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Acyclic *N*-(azacycloalkyl)bisindolylmaleimides: Isozyme Selective Inhibitors of PKCβ

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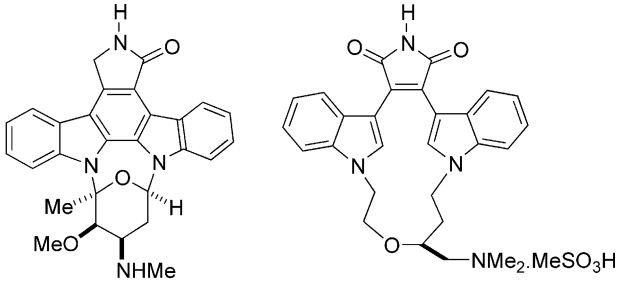
Abstract—The synthesis and structure–activity relationship (SAR) trends of a new class of *N*-(azacycloalkyl)bisindolylmaleimides **1**, acyclic derivatives of staurosporine, is described. The representative compound for this series (**1e**) exhibits an IC_{50} of 40–50 nM against the human $PKC\beta_1$ and $PKC\beta_2$ isozymes and selectively inhibits the PKCβ isozymes in comparison to other PKC isozymes (α, γ, δ, ε, λ, and η). The series is also kinase selective for PKC in comparison to other ATP-dependent kinases. A comparison of the PKC isozyme and kinase activity of the series is made to the kinase inhibitor staurosporine.

Protein kinase C (PKC), a family of serine/threonine specific kinases,[1] composed of at least 12 isozymes, are involved in signal transduction pathways that govern a wide range of physiological processes including differentiation, proliferation, gene expression, brain function, membrane transport and the organization of cytoskeletal and extracellular matrix proteins which regulate vascular function.[2–4] Therapeutically, antagonists that possess kinase selectivity for PKC in addition to PKC isoenzyme selectivity, are potentially useful pharmacological agents for treatment of a variety of diseases including diabetes and cancer.[5,6]

The natural product staurosporine, although a potent inhibitor of PKC, has limited selectivity in vitro for both ATP-dependent kinases and individual PKC isozymes (Fig. 1).[7] Recently, our group identified a novel class of macrocyclic bisindolylmaleimides, represented by ruboxistaurin mesylate (LY333531), that were competitive reversible inhibitors of PKC β_1 and β_2.[3,8–10] In addition, ruboxistaurin was found to be several orders of magnitude more selective for inhibition of PKCβ relative to the other kinases, and is currently being evaluated in the clinic for treatment of diabetic complications.

In our continued evaluation of the biological activity of compounds in this area, we identified a series of acyclic (*N*-azacycloalkyl)bisindolylmaleimides **1a–k**, that are



Staurosporine **Ruboxistaurin Mesylate**

Figure 1. Chemical structures for (+)-staurosporine and ruboxistaurin mesylate.

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also more selective kinase inhibitors than staurosporine and have selectivity for PKCβ comparable to ruboxistaurin. This manuscript will describe the results of this SAR that has resulted in identification of **1e** as a selective inhibitor of PKCβ. This compound is currently being evaluated in the clinic as an antiangiogenic agent for the treatment of cancer, either as stand-alone therapy or in combination with traditional oncolytic therapies.

Acyclic (*N*-azacycloalkyl) bisindolylmaleimides **1b–k** were prepared in 6–35% yield (unoptimized) by a two-step sequence involving (i) conversion of the *N*-(azacycloalkyl)indole **2b–k** into the corresponding glyoxyl chloride **3b–k**; (ii) reaction of **3b–k** with the appropriately substituted indolyl-3-acetimidate **4b–k** in the presence of a suitable base to generate an intermediate hydroxypyrroline that upon dehydration with *p*-TsOH or TFAA/pyridine afforded **1b–k** (Scheme 1).[11,12] The unsubstituted piperidine derivative **1a**, was prepared from **1g** by removal of the Boc-protecting group under acidic conditions.[13] An improved procedure for the synthesis of these compounds has been developed and will be reported in the future.[14]

In analogy to our work on the macrocyclic series, a panel of eight cloned human PKC isozymes (α, β_1, β_2, γ, δ, ε, ζ, and η) were employed to optimize this series of PKCβ selective antagonists.[8] The panel is representative of the calcium dependent PKC isoforms (α, β and γ), the calcium independent isoforms (δ, ε and η) and the atypical isoforms (ζ). Using assay conditions that simulate the endogenous activation of PKC with DAG and phosphatidylserine, compounds **1a–k** were found to be PKCβ selective in comparison to most of the other isozymes in the panel. Acyclic bisindolylmalimides have previously been reported to be competitive inhibitors of ATP-binding, presumably by interacting at the ATP-binding site.[15] An additional criterion for selectivity is thus the preferential inhibition of the PKCβ isozymes versus other ATP-dependent kinases. An ATP dependent kinase panel consisting of four kinases (PKA, calcium calmodulin, casein kinase and src-tryosine kinase) was used in tandem with the PKC isozyme panel to demonstrate the kinase selectivity of the acyclic (*N*-azacycloalkyl)bisindolylmaleimides **1a–k**.

Staurosporine is a potent, non selective PKC inhibitor (Table 1). However, as reported previously,[8] ruboxistaurin shows remarkable selectivity for the β isozymes, especially over the other calcium dependent isozymes (PKCα and PKCγ), which have a high degree of shared homology in the ATP binding region.

The acyclic (*N*-azacycloalkyl)bisindolylmaleimides **1a–k** produced selective inhibition of PKCβ when assayed in the isozyme panel, but were about 10-fold less active than ruboxistaurin for the PKCβ isozymes. These compounds showed good PKCβ selectivity with regard to PKCβ inhibition and PKC isozyme selectivity. The SAR comparing the 5-membered pyrrolidine derivative **1d** with the 6-membered piperidine **1c**, showed that comparable results were obtained for PKCβ inhibition and PKC selectivity, thus the SAR was completed with the achiral piperidine derivatives. The SAR around the R1 groups attached to the piperidine ring shows that the larger groups (benzyl **1c**, pyridyl **1e** and **1h**, Boc **1g**) are well tolerated compared to the smaller groups (H **1a**, Me **1b** and **1i**) and these large substituents do not change the PKCβ activity but decrease PKCα and PKCγ inhibition. The benzyl group also decreases activity across the panel of calcium independent PKCs (δ, ε and η) (e.g., **1c**).

A large increase in kinase selectivity is observed in going from staurosporine to the (*N*-azacycloalkyl)bisindolylmaleimides **1a–k**. Larger R1 groups (**1c**, **1e**, **1g**, and **1h**) increase the selectivity versus pK_A and src-tryosine kinase, while the smaller R1 groups (**1a**, **1b**, and **1f**) showed decreased or similar selectivity for these isozymes. In terms of kinase selectivity we were most con-

Table 1. PKC isozyme IC_{50} values[a]

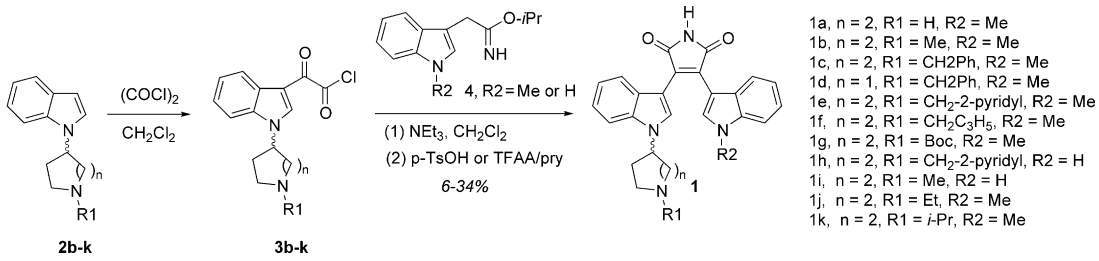
Compd	PKC isozyme IC_{50} (μM)							
	α	βI	βII	γ	δ	ε	ζ	η
1a	0.3	0.03	0.03	2	0.4	1	4	0.2
1b	0.4	0.02	0.01	0.5	0.4	0.4	4	0.05
1c	6	0.05	0.03	7	6	4	>100	0.5
1d	3.8	0.022	0.024	3.5	4.2	9.4	>100	0.042
1e	0.8	0.03	0.03	2	1	0.3	8	0.4
1f	0.3	0.03	0.01	0.4	0.4	0.9	9	0.05
1g	6	0.2	0.08	5	7	6	>100	8
1i	0.2	0.02	0.01	0.5	0.4	0.05	5	0.04
1j	0.4	0.02	0.005	0.3	0.3	0.4	7	0.04
1k	0.34	0.019	0.026	1.3	0.97	0.39	9.3	0.13
Staurosporine	0.045	0.023	0.019	0.11	0.028	0.018	>1.5	0.005
Ruboxistaurin	0.36	0.0047	0.0059	0.3	0.25	0.6	>100	0.052

[a]Measurements are the average of at least three independent determinations from eight-point titration curves. The typical standard deviation was 30% of the IC_{50} value. Cloned human isozymes were used in the assay that were activated by DAG using phosphatidylserine vesicles incubated with [^{32}P]ATP and histone or myelin basic protein as a substrate.

Table 2. ATP dependent kinase IC_{50} values and IC_{50} values for inhibition of rat brain PKC[a]

Compd	Kinase IC_{50} (μM)				
	pK_A[b]	Ca calmod[c]	Casein K[d]	src-Tk[e]	RB-PKC[f]
1a	1	2	>100	nt	0.4
1b	7	2	>100	>100	0.2
1c	>100	>40	>40	>100	4
1d	>100	59	>100	nt	2.8
1e	>100	10	>100	>100	0.7
1f	>100	25	>100	68	0.3
1i	4	3	91	83	0.2
1j	8	7	>100	11	0.2
1k	>100	7.5	>100	nt	0.25
Staurosporine	0.10	0.004	14	0.001	0.19
Ruboxistaurin	>100	6.2	>100	>100	0.32

[a]The values are the average of at least three independent determinations from eight-point titration curves.
[b]Bovine heart cAMP dependent protein kinase catalytic subunit kinase assay.
[c]Purified mammalian brain calcium calmodulin dependent protein kinase assay.
[d]Purified rat brain casein protein kinase II assay.
[e]src protein tryrosine kinase assay.
[f]Purified rat brain protein kinase C assay.



Scheme 1. Method employed for the synthesis of acyclic (*N*-azacycloalkyl)bisindolylmaleimide.

cerned with the inhibition of calcium calmodulin because of the possibility of toxicological problems and wanted to advance a compound that was at least as selective for calcium calmodulin as ruboxistaurin.

The second criterion employed in evaluating our compounds was rat brain PKC inhibition. Although we were most interested in inhibition of the human isozymes, a compound that approximated the activity of ruboxistaurin versus rat brain PKC would help us to decide which compound to carry forward.[16] Comparison of rat brain PKC inhibition data and kinase selectivity data (Table 2), indicated that only compound **1e** produced the required kinase selectivity, PKC selectivity, and rat brain PKC inhibition and was selected as the clinical candidate.

In conclusion, a new series of acyclic (*N*-azacycloalkyl) bisindolylmaleimides **1a**–**k** have been synthesized. The piperidinyl derivative **1e** exhibits nanomolar activity against PKCβ and at least >26-fold selectivity for PKCβ versus PKCα. Kinase selectivity is also maintained in this series and **1e** is >300-fold more selective for PKCβ relative to calcium calmodulin and >3000-fold more selective for src-tyrosine kinase. Therefore, **1e** was identified as a clinical candidate and is currently under evaluation in the clinic for the treatment of cancer.

Acknowledgements

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12. Preparation of **1b**–**k**: These compounds were prepared by a slight modification of the process described in ref 11. To an ice-cooled solution of the indole derivative **2b**,**c**,**d**,**e**,**f**,**i**, **j** and **k** (2.5 mmol) in 6 mL of absolute Et_2O under argon was added oxalyl chloride (1.1 equiv) dropwise. Stirring was continued for 30 min at 0 °C. The crystalline precipitate of the corresponding indol-3-yl glyoxyl chloride thus obtained was filtered off under an argon atmosphere with exclusion of moisture and rinsed with a small amount of Et_2O. The precipitate then was redissolved or suspended in 15 mL of dry DCM and 2.5 mmol (1.0 equiv) of the imidate ester hydrochloride **4** added. The mixture was cooled to 0 °C and a solution of 12.6 mmol (5 equiv) of Et_3N in 4 mL of DCM added under stirring, maintaining the temperature at 0 °C. The mixture was warmed to rt and stirring was continued for 4 h. Excess of TsOH monohydrate (7 equiv) was added under cooling (slightly exothermic) and the reaction stirred for 3.5 h. The organic solution was washed successively with saturated aqueous Na_2CO_3, brine and water and dried over Na_2SO_4. After evaporation the residue was further purified via column chromatography or by recrystallization from dioxane, ethyl acetate or Et_2O.
13. Preparation of **1a**: Boc-protecting group was cleaved as follows: 0.13 mmol of the maleimide was added to a mixture of 0.5 mL of TFA and 0.05 mL ethane thiol and stirred for 7 min while monitored by TLC. A saturated aqueous solution of Na_2CO_3 was added carefully, followed by DCM. The precipitate obtained was filtered, thoroughly rinsed with water and dried.
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16. PKCs are ubiquitously expressed, although the levels of individual isoaymes vary considerable among different cells and tissues. PKCα is found in most cells and tissues. PKCβ is mainly expressed in immune function cells as well as some epithelial cells.