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Synthesis and PKCθ inhibitory activity of a series of 5-vinyl phenyl sulfonamide-3-pyridinecarbonitriles

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ABSTRACT

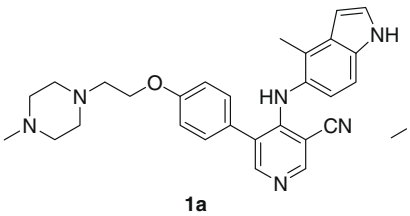
A series of 5-vinyl phenyl sulfonamide-3-pyridinecarbonitriles were prepared and evaluated as PKCθ inhibitors. Optimization resulted in the identification of compound **15** with an IC_{50} value 0.44 nM for the inhibition of PKCθ with 150-fold selectivity over PKCδ.

The protein kinase C (PKC) family of serine–threonine kinases share sequence homology but vary in their activation requirements, expression and regulation.[1] Conventional PKCs (α, β, and γ) require calcium and diacylglycerol as secondary messengers, the novel PKCs (δ, ε, η, and θ) do not require calcium, and the atypical isoforms (ζ and λ) require neither calcium nor diacylglycerol.[2] PKCθ was first characterized in 1993 and is predominantly expressed in T cells.[3] Studies using PKCθ knock-out (KO) mice have established the role of this kinase in diseases such as multiple sclerosis, arthritis, asthma, inflammatory bowel disease and organ transplantation.[4] PKCθ displays particularly high homology to PKCδ. PKCδ deficiency in mice led to a hyperproliferation of B cells and overproduction of inflammatory cytokines.[5] Therefore we used PKCδ as the primary counter assay in our inflammation project.

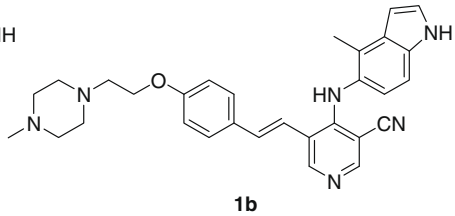
Several chemical series have been shown to be ATP-competitive inhibitors of PKCθ including 2,4-diamino pyrimidines,[6] thieno[2,3-*b*]pyridinecarbonitriles[7–9] and 3-pyridinecarbonitriles.[10–15] We earlier reported that the 5-phenyl-3-pyridinecarbonitrile **1a** had an IC_{50} value of 7.4 nM for the inhibition of PKCθ.[12]

Insertion of a vinyl group at C-5 to provide **1b** increased the PKCθ inhibitory activity (IC_{50} = 3.6 nM) and also increased the selectivity for PKCθ over PKCδ from sevenfold to 11-fold (Table 1).[15] This increased potency and selectivity of **1b** compared to **1a** prompted a study of replacing the ethoxy tether between the phenyl ring and the amine with additional groups. This Letter focuses on sulfonamide replacements for the ethoxy moiety that would have the potential of forming multiple hydrogen bonding interactions.[16]

The desired sulfonamides were prepared by Heck coupling of the corresponding bromobenzenesulfonamides with the 5-vinyl-3-pyridinecarbonitrile **2a**[15] (Scheme 1). The bromobenzenesulfonamides were prepared from the corresponding sulfonyl chlorides and amines. The *p*-*N*-Methylpiperazinesulfonamide analog **3** inhibited PKCθ with an IC_{50} of 23 nM with 6.1-fold selectivity over PKCδ Table 1. The pyrrolidine and piperidine analogs **4** and **5** had



1a



1b

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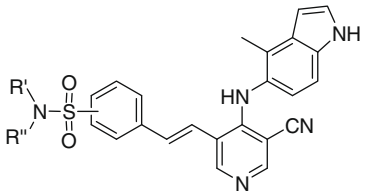
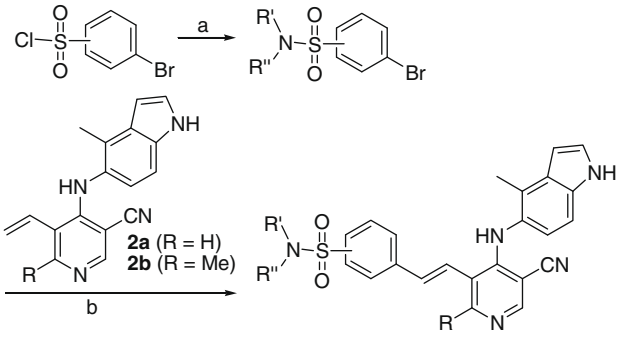
Table 1
PKCθ and PKCδ inhibitory activity of 5-vinyl phenyl sulfonamide-3-pyridinecarbonitriles



Compd	NR′R″	PKCθ IC_{50}[a], nM	PKCδ IC_{50}[a], nM	δ/θ
1a		7.4	51	6.9
1b		3.6	41	11
3	*p*-*N*-Methylpiperazine	23	140	6.1
4	*p*-Pyrrolidine	6.0	450	75
5	*p*-Piperidine	12	20,000	1700
6	*p*-NH_2	5.4	114	21
7	*p*-NMe_2	11	1,300	120
8	*p*-NEt_2	8.7	3,300	380
9	*p*-N(*n*-Pr)$_2$	350	51,000	150
10	*p*-N(*iso*-Pr)$_2$	410	9,400	23
11	*p*-NH(*sec*-Bu)	121	NT[b]	—
12	*m*-Pyrrolidine	6.0	120	20
13	*m*-Piperidine	3.0	390	130
14	*m*-*N*-Methylpiperazine	0.49	18	37

Assay protocols are described in Ref. 10.
[a] Values are the mean of at least two determinations.
[b] NT = not tested.



Scheme 1. Reagents and conditions: (a) R′R″NH, CH_2Cl_2, 23 °C (b) P(*o*-tol)$_3$, Pd(OAc)$_2$, NEt_3, DMF, 100 °C.

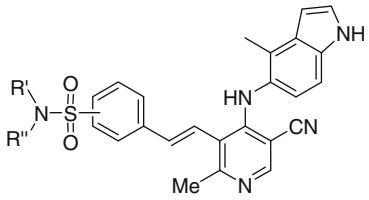
improved PKCθ activity and selectivity over PKCδ, with **5** exhibiting an exceptional 1700-fold selectivity. In the case of acyclic sulfonamides (compounds **6–11**), the activity fell off sharply with the increasing size of the amine. The best profile was seen with the diethylamino analog **8** which had an IC_{50} value of 8.7 nM for the inhibition of PKCθ with 380-fold selectivity over PKCδ. It was notable that the acidic unsubstituted sulfonamide **6** showed similar potency to **3** which contains a basic amine as polar group.

When the substitution pattern was changed from *para* to *meta* with cyclic amines, a general improvement in PKCθ activity resulted with a concomitant increase in PKCδ activity. The *m*-*N*-methylpiperazine analog, **14**, was particularly active with an IC_{50} value of 0.49 nM against PKCθ and 37-fold selectivity over PKCδ.

Compounds in Table 1 showed half lives of less than 10 min in rat liver microsome stability assays. Metabolism studies of **14** identified a major site of oxidation to be the 6-position of the 3-pyridinecarbonitrile core.

In order to block the site of metabolism, a 6-methyl moiety was introduced by employing compound **2b**[18] (R = Me) in the Heck coupling with the bromobenzenesulfonamides. The SAR of the 6-methyl analogs (Table 2) generally corresponded to that of the unsubstituted derivatives. In all cases, except **20**, the selectivity of PKCθ over PKCδ was greatly enhanced. Compound **15** inhibited

Table 2
PKCθ and PKCδ inhibitory activity of 5-vinyl phenyl sulfonamide-6-methyl-3-pyridinecarbonitriles



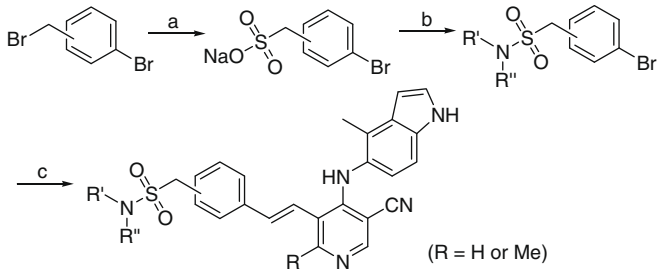
Compd	NR′R″	PKCθ IC_{50}[a], nM	PKCδ IC_{50}[a], nM	δ/θ
15	*m*-*N*-Methylpiperazine	0.44	65	150
16	*m*-Pyrrolidine	2.4	850	350
17	*m*-Piperidine	4.8	2,000	420
18	*p*-*N*-Methylpiperazine	9.3	4,400	470
19	*p*-Pyrrolidine	21	7,100	340
20	*p*-Piperidine	68	21,000	310

[a] Values are the mean of at least two determinations.

PKCθ with an IC_{50} value of 0.44 nM with 150-fold selectivity over PKCδ. Unfortunately, the microsomal half lives were not improved. Compound **15** showed half lives of less than 30 min in mouse, rat, and human microsomal stability assays.

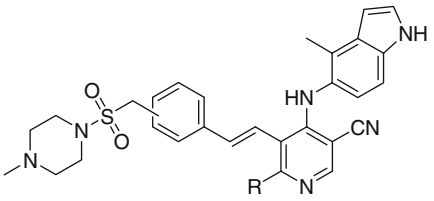
Another variation on the existing template was carried out by inserting a methylene unit between the sulfonamide and aryl moieties. Bromobenzyl bromides were refluxed with sodium sulfite and a catalytic amount of *N,N,N,N*-tetrabutylammonium iodide. Phosphorus pentachloride in phosphorus oxychloride was used to form the sulfonyl chlorides which were converted to the corresponding sulfonamides.[17] The sulfonamides were coupled with **2a** and **2b** via a Heck reaction (see Scheme 2).

The effect of inserting a methylene between the phenyl and 4-methylpiperaziny-1-ylsulfonyl groups is shown in Table 3. In general, the activity against PKCθ and the selectivity against PKCδ



Scheme 2. Reagents and conditions: (a) Na_2SO_3, (*n*-Bu)$_4$NI, water, reflux (b) PCl_5, $POCl_3$, 120 °C; R′R″NH, CH_2Cl_2 (c) **2a** or **2b**, P(*o*-tol)$_3$, Pd(OAc)$_2$, NEt_3, DMF, 100 °C.

Table 3
PKCθ and PKCδ inhibitory activity of 5-vinyl benzyl sulfonamide-3-pyridinecarbonitriles



Compd	R	*m*/*p*-	PKCθ IC_{50}[a], nM	PKCδ IC_{50}[a], nM	δ/θ
21	H	*p*	7.9	320	41
22	H	*m*	6.2	94	15
23	Me	*p*	4.3	110	26
24	Me	*m*	4.4	97	22

[a] Values are the mean of at least two determinations.

Table 4
Kinase selectivity of **14** and **15**

Kinase	IC_{50}[a] (nM) of **14**	IC_{50}[a] (nM) of **15**
PKCε	1.6	36
PKCη	100	490
PKCβ	41,000	>50,000
PKCζ	>100,000	>100,000
PKA	130	1300
HCK	550	>50,000
LYN A	290	>50,000
SRC	220	>50,000
FYN	140	>50,000
GCK	1,300	>50,000
VEGFR2	2,400	>50,000
CDK1/cyclinB	>50,000	>50,000
CDK2/cyclinA	44,000	>50,000
Aurora B	>50,000	>50,000
ROCK1	>50,000	>50,000
CK1γ1	>50,000	>50,000
MK2	>50,000	>50,000
P38α	>50,000	>50,000
ERK2	>50,000	>50,000
CHK1	>50,000	>50,000
RSK1	>50,000	>50,000
PDGFRα	>50,000	>50,000

[a] Values are the mean of at least two determinations.

were both diminished. Methylene insertion did not change the pattern of low microsomal stability.

Table 4 illustrates the kinase selectivity profile for the two most active compounds **14** and **15**. While compound **14** was found to exhibit a high affinity for PKCε, another novel PKC, both were very selective (more than 100-fold) over PKCη a novel PKC, PKCβ a conventional PKC and PKCζ an atypical PKC. Enhanced selectivity across the board was exhibited for the C-6 methyl analog **15**.

In summary, a series of 3-pyridinecarbonitriles containing a C-5 vinyl phenyl sulfonamide group were found to be potent and selective inhibitors of PKCθ. Small changes in either the amino group, the position of the sulfonamide on the phenyl ring or the presence of a methyl group at C-6 affected activity and selectivity. The source of the selectivity observed with these analogs is unclear given our current structural biology knowledge. There is very high identity in the ATP binding sites of PKCθ and PKCδ. The amino acid residues relatively close to the binding cleft that vary include the Tyr460 of PKCθ that corresponds to a Phe in PKCδ and the Ile510 of PKCθ that corresponds to a Val in PKCδ, neither of which makes an apparent direct contact with the inhibitors.

Acknowledgments

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