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C-5 Substituted heteroaryl 3-pyridinecarbonitriles as PKCθ inhibitors: Part I

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ABSTRACT

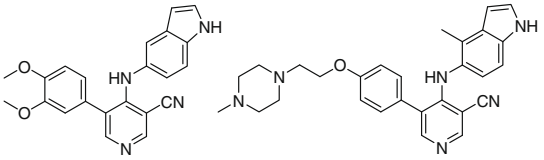
We earlier reported that 3-pyridinecarbonitriiles with a 4-methylindolyl-5-amino group at C-4 and a phenyl group at C-5 were inhibitors of PKCθ. Keeping the group at C-4 of the pyridine core constant, we varied the water solubilizing group on the phenyl ring at C-5 and then replaced the C-5 phenyl ring with several monocyclic heteroaryl rings, including furan, thiophene and pyridine. Analog **6e** with a 4-methylindol-5-ylamino group at C-4 and a 5-[(4-methylpiperazin-1-yl)methyl]-2-furyl group C-5 had an IC_{50} value of 4.5 nM for the inhibition of PKCθ.

The members of the protein kinase C (PKC) family of serine threonine kinases are subdivided into three classes based on the activation requirements of their N-terminal regulatory domains.[1,2] The conventional or classical isoforms require both calcium and diacylglycerol (DAG), the novel isoforms require only DAG and the atypical isoforms require neither. The PKC inhibitors currently in clinical development target either the conventional isoforms or both the conventional and novel isoforms.[3]

PKCθ, a novel isoform, is primarily expressed on T cells and plays a key role in various signaling pathways of the immune response.[4–6] PKCθ activates T cell cytokine production, specifically that of IL-2, which regulates T cell proliferation. Mice engineered to have a deficiency in PKCθ have a diminished development of several inflammatory diseases including asthma, multiple sclerosis, arthritis, inflammatory bowel disease, and transplant rejection, making this kinase a desirable target for therapeutic intervention.

The PKC isoforms have a strong structural similarity in their C-terminal catalytic domains, with PKCδ having the closest homology to PKCθ.[7] The ATP binding sites of these two kinases differ by only one amino acid. In contrast to PKCθ, mice where the gene for PKCδ was deleted or 'knocked out' developed autoimmune disease as a result of B cell hyperproliferation and an accompanying overproduction of inflammatory cytokines.[8,9] PKCδ is therefore often used as a counter assay where the desired target is PKCθ.

There are only a few reports in the literature of efforts to identify a selective inhibitor of PKCθ.[10–16] We earlier reported that optimization of the group at C-4 and of the substituents on the C-5 phenyl ring of **1a**[14] (IC_{50} = 70 nM) led to the identification of **1b** (IC_{50} = 7.4 nM) as a more potent inhibitor of PKCθ.[15,16] In efforts to optimize this series, we kept the C-4 group of **1b**, and varied the water solubilizing group on the phenyl ring at C-5.



1a **1b**

The analogs in Table 1 were prepared as shown in Scheme 1. Suzuki coupling of **2**[16] and the corresponding formylphenylboronic acid followed by reductive amination of the aldehyde gave **4a**, **4b**, and **4d–4g**. Commercially available 2-[(dimethylamino)-methyl]phenylboronic acid was subjected to a Suzuki coupling reaction with **2** to provide **4c**. As depicted in Table 1, since the *para* and *meta* isomers (**4a** and **4b**) exhibited greater potency against PKCθ than the *ortho* isomer (**4c**), additional optimization by varying the amine was only done on the *para* and *meta* isomers.

The *N*-methylpiperazine and morpholine analogs **4d–4g** were prepared by reductive amination of **3a** and **3b** as shown in Scheme 1. The analogs with an *N*-methylpiperazine group (**4d** and **4e**) (Table 1) exhibited increased PKCθ inhibition compared to those with a dimethylamine (**4a** and **4b**) or a morpholine substituent (**4f** and **4g**). Therefore, the *N*-methylpiperazine group was retained for further study.

Replacement of the C-5 phenyl ring with various heteroaryl rings was carried out as depicted in Scheme 2. Intermediate **2** was subjected to Suzuki coupling with various formylheteroaryl boronic acids to give **5a–5e**. The aldehydes, **5a–5e**, then underwent reductive amination with *N*-methylpiperazine to provide **6a–6e**.

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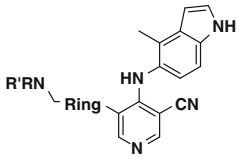
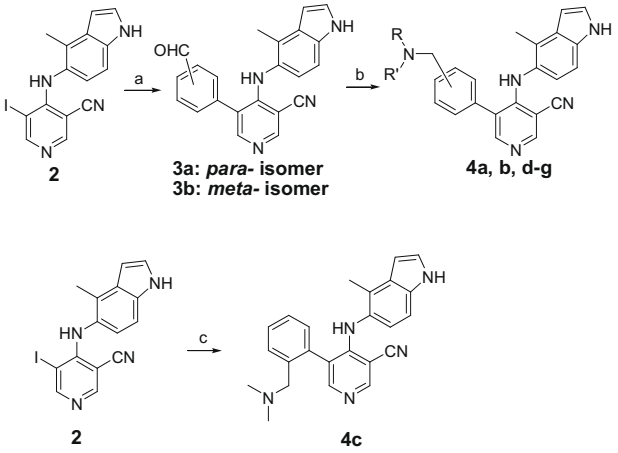
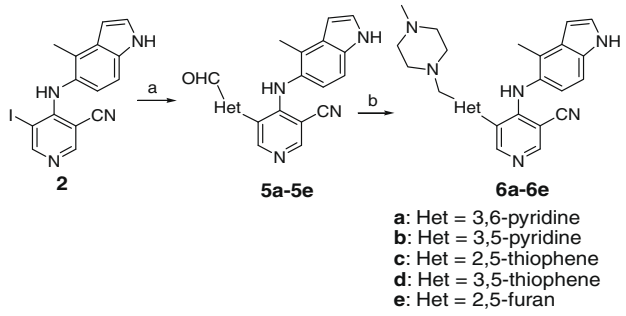
Table 1
PKCθ and PKCδ inhibitory activity of C-5 phenyl and heteroaryl analogs



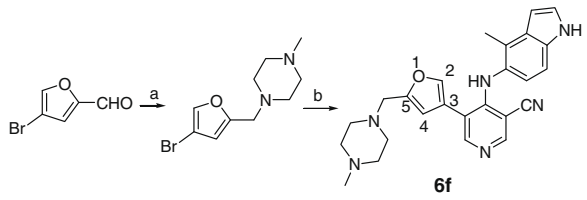
Compound number	NRR′	Ring	PKCθ IC_{50}[18] (nM)	PKCδ IC_{50}[18] (nM)	δ/θ
4a	NMe_2	1,4-Phenyl	8.4	58	6.9
4b	NMe_2	1,3-Phenyl	6.9	25	3.6
4c	NMe_2	1,2-Phenyl	200	2400	12
4d	*N*-Methylpiperazine	1,4-Phenyl	5.0	25	5.0
4e	*N*-Methylpiperazine	1,3-Phenyl	3.0	22	7.3
4f	Morpholine	1,4-Phenyl	9.0	70	7.8
4g	Morpholine	1,3-Phenyl	23	84	3.7
6a	*N*-Methylpiperazine	3,6-Pyridine	38	250	6.6
6b	*N*-Methylpiperazine	3,5-Pyridine	190	NT[18]	NA[18]
6c	*N*-Methylpiperazine	2,5-Thiophene	6.1	50	8.2
6d	*N*-Methylpiperazine	3,5-Thiophene	4.9	9.7	2.0
6e	*N*-Methylpiperazine	2,5-Furan	4.5	45	10
6f	*N*-Methylpiperazine	3,5-Furan	12	36	3.0



Scheme 1. Reagents and conditions: (a) for **3a**: 1.2 equiv 4-CHO-Ph-$B(OH)_2$, 0.05 equiv $Pd(PPh_3)_4$, DME, satd aq $NaHCO_3$, 95 °C, overnight; for **3b**: 1.2 equiv 3-CHO-Ph-$B(OH)_2$, 0.05 equiv $Pd(PPh_3)_4$, DME, satd aq $NaHCO_3$, 95 °C, overnight; (b) 10 equiv RR′NH, 5 equiv $NaBH(OAc)_3$, THF, room temperature, overnight; (c) 1.2 equiv 2-(CH_2NMe_2)-Ph-$B(OH)_2$, 0.05 equiv $Pd(Ph_3P)_4$, DME, satd aq $NaHCO_3$, 100 °C, overnight.



Scheme 2. Reagents and conditions: (a) 1.5–2 equiv formylheteroarylboronic ester or acid, 0.05 equiv $Pd(PPh_3)_4$, DME, satd aq $NaHCO_3$, reflux, 4 h, overnight; (b) for **6a**,**b**,**e**: 4–5 equiv *N*-methylpiperazine, 4–5 equiv $NaBH(OAc)_3$, THF, room temperature, overnight; for **6c**: 1.1 equiv *N*-methylpiperazine, 1.3 equiv $NaBH_3CN$, 1.2 equiv HOAc, EtOH, room temperature, 7 h; for **6d**: 3.2 equiv *N*-methylpiperazine, 5 equiv $NaBH(OAc)_3$, cat HOAc, CH_2Cl_2, NMP, room temperature, overnight.

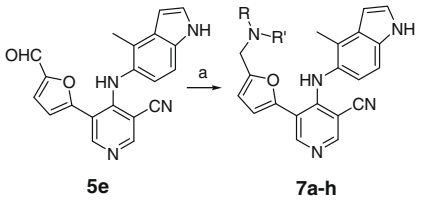


Scheme 3. Reagents and conditions: (a) 5 equiv *N*-methylpiperazine, 5 equiv $NaBH(OAc)_3$, 2 equiv HOAc, CH_2Cl_2, room temperature, overnight; (b) (1) 2.5 equiv triisopropyl borate, 2.7 equiv *n*-BuLi, THF, −78 °C 3 h, room temperature 1 h; (2) 0.5 equiv **2**, 0.05 equiv $Pd(dppf)_2Cl_2{\cdot}CH_2Cl_2$, DME, satd aq Na_2CO_3, 85 °C, 1.5 h.

As depicted in Scheme 3, 4-bromo-2-furaldehyde was subjected to reductive amination with *N*-methylpiperazine followed by treatment with *n*-butyl lithium and triisopropyl borate to give the furanylboronic ester. The ester was then coupled with **2** under Suzuki conditions to give **6f**.

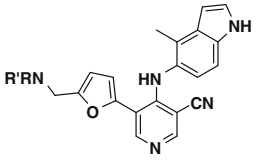
Selectivity for PKCθ over PKCδ with **6a–f** varied from 2- to 10-fold, as shown in Table 1. When compared to the phenyl analogs, **4a** and **4b**, loss of potency was observed with the C-5 pyridine analogs, **6a** and **6b**, while the thiophene and furan analogs **6c–6f** retained the potency. Overall **6e** exhibited the highest potency for the inhibition of PKCθ as well as the best selectivity over PKCδ. Therefore, the 2,5-furan ring of **6e** was retained for further optimization.

Compounds **7a–h** were prepared via reductive amination of **5e** with the corresponding amine (Scheme 4). While replacement of the *N*-methylpiperazine group of **6e** with a morpholine (**7a**) or thiomorpholine (**7b**) group retained much of the activity of the parent compound, replacement with a piperidine, pyrrolidine or a diethylamino group (**7c**–**e**) led to decreased inhibition of PKCθ (Table 2). Interestingly, addition of a 4-dimethylamino group to the piperidine ring of **7c**, namely **7f**, provided increased activity against both PKC isoforms; however the selectivity against PKCδ dropped. Variation of the methyl group on **6e** with either an isopropyl or a cyclopentyl group, **7g** and **7h**, also resulted in slightly increased potency against both PKC isoforms with decreased selectivity against PKCδ.



Scheme 4. Reagents and conditions: (a) 10 equiv RR′NH, 5 equiv $NaBH(OAc)_3$, THF, room temperature, overnight.

Table 2
PKCθ and PKCδ inhibitory activity of **6e** analogs



Compound number	NRR′	PKCθ IC_{50}[18] (nM)	PKCδ IC_{50}[18] (nM)	δ/θ
6e	*N*-Methylpiperazine	4.5	45	10
7a	Morpholine	7.8	86	11
7b	Thiomorpholine	7.5	94	13
7c	Piperidine	41	390	9.5
7d	Pyrrolidine	35	300	8.6
7e	*N,N*-Diethylamine	42	190	4.5
7f	4-Dimethylaminopiperidine	1.3	8.2	6.3
7g	*N*-Isopropylpiperazine	2.4	14	5.8
7h	*N*-Cyclopentylpiperazine	3.5	18	5.1

When **6e** was tested against other PKC family members, the IC_{50} values for the novel PKC isoforms PKCε and PKCη were 12 and 140 nM, respectively. As was seen with **1b**,[16] while **6e** inhibited the novel PKC isoforms it had greatly reduced activities against PKCβ IC_{50} = 2.5 μM), a classical isoform, and PKCζ (IC_{50} >100 μM) an atypical isoform. Additional kinase profiling of **6e** provided IC_{50} values of greater than 1 μM for Lck and greater than 5 μM for Lyn, PDGFR, MK2, p38, and ROCK1.

To assay the cell activity of **6e**, T cells isolated from both wild-type (WT) and PKCθ knock-out (KO) mice were treated with anti-CD3 and anti-CD28 to stimulate the production of IL-2. In the WT cells, **6e** exhibited good inhibition of IL-2 production (IC_{50} = 86 nM), making it about twofold more potent than **1b** (IC_{50} = 160 nM). As would be expected for a PKCθ inhibitor, much lower activity in the PKCθ KO cells was seen for both compounds (**6e**: IC_{50} = 1900 nM; **1b**: IC_{50} >15,000 nM). While **6e** had a 20-fold loss of activity in the PKCθ deficient cells compared to the normal cells, activity in both these cell assays can be the result of the inhibition of another kinase that contributes to IL-2 signaling.

Compound **6e** exhibited acceptable permeability (3.20×10^{-7} cm/s as measured in a PAMPA assay) and good solubility at pH 7.4 (>100 μg/mL). However, it had very poor stability in mouse, rat, and human liver microsomes (half lives of less than 10 min).

Our continuing optimization of this series of 3-pyridinecarbonitriles is described in the subsequent Letter.[17] Replacement of the heteroaryl ring at C-5 with a bicyclic heteroaryl ring resulted in improved potency against PKCθ, improved selectivity over PKCδ and improved metabolic stability.

Acknowledgments

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References and notes

1. Spitaler, M.; Cantrell, D. A. *Nat. Immunol.* **2004**, *5*, 785.
2. Mackay, H. J.; Twelves, C. J. *Nat. Rev. Cancer* **2007**, *7*, 554.
3. Baier, G.; Wagner, J. *Curr. Opin. Cell Biol.* **2009**, *21*, 262.
4. Hayashi, K.; Altman, A. *Pharmacol. Res.* **2007**, *55*, 537.
5. Marsland, B. J.; Kopf, M. *Trends Immunol.* **2008**, *29*, 179.
6. Chaudhary, D.; Kasaian, M. *Curr. Opin. Investig. Drugs* **2006**, *7*, 432.
7. Baier, G.; Telford, D.; Giampa, L.; Coggeshall, K. M.; Baier-Bitterlich, G.; Isakov, N.; Altman, A. *J. Biol. Chem.* **1993**, *268*, 4997.
8. Mecklenbräuker, I.; Saijo, K.; Zheng, N. Y.; Leitges, M.; Tarakhovsky, A. *Nature* **2002**, *416*, 860.
9. Miyamoto, A.; Nakayama, K.; Imaki, H.; Hirose, S.; Jiang, Y.; Abe, M.; Tsukiyama, T.; Nagahama, H.; Ohno, S.; Hatakeyama, S.; Nakayama, K. I. *Nature* **2002**, *416*, 865.
10. Cywin, C. L.; Dahmann, G.; Prokopowicz, A. S.; Young, E. R. R.; Magolda, R. L.; Cardozo, M. G.; Cogan, D. A.; DiSalvo, D.; Ginn, J. D.; Kashem, M. A.; Wolak, J. P.; Homon, C. A.; Farrell, T. M.; Grbic, H.; Hu, H.; Kaplita, P. V.; Liu, L. H.; Spero, D. M.; Jeanfavre, D. D.; O'Shea, K. M.; White, D. M.; Woska, J. R.; Brown, M. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 225.
11. Boschelli, D. H.; Wu, B.; Barrios Sosa, A. C.; Chen, J.; Asselin, M.; Cole, D. C.; Lee, J.; Yang, X.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2850.
12. Wu, B.; Boschelli, D. H.; Lee, J.; Yang, X.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 766.
13. Tumey, L. N.; Boschelli, D. H.; Lee, J.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4420.
14. Cole, D. C.; Asselin, M.; Brennan, A.; Czerwinski, R.; Ellingboe, J. W.; Fitz, L.; Greco, R.; Huang, X.; Joseph-McCarthy, D.; Kelly, M. F.; Kirisits, M.; Lee, J.; Li, Y.; Morgan, P.; Stock, J. R.; Tsao, D. H. H.; Wissner, A.; Yang, X.; Chaudhary, D. *J. Med. Chem.* **2008**, *51*, 5958.
15. Dushin, R.; Nittoli, T.; Ingalls, C.; Boschelli, D. H.; Cole, D. C.; Wissner, A.; Lee, J.; Yang, X.; Morgan, P.; Brennan, A.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2461.
16. Boschelli, D. H.; Wang, D.; Prashad, A. S.; Subrath, J.; Wu, B.; Niu, C.; Lee, J.; Yang, X.; Brennan, A.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3623.
17. Prashad, A. S.; Wang, D.; Subrath, J.; Wu, B.; Lin, M.; Zhang, M.-Y.; Kagan, N.; Lee, J.; Yang, X.; Brennan, A.; Chaudhary, D.; Xu, X.; Leung, L.; Wang, J.; Boschelli, D. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*.
18. For assay protocols see Ref. 14. IC_{50} values represent the mean of at least two determinations. NT: Compound was not tested. NA: Selectivity ratio is not available.