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Synthesis and PKCθ inhibitory activity of a series of 4-(indol-5-ylamino)thieno[2,3-*b*]pyridine-5-carbonitriles

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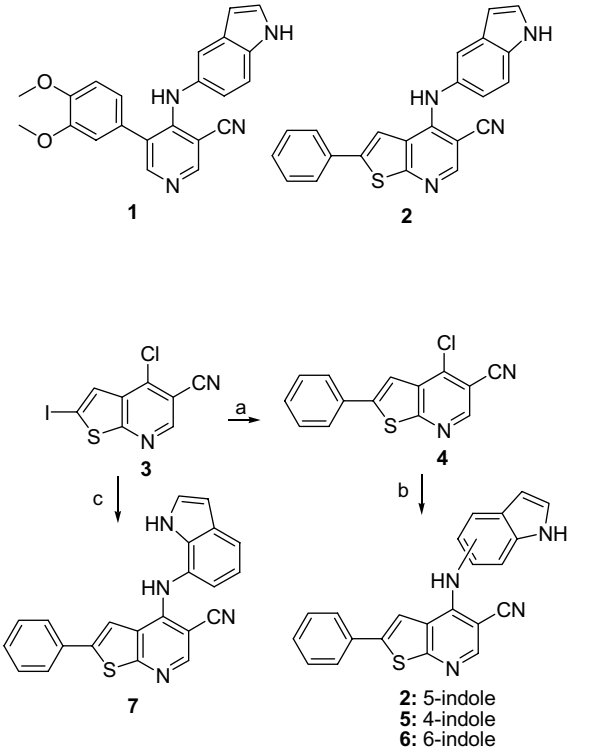
Abstract—The thieno[2,3-*b*]pyridine-5-carbonitrile with a 5-indolylamine at C-4 and a phenyl group at C-2 had a moderate activity against PKCθ. Optimization of the groups at C-4 and C-2 led to analog **29**, which has an IC_{50} value of 7.5 nM for the inhibition of PKCθ.

The protein kinase Cs (PKCs) are a family of serine threonine kinases that share sequence and structural homology and vary in their activation requirements and tissue expression.[1] Biochemical regulation of the classical PKC isoforms, α, β, and γ, requires the second messengers, calcium and diacylglycerol. The novel isoforms, δ, ε, η, and θ, do not require calcium and the atypical isoforms, ζ and λ, do not require either calcium or diacylglycerol. The three PKC inhibitors currently in late stage clinical trials, midostaurin,[2] enzastaurin,[3] and ruboxistaurin,[4] all target the classical PKCs.

PKCθ, a novel isoform, was first characterized in 1993 and plays a key role in the activation and survival of T-cells.[5,6] Studies with mice that have the PKCθ gene deleted or knocked out (KO) showed these animals to be resistant to the development of several T-cell mediated diseases including multiple sclerosis,[7,8] arthritis,[9] and asthma.[10,11] Therefore, the inhibition of PKCθ could be of therapeutic benefit in a variety of disease states. Interestingly, studies with PKCδ KO mice revealed that the inhibition of this kinase results in the increased proliferation of B-cells making these animals susceptible to autoimmune disease.[12,13]



Scheme 1. Reagents: (a) phenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$; (b) for **2**: 5-aminoindole, $EtOCH_2CH_2OH$; for **5**: 4-aminoindole, $Pd_2(dba)_3$, K_3PO_4, DME; for **6**: 6-aminoindole, EtOH; (c) (1) 7-aminoindole, EtOH (2) phenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$.

Keywords: PKCθ; Thieno[2,3-*b*]pyridine-5-carbonitrile.
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2,4-Diaminopyrimidines[14,15] and pyridine-3-carbonitriles[16] have been reported to be templates for PKCθ inhibitors. The pyridine-3-carbonitrile **1** shares common structural features with the thieno[2,3-*b*]pyridine-5-carbonitriles, compounds previously studied as Src kinase inhibitors.[17] To ascertain if this bicyclic core could also provide inhibitors of PKCθ, compound **2** was prepared as shown in Scheme 1. The reaction of **3**[17] with phenylboronic acid under Suzuki conditions provided **4**. The subsequent treatment of **4** with 5-aminoindole gave **2**, which had an IC_{50} value of 460 nM for the inhibition of PKCθ activity. Since this series originated from a Src kinase program, **2** was tested for activity against Lyn, a member of the Src family of kinases. Activity against Lyn is undesirable due to the finding that Lyn KO mice develop autoimmune disease as a result of a hyperresponsive B-cell phenotype.[18,19] Fortunately, **2** had weak activity against Lyn (IC_{50} = 33 μM). However, as shown in Table 1, **2** inhibited PKCδ with an IC_{50} value of 2.0 μM. Due to the less than fivefold selectivity for PKCθ over PKCδ and the potential deleterious effect of inhibiting this isoform, all compounds with IC_{50} values of less than 500 nM for PKCθ were assayed for PKCδ activity.

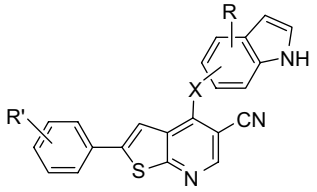
This route used to prepare **2** was used for the preparation of **5** and **6**, the 4 and 6-indolyl isomers of **2** (Scheme 1). An alternate route was used to prepare **7**, the 7-indolyl isomer, in that 7-aminoindole was added to **3**, followed by coupling with phenylboronic acid. Of these, **5**, the 4-indolyl isomer, had the best activity against PKCθ; however, it also had decreased selectivity against PKCδ. Of the four indolyl isomers, **2** had the best combination of potency and selectivity, and was therefore chosen for further SAR study.

To investigate the effect of variation of the linker between the 5-indoyl headpiece and the thieno[2,3-*b*]pyridine-5-carbonitrile core, key intermediate **4** was treated with 5-methylaminoindole,[20] 5-hydroxyindole, indole-5-methanamine, and indole-5-carboxamide, to provide **8**–**11**. As shown in Table 1, all these compounds had reduced PKCθ inhibitory activity compared to **2**.

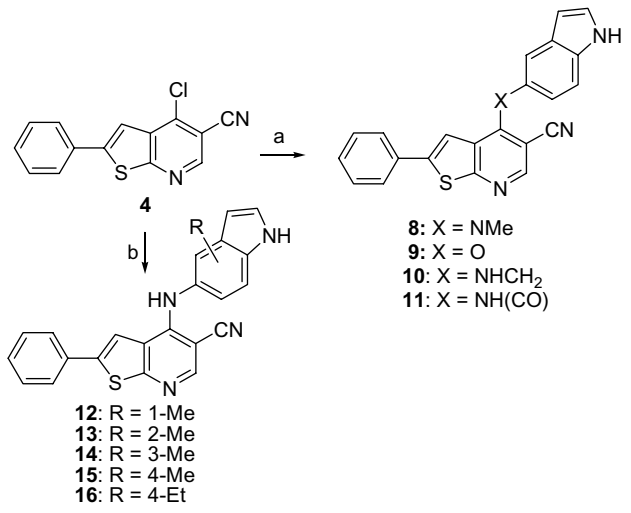
To determine if the PKCθ inhibitory activity of **2** could be increased by the presence of a substituent on the indole ring, **4** was reacted with various 5-aminoindoles containing a methyl group at C-1, 2, 3, or 4 to provide **12**–**15** (Scheme 2). 5-Amino-4-methylindole was prepared by the previously reported route.[21] 5-Amino-3-methylindole was obtained by the hydrogenation of 3-methyl-5-nitroindole.[22] As shown in Table 1, the 4-methyl isomer **15** had increased PKCθ inhibitory activity compared to **2**, but also had a corresponding increase in PKCδ activity. Lyn inhibition also increased, with **15** having an IC_{50} value of 3.4 μM against this kinase. Interestingly, the 4-ethyl analog **16** was much less active than **15** against both PKC isoforms.[23]

Concurrent with the optimization of the headpiece, studies were underway to optimize the tailpiece at C-2. In order to facilitate the preparation of these analogs, **3** was converted to the 2-iodo derivative **17** as shown in Scheme 3. The reaction of **17** with 4-formylphenylbo-

Table 1. PKCθ and PKCδ inhibitory activity[25]

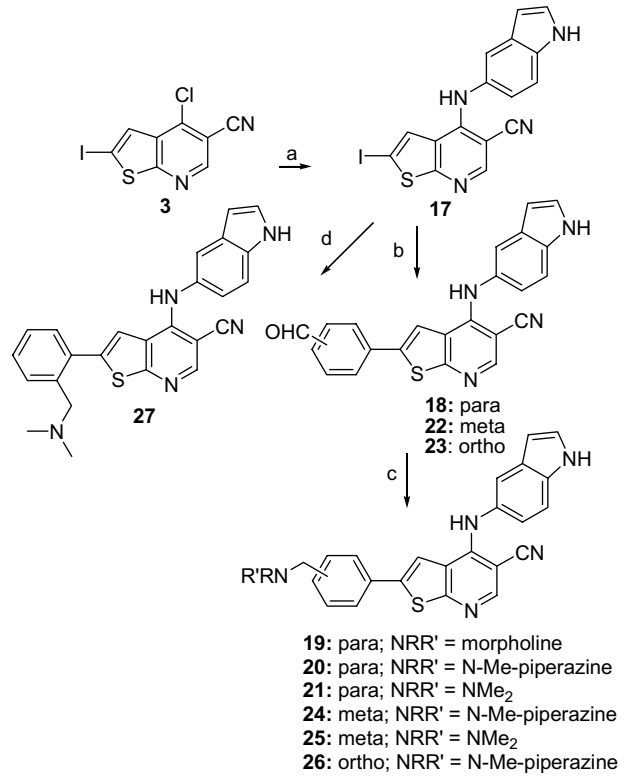


Ex	Indole isomer	X	R	R′	PKCθ IC_{50} (nM)	PKCδ IC_{50} (nM)
2	5	NH	H	H	460	2000
5	4	NH	H	H	230	310
6	6	NH	H	H	>5000	
7	7	NH	H	H	>5000	
8	5	NMe	H	H	>5000	
9	5	O	H	H	>5000	
10	5	$NHCH_2$	H	H	3700	
11	5	NH(CO)	H	H	>5000	
12	5	NH	1-Me	H	>5000	
13	5	NH	2-Me	H	1800	
14	5	NH	3-Me	H	1300	
15	5	NH	4-Me	H	52	370
16	5	NH	4-Et	H	830	3300
19	5	NH	H	4-CH_2-morpholine	450	1100
20	5	NH	H	4-CH_2-N-Me-piperazine	200	730
21	5	NH	H	4-CH_2-NMe_2	130	200
24	5	NH	H	3-CH_2-N-Me-piperazine	100	93
25	5	NH	H	3-CH_2-NMe_2	75	85
26	5	NH	H	2-CH_2-N-Me-piperazine	730	
27	5	NH	H	2-CH_2-NMe_2	570	
29	5	NH	4-Me	3-CH_2-NMe_2	7.5	26



Scheme 2. Reagents: (a) for **8**: 5-methylaminoindole, EtOH, for **9**: 5-hydroxyindole, K_2CO_3, acetonitrile, for **10**: indole-5-methanamine, Hunig's base, 2-ethoxyethanol, for **11**: indole-5-carboxamide, NaH, DMF; (b) substituted 5-aminoindole, EtOH.

ronic acid provided **18**, with subsequent reductive amination with morpholine, *N*-methylpiperazine and dimethylamine resulting in analogs **19**–**21**, respectively. As shown in Table 1, the *N*-methylpiperazine and dimethylamine derivatives **20** and **21** had increased PKCθ inhibitory activity compared to **2**. The *meta* and *ortho* isomers of **20** and **21** were prepared as shown in Scheme 3. While the *meta* isomers **24** and **25** had improved inhibitory activity against PKCθ, the *ortho* isomers **26** and **27** had reduced activity.



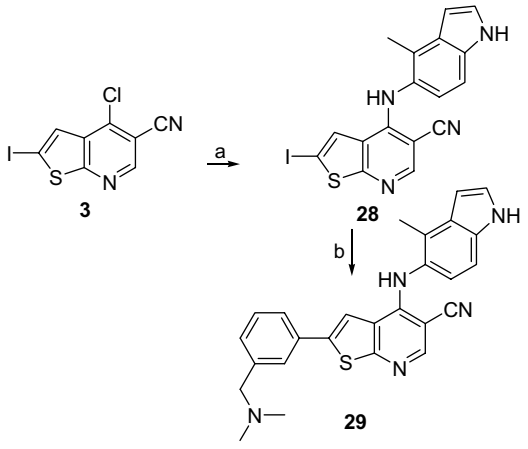
Scheme 3. Reagents: (a) 5-aminoindole, EtOH; (b) 4, 3, or 2-formylphenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$; (c) RR′NH, $Na(OAc)_3BH$, CH_2Cl_2, NMP; (d) 2-[(dimethylamino)methyl]-phenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$.

The optimized tailpiece and headpiece were added to the core as shown in Scheme 4. The reaction of **3** with 5-amino-4-methylindole resulted in **28**. The treatment of **28** with 3-[(*N*,*N*-dimethylamino)methyl]phenylboronic acid pinacol ester gave **29**, which had an IC_{50} value of 7.5 nM for the inhibition of PKCθ. Based on the earlier SAR, it was not surprising that **29** also showed a corresponding increase in the inhibition of PKCδ (IC_{50} = 26 nM), and of Lyn (IC_{50} = 520 nM).

Analog **29** was profiled against other PKC family members. While **29** only weakly inhibited PKCβ, (IC_{50} = 1.6 μM), more potent inhibition of PKCη and PKCε, two novel PKCs, was observed, with **29** having IC_{50} values of 62 and 17 nM, respectively. Limited additional kinase profiling of **29** provided IC_{50} values of 28 nM for Lck and of 2.2 μM for PKA. Weaker activity against MK2 and AKT was observed (IC_{50} values of >20 μM). **29** demonstrated ATP-competitive binding with a K_i of 9 nM for PKCθ and 96 nM for PKCδ.[16,24]

To assay cell activity, T-cells isolated from both PKCθ wild type (WT) and KO mice were stimulated with anti-CD3 and anti-CD28 to produce IL-2.[16] Compound **29** blocked the production of IL-2 from the WT cells with an IC_{50} value of 230 nM with decreased potency in the PKCθ KO cell assay (IC_{50} = 1300 nM) as would be expected for a PKCθ inhibitor. It should be noted that some of the activity in these cell assays may be due to the inhibition of Lck by **29**.

In pharmaceutical profiling assays, **29** had a permeability of 4.6×10^6 cm/s in a PAMPA assay, with low solubility at neutral pH. However, as expected due to the presence of the dimethylamine group, decreasing the pH to 3.0 increased the solubility of **29** to >100 μg/mL. In a stability study with rat liver microsomes, **29** had an estimated half-life of 17 min. These results indicate that this new class of PKCθ inhibitors should have acceptable physical chemical properties.



Scheme 4. Reagents: (a) 5-amino-4-methylindole, EtOH; (b) 3-[(*N*,*N*-dimethylamino)methyl]phenylboronic acid pinacol ester, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$.

The major challenge is achieving selectivity with this new scaffold. Good selectivity for PKCθ over Lyn (>100-fold) has been reported for the majority of the 2,4-diaminopyrimidine[14] and pyridine-3-carbonitrile[16] PKCθ inhibitors. Some of the pyrimidine analogs have >100-fold selectivity over PKCδ.[15] Additional structural modifications of the thieno[2,3-*b*]pyridine-5-carbonitriles are underway in attempts to retain the potent PKCθ inhibitory activity while reducing off target activity against PKCδ and the Src family kinases.

Acknowledgments

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