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Synthesis of anilino-monoindolylmaleimides as potent and selective PKCβ inhibitors

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Abstract—We report herein synthesis of PKCβ-selective inhibitors possessing the novel pharmacophore of anilino-monoindolylmaleimide. Several compounds of this series exhibited IC_{50}'s as low as 50 nM against human PKCβ2. One of the most potent compounds, **6l**, inhibited PKCβ1 and PKCβ2 with IC_{50} of 21 and 5 nM, respectively, and exhibited selectivity of more than 60-fold in favor of PKCβ2 relative to other PKC isozymes (PKCα, PKCγ, and PKCε).

There have been reported 12 isozymes in a protein kinase C (PKC) family, and they play indispensable roles in organisms regulating cellular signal transduction pathways although specific physiological roles of each isozyme remain unclear.[1] Since the discovery of staurosporine, a nonselective PKC inhibitor produced by *Streptomyces* sp., a number of its analogues possessing various inhibitory activity have been synthesized.[2] Among these analogues, ruboxistaurin (PKCβ1; IC_{50} of 4.7 nM, PKCβ2; IC_{50} of 5.9 nM), a macrocyclic bisindolylmaleimide, is widely known because of its marked PKCβ selectivity.[3] More importantly, it was reported that this compound showed ameliorative effects on diabetic vascular dysfunction including retinopathy in the clinical studies through inhibition of activated PKCβ2 in diabetic hyperglycemic conditions.[4] More recently enzastaurin, another bisindolylmaleimide, was reported as a PKCβ-selective inhibitor, which is now evaluated for the treatment of cancer.[5] Thus, PKCβ has been now proven to be a novel target for the treatment of diabetic complications and cancer,[6] but no highly potent PKCβ-selective inhibitors have heretofore been reported other than ones with the Ro 31-6233 (**1**)[7] pharmacophore (Fig. 1). In this paper, we report a new class of staurosporine-related PKCβ-selective inhibitors, anilino-monoindolylmaleimides represented by the structure of **4** and **6**.[8] One of our most potent compounds, **6l**, showed an IC_{50} of 21 and 5 nM for human PKCβ1 and PKCβ2, respectively, with selectivity for PKCβ2 of more than 60-fold compared to the α and ε isozymes.

Anilino-monoindolylmaleimides **4** were prepared via two steps as shown in Scheme 1. 3-Hydroxymaleimide **3**, which was obtained by treatment of 3-indolylacetamide **2** with $(CO_2Me)_2$ in the presence of *t*-BuOK,[9] was converted into **4** by heating with an excess amount of amine (R^1R^2NH) in AcOH.[10] The yield of representative compound **4c** (R^1 = Ph, R^2 = H) was 37% from **2**.[11] One of the most potent compounds, **6l**, was prepared as shown in Scheme 2. Alkylation of **2** with $Br(CH_2)_3OTBDMS$ gave **5**, which was then converted into **6e** via the anilino-maleimide formation step with the similar conditions used in the preparation of **4** and deprotection step. The terminal hydroxyl group was replaced with imidazole via bromination step to afford compound **6l**.[12] Compounds **6a**–**d**,**f**–**k** were similarly obtained in 10–38% from **2**.[10]

Although no crystal structure of the catalytic domain of PKC is available to date, the crystal structure of PKA bound to staurosporine[13] allowed us to speculate how staurosporine inhibits PKC since PKA and PKC are both inhibited by staurosporine in an ATP-competitive manner and the ATP binding catalytic domains of these kinases are well conserved. This crystal structure suggests that the minimal structural elements required for

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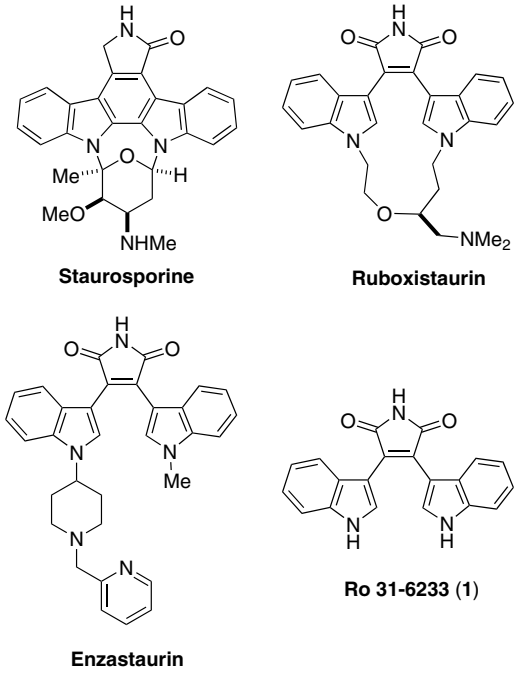
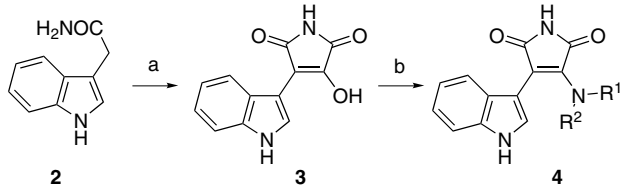
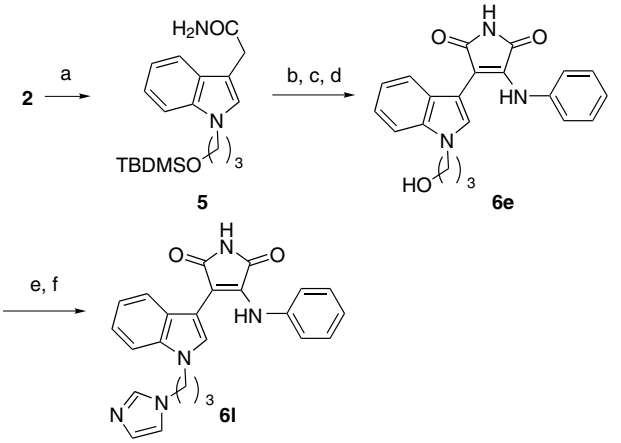


Figure 1. Structure of staurosporine, ruboxistaurin, enzastaurin, and Ro 31-6233.



Scheme 1. Reagents and conditions: (a) $(CO_2Me)_2$, *t*-BuOK, DMF, 0 °C to rt; (b) R^1R^2NH, AcOH, Δ.



Scheme 2. Reagents and conditions: (a) $Br(CH_2)_3OTBDMS$, NaH, DMF, 0 °C to rt, 92%; (b) $(CO_2Me)_2$, *t*-BuOK, THF, 0 °C to rt; (c) $PhNH_2$, AcOH, Δ; (d) TBAF, THF; (e) NBS, PPh_3, THF; (f) imidazole, NaH, DMF, 31% overall from **5**.

PKC inhibition are (i) maleimide or five-membered lactam part, which forms rigid hydrogen bonds to the peptide backbone of the enzyme (Glu121 and Val123 in PKA numbering), and (ii) widely spread two aromatic rings bound to the 3- and 4-positions of the maleimide (or the five-membered lactam) filling hydrophobic regions of the enzyme. Based on these requirements, we initially investigated the inhibitory activity of bisindolylmaleimide **1** against PKC β1, β2, α, and ε because **1** was the smallest inhibitor that meets the pharmacophoric requirements, and is a substructure of staurosporine-related PKC inhibitors including ruboxistaurin and enzastaurin.[7] In our assay conditions, **1** exhibited moderate PKCβ inhibitory activity (PKCβ1; IC_{50} of 304 nM, PKCβ2; IC_{50} of 212 nM). Taking this activity as a criterion, one of the indoles of **1** was replaced with aliphatic amines or aniline in order to find novel pharmacophores with the guide of PKCβ2 inhibitory activity as shown in Scheme 1 and Table 1.[14]

Compound **4a**, obtained using benzylamine, did not inhibit the enzyme up to a concentration of 10 μM. The inhibitory activity of cyclohexyl analogue **4b** was also 10-fold less potent than **1**.[15] In marked contrast to these aliphatic amines, anilino-monoindolylmaleimide **4c** showed an IC_{50} of 223 nM and this was found to be as potent as **1**. More importantly, **4c** was significantly more selective than **1** over PKC α and ε (Table 2).[16] It is speculated that the inhibitory activity and selectivity of **4c** are imparted by the sp^2-like character of the nitrogen-linker, which allows the plane of the aromatic system to take an appropriate angle, moderately deviated from the plane of the maleimide. The cocrystal structure of PKA-staurosporine suggests that the two indoles in staurosporine, which are strictly placed on the same plane of the five-membered lactam, are important to exert its PKA inhibitory activity. As for PKCβ inhibition, however, this planarity requirement is not likely to be very strict since nonplanar ruboxistaurin and enzastaurin also show good affinity to the enzyme. The nonplanarity should be rather speculated to be preferable from the point of view of PKCβ selectivity. Thus, a nonplanar conformation of **4c**, in which the phenyl ring adopts

Table 1. PKCβ2 inhibitory activity of compounds **4**

Compds	R^1	R^2	PKCβ2 IC_{50} (nM)
4a	$-CH_2Ph$	H	>10,000
4b	Cyclohexyl	H	2448
4c	Phenyl	H	223
4d	Phenyl	$-CH_3$	939
4e	2-Methylphenyl	H	2146
4f	3-Methylphenyl	H	151
4g	4-Methylphenyl	H	480
4h	3-Fluorophenyl	H	249
4i	3-Chlorophenyl	H	198
4j	3-Methoxyphenyl	H	747
4k	3-Trifluoromethylphenyl	H	1450
4l	3-(*i*-Propyl)phenyl	H	2863

Table 2. Comparison of PKCβ selectivity of **1** and **4c**

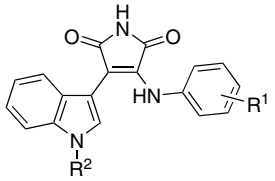
Compds	PKC IC_{50} (nM)			
	β1	β2	α	ε
1	304	212	1599	7516
4c	395	223	7233	>10,000

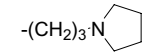
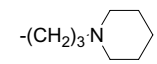
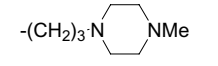
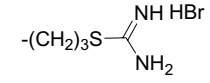
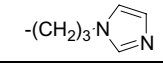
an appropriately deviation from the plane of the maleimide, is considered to be crucial not only for the inhibitory activity but also for the selectivity. Methylation of the nitrogen atom of **4c** might oust the phenyl group too far from the maleimide plane resulting in lower inhibitory activity (see compound **4d**, Table 1).

Next, the effect of the substituents on the benzene ring was investigated (Table 1). Although no significant increase in potency was found in this approach, compounds possessing a small substituent at 3-position (**4f**, **4h**, **4i**) were as potent as nonsubstituted **4c**. The larger substituents at this position, however, were not well tolerated (**4j**, **4k**, **4l**). Introduction of a methyl group at the 2-position (**4e**) also resulted in loss of activity by 10-fold reflecting the importance of the torsional angle of the phenyl plane relative to the maleimide plane as seen in **4d**.

In order to increase the activity of **4c**, hydrophilic groups were attached to the molecule using the indole NH as a handhold (Scheme 2 and Table 3). It could be predicted from earlier SAR studies on staurosporine-related compounds[3b,17] that attaching hydrophilic substituent(s) on the appropriate position of the modest PKCβ2 inhibitor **4c** would generate much more potent inhibitors. Initially dimethylamine was introduced with linkers of varying lengths to afford **6a**–**d**. As expected, the potency was increased dramatically by this approach. Compounds **6b** and **6c** showed IC_{50} values of 12 and 15 nM, respectively, indicating linkers of C3 and C4 length were appropriate for PKCβ2 inhibition.

Table 3. Enzyme inhibitory activity of compounds **6**



Compds	R^1	R^2	PKC IC_{50} (nM)		
			β2	α	ε
6a	H	$-(CH_2)_2NMe_2$	28	2157	>10,000
6b	H	$-(CH_2)_3NMe_2$	12	460	3456
6c	H	$-(CH_2)_4NMe_2$	15	730	5866
6d	H	$-(CH_2)_5NMe_2$	41	2232	3905
6e	H	$-(CH_2)_3OH$	30	1620	6322
6f	3-Me	$-(CH_2)_3NMe_2$	15	727	3455
6g	3-Cl	$-(CH_2)_3NMe_2$	10	558	2605
6h	H		18	662	>10,000
6i	H		17	726	3425
6j	H		23	968	>10,000
6k	H		2	27	58
6l	H		5	331	2807

More importantly the selectivity in favor of PKCβ2 versus PKC α and ε was as high as that of **4c**. Introduction of a hydroxyl group was also effective in raising the potency, although **6e** has half the potency of **6b**. Modification of 3-position of the aniline part of **6b** was well tolerated, and **6f** and **6g** were as potent as **6b**. These results were consistent with the SAR found in **4**. Replacement of the dimethylamine part of **6b** with cyclic amines was also conducted. However, **6h**, **6i**, and **6j** had IC_{50} around 20 nM, which have half the potency of **6b**. Aiming at finding more potent compounds, the isothiourea salt **6k** was prepared. This functional group had been used as a hydrophilic part of bisindolylmaleimide type PKC inhibitors by the Roche group and reported to be effective in increasing the PKC inhibitory activity remarkably.[17] As anticipated, **6k** showed IC_{50} of 2 nM for PKCβ2, but the selectivity over PKC α and ε was significantly decreased. The most potent compound with remarkable isozyme selectivity was the imidazole derivative **6l**, which showed IC_{50} of 5 nM for PKCβ2 (21 nM for PKCβ1) and selectivity over PKC α and ε was 66-fold and 560-fold, respectively. IC_{50}'s of **6b** and **6l** against PKCγ, which is one of the conventional PKC isozymes, were found to be greater than 1 μM.

In conclusion, a new class of anilino-monoindolylmaleimides was synthesized and evaluated for their PKCβ2 inhibitory activity and selectivity over other isozymes. Representative compounds **6b** and **6l** exhibited IC_{50} of 12 and 5 nM for PKCβ2, respectively, with remarkable selectivity over the other calcium-dependent isozymes (PKCα and PKCγ) and the calcium-independent isozyme (PKCε). The pharmacokinetic studies of these compounds remain to be conducted for further evaluation of their ameliorative effects in diabetic animals.

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11. 3-Anilino-4-(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (**4c**): ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.64–6.95 (6H, m), 7.22 (2H, d, J = 8.1 Hz), 7.32 (2H, d, J = 8.0 Hz), 9.04 (1H, s), 10.60 (1H, s), 11.16 (1H, br s). Anal. Calcd for $C_{18}H_{13}N_3O_2$: C, 71.28; H, 4.32; N, 13.85. Found: C, 71.10; H, 4.70; N, 13.86.
12. 3-[1-(3-Imidazol-1-ylpropyl)-1*H*-indol-3-yl]-4-anilino-1*H*-pyrrole-2,5-dione (**6l**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (2H, quintet, J = 4.0 Hz), 3.77 (2H, t, J = 4.0 Hz), 3.97 (2H, t, J = 4.0 Hz), 6.71–6.80 (4H, m), 6.85–7.03 (4H, m), 7.13–7.18 (4H, m), 7.42 (1H, s), 7.54–7.61 (2H, m). Anal. Calcd for $C_{24}H_{21}N_5O_2$: C, 70.06; H, 5.14; N, 17.02. Found: C, 69.89; H, 5.38; N, 17.07.
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