogs from displaying cellular activities. In an attempt to eliminate this problem, a number of compounds were prepared as prodrugs. The most encouraging examples are the pivaloyloxymethyltetrazole derivative (**30**)[63], the phosphonatobenzyl ester (**31**) and the methyl ester (**32**)[64] (Figure 4, Table 2). These compounds were shown to inhibit PMA induced superoxide release in human neutrophils at submicromolar concentrations whereas their parent compounds were inactive.

Our kinetic studies[65] suggested a competitive inhibition of PKC by balanol analogs with respect to ATP, which is consistent with studies performed by others[47,54].

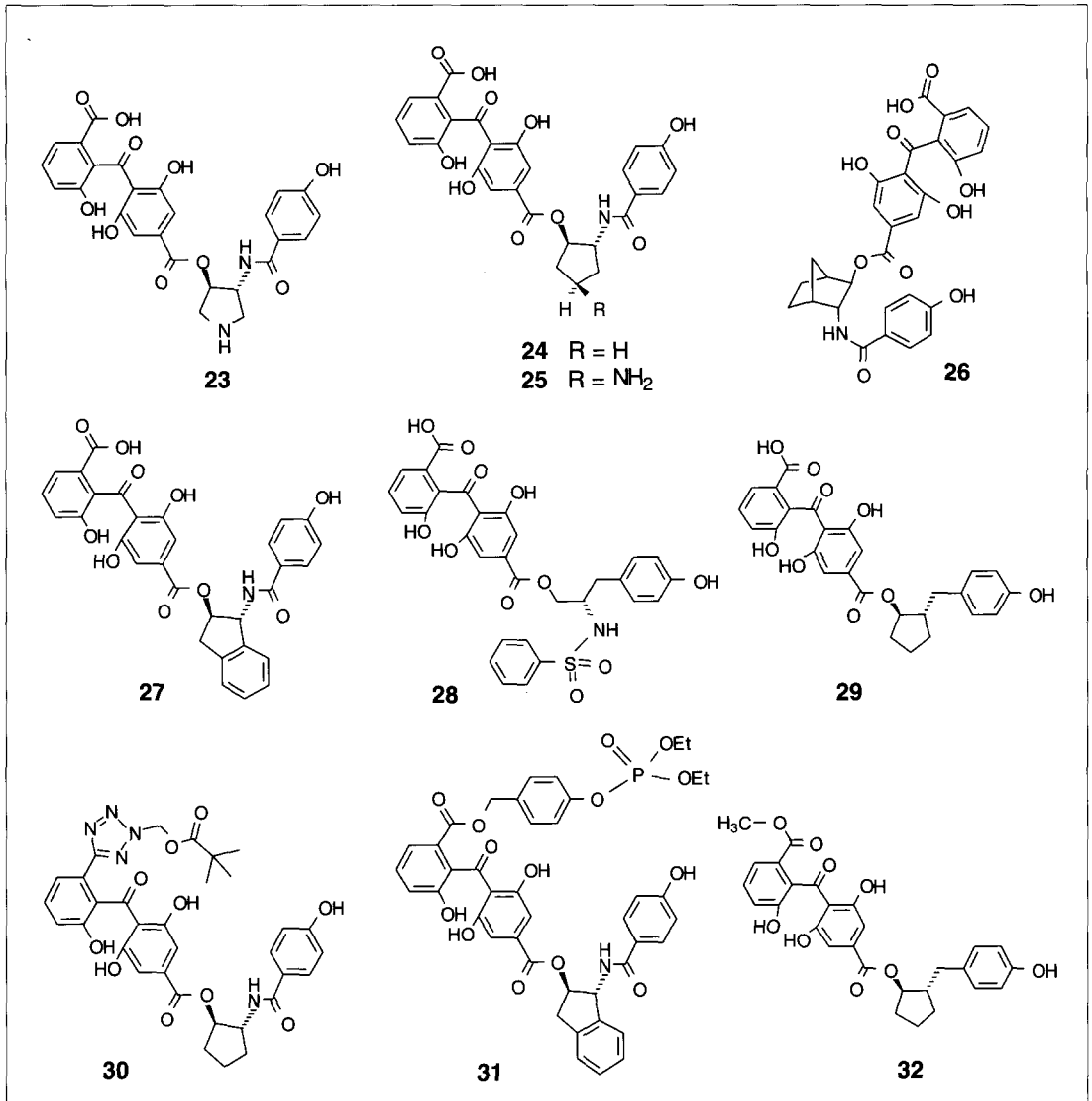

Figure 4. *Chemical structures of balanol analogs.*

Sphingolipids

The natural product sphingosine (**33**, Figure 5), a long-chain lipid base containing an aminodiol functionality, and other related sphingolipids are

known to inhibit PKC (Ref. 66) and to display anti-inflammatory activity in human neutrophils[67]. Sphingosine also reduced phorbol-ester-induced inflammation and epidermal hyperplasia *in vivo*[68]. A series of aryl-fused sphingolipids such as **35–37** (Figure 5), in which aryl/heteroaryl moieties are incorporated into the allylic 4,5,6-position of sphingosine, was reported to possess moderate *in vitro* PKC inhibitory activity and *in vivo* topical anti-inflammatory activity comparable to sphingosine[69]. As seen with sphingosine enantiomers, the individual enantiomers of **35**, **36** and **37** were shown not to be more potent than their respective racemates[70]. Thus there appears to be no stereochemical specificity for these analogs in terms of interaction with PKC.

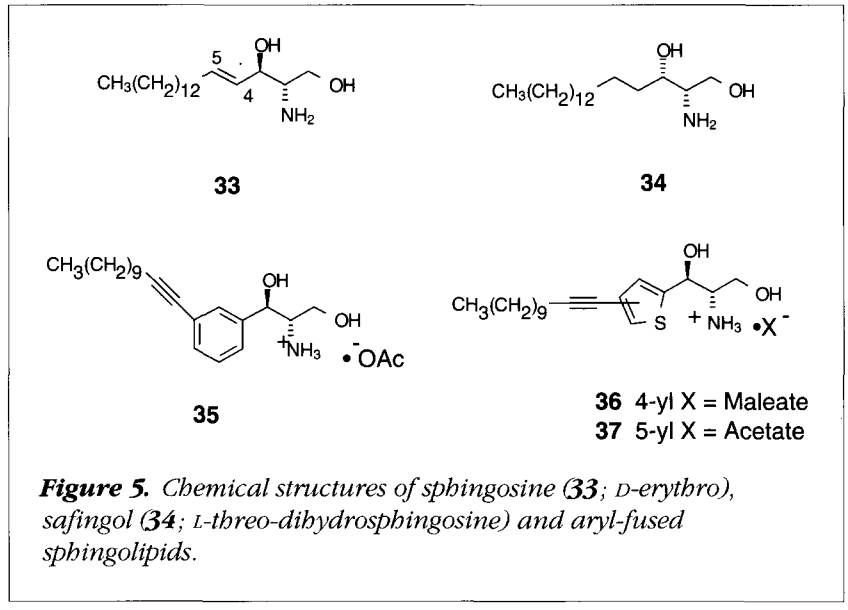

Figure 5. *Chemical structures of sphingosine (**33**; D-erythro), safingol (**34**; L-threo-dihydrosphingosine) and aryl-fused sphingolipids.*

Safingol (**34**, Figure 5), a dihydrosphingosine analog, is a specific inhibitor of PKC with IC_{50} values in the micromolar range. It possesses minimal or no activity against other serine/threonine kinases, such as PKA, casein kinase II and calcium/calmodulin kinase [Lawrence M. Ballas (Sphinx Pharmaceuticals), pers. commun.]. Inhibition of PKC by safingol is believed to be competitive with DAG, calcium and phosphatidylserine at the regulatory domain of PKC (Ref. 66). Safingol was shown to enhance cytotoxic drug accumulation in tumor cell lines, to attenuate resistance in multidrug-resistant cells, and to substantially potentiate the effects of doxorubicin and cisplatin on tumor growth delay in *in vivo* models[71,72]. It has become the first PKC-specific inhibitor to enter into clinical trials in combination with chemotherapeutic agents[73]. Toxicological studies in rats and dogs showed that, when administered intravenously, safingol is well tolerated and may represent a safe adjunct therapy in combination with chemotherapeutic agents for cancer treatment[74]. The mechanism by which safingol potentiates the activity of cancer chemotherapeutic agents is unclear, although inhibition of P-glycoprotein phosphorylation and consequent reversal of the multidrug-resistant phenotype have been suggested[72,75]. The potentiation of chemotherapy-induced apoptosis by safingol in combination with mitomycin C in *in vitro* studies with gastric cancer cells has been described recently as an alternative mechanism[76].

Isoxazolones

3-Aryl-4-arylidene-5-isoxazolones (**38** and **39**, Figure 6), a known class of compounds in the literature, have been recognized recently as submicromolar inhibitors of PKC with moderate selectivity for PKC over other protein kinases[77]. These compounds are also active at micromolar concentrations in *in vitro* and *in vivo* models of graft-versus-host disease, which is thought to be a PKC-linked immunodisorder. The fact that structures **38** and **39** incorporate electrophilic functionality suggests inhibition of PKC via covalent modification of the enzyme, but no evidence for this has yet been provided.

Natural products

Two novel spirosesquiterpene aldehydes, corallidictyals A and B (**40** and **41**, Figure 6), were isolated as a mixture from the marine sponge *Aka* (= *Siphonodictyon*) *coralliphagum*[78]. In an assay with four purified recombinant human PKC isozymes (α, ε, η and ζ), corallidictyals were shown to be selective for inhibition of the α isozyme (IC_{50} = 30 μM) over ε, η and ζ (IC_{50}: 89, >300 and >300 μM, respectively). In addition, corallidictyals appeared to have a >10-fold selectivity for the inhibition of PKC relative to PKA (IC_{50} >300 μM). Their corresponding methyl ethers were inactive against PKC.

The sphingolipid-like molecule penazetidine A (**42**, Figure 6), a constituent of the Indo-Pacific marine sponge *Penares sollasi*, is one of the most potent PKC inhibitors isolated to date in the series. It exhibited 50% inhibition of PKC at 1 μM and showed no inhibitory activity at the same concentration against tyrosine kinase, which suggests a binding to the regulatory site of PKC (Ref. 79).

Rottlerin (**43**, Figure 6), a natural product from *Mallotus philippinensis*, was found to be a more potent inhibitor

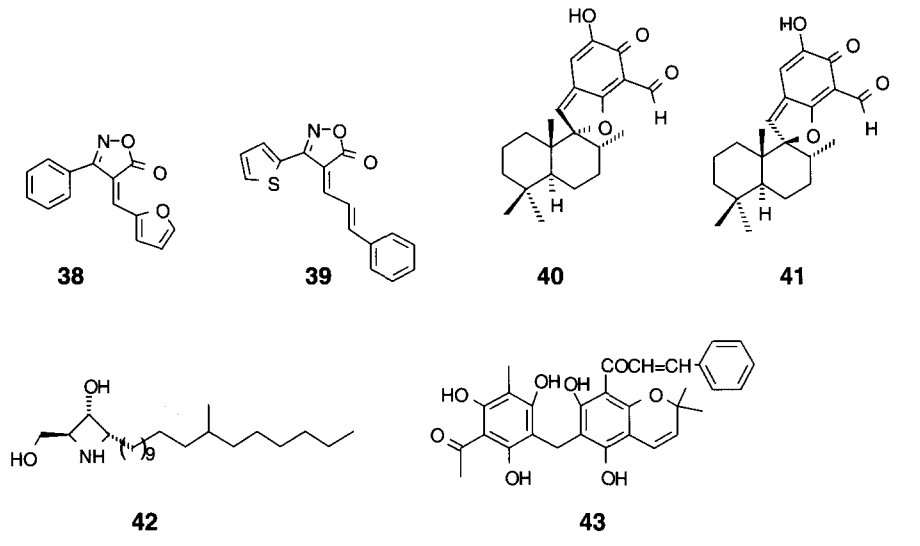

Figure 6. *Chemical structures of miscellaneous PKC inhibitors.*

of PKC-δ (IC_{50} = 3-6 μM) than of other isozymes (IC_{50} = 30–42 μM for PKC-α, -β, -γ; IC_{50} = 80–100 μM for PKC-ε, -η, -ζ)[80]. Except for inhibition of calcium calmodulin kinase III (Ref. 81), in which it was as effective as against PKC-δ, rottlerin inhibited PKA and Src kinase at at least one order of magnitude higher concentrations. Inhibition of PKC appeared to be competitive with ATP in these cases.

Summary

As increasing numbers of potent and kinase/isozyme selective PKC inhibitors become available, further elucidation of the role of specific isozymes in various physiological and pathophysiological processes may be forthcoming.

Development of the PKC-β isozyme-specific inhibitor LY 333531 (**14**) has presented a promising future for PKC inhibitors to be employed in the management of PKC-mediated human diseases. Balanol represents a novel and unique chemical class among the known PKC inhibitors. Identification of potent and kinase-selective balanol analogs has provided new opportunities for developing novel therapeutic agents and for probing PKC functions. These new entries in the list of potent and selective PKC inhibitors will also allow re-evaluation of the biology of PKC and the involvement of PKC in pathological processes, since some of the earlier studies were conducted with potentially misleading non-kinase-selective PKC inhibitors such as staurosporine.

Staurosporine congeners and balanol analogs are ATP-competitive inhibitors. It remains desirable to develop potent and non-ATP-competitive inhibitors of PKC. Inhibitors of this type will be useful in understanding PKC-mediated cellular events, because inhibition of PKC by ATP-competitive PKC inhibitors is sometimes suppressed by high concentrations of cellular ATP.

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