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Optimization of 5-vinylaryl-3-pyridinecarbonitriles as PKCθ inhibitors

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ABSTRACT

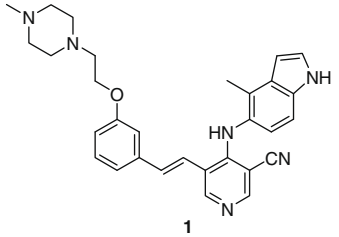
Analog **8**, a 3-pyridinecarbonitrile with an (*E*)-2-{6-[(4-methylpiperazin-1-yl)methyl]pyridin-2-yl}vinyl group at C-5, had an IC_{50} value of 1.1 nM for the inhibition of PKCθ and potently blocked the production of IL-2 in both stimulated murine T cells (IC_{50} = 34 nM) and human whole blood (IC_{50} = 500 nM).

PKCθ is a serine/threonine kinase expressed primarily in T cells. This kinase is a critical regulator of T cell function, acting in signaling pathways controlling IL-2 production, a trigger for T cell proliferation.[1] Extensive studies with PKCθ deficient mice propose that PKCθ inhibitors could be useful in the treatment of various inflammatory disease states including arthritis, asthma, multiple sclerosis and colitis.[2] Additional reports suggest that inhibition of PKCθ could be an effective therapy for systemic lupus erythematosus (SLE).[3,4] The current treatments for lupus, a complex autoimmune disease, are limited and can cause severe side effects.[5]

PKCθ is a member of a family of highly structurally related kinases and shares close homology with PKCδ.[6] Studies with PKCδ deficient mice revealed a role for this kinase in the development of autoimmune disease as a result of increased B cell proliferation.[7,8] Both PKCθ and PKCδ belong to the novel class of PKCs that also includes PKCε and PKCη. The other classes of PKCs are the classical isoforms such as PKCα and PKCβ and the atypical isoforms, which includes PKCζ.

Although there have been several reports of small molecules targeted as selective inhibitors of PKCθ, none of these compounds have entered the clinic.[9] While Sotrastaurin (AEB071), a PKCθ inhibitor from Novartis, is currently in phase II trials for the prevention of renal transplant rejection and the treatment of psoriasis, this compound also inhibits both the additional novel and the classical PKC isoforms.[10–12]

In 2008, Wyeth reported a series of 4-indolylamino-5-phenyl-3-pyridinecarbonitriles as inhibitors of PKCθ with selectivity over PKCδ.[13] Replacement of the phenyl ring at C-5 with a vinyl group led to new potent inhibitors of PKCθ.[14–16] However for various reasons, none of these analogs met our criteria for advancement into in vivo efficacy studies. For example, while **1** had an IC_{50} value of 4.7 nM for the inhibition of PKCθ, it had a moderate in vitro half-life in rat liver microsomes (21 min) and poor aqueous solubility (5 μg/mL).[14] Hoping to retain the desired in vitro activity while increasing the stability and solubility of this series of inhibitors, additional analogs of **1** were targeted.



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Analogs where the OCH_2CH_2 linker of **1** was replaced by a CH_2 group were prepared as shown in Scheme 1. Palladium catalyzed coupling of the 5-vinyl-3-pyridinecarbonitrile **2** with the three isomers of 1-(bromobenzyl)-4-methylpiperazine provided **3**, **4** and **5**.

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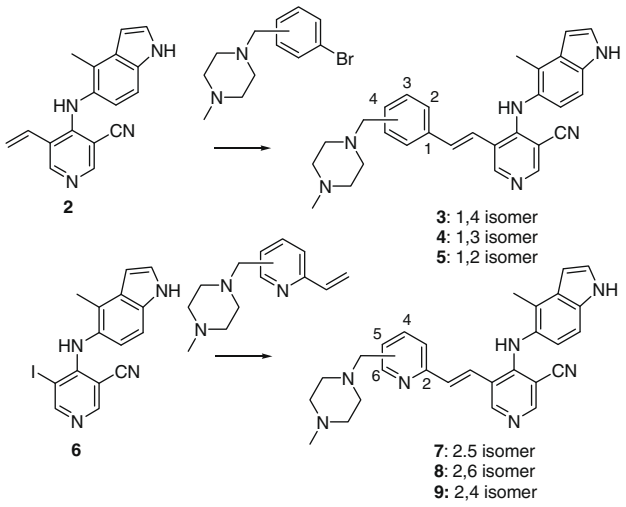
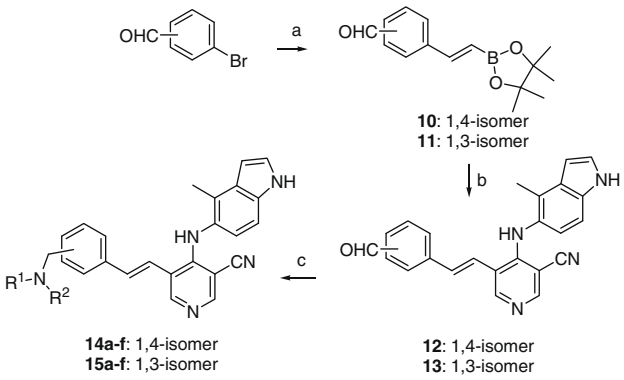


Scheme 1. Reagents and conditions: $Pd(OAc)_2$, P(*o*-tolyl)$_3$, DMF, Et_3N, 95–120 °C.

As shown in Table 1, the 1,3-phenyl analog **4** was the most potent inhibitor of PKCθ, followed by the 1,4-isomer **3**, with the 1,2-isomer **5** being the least active. While all three analogs were at least 10-fold selective over PKCδ, all had poor rat liver microsomal stability.

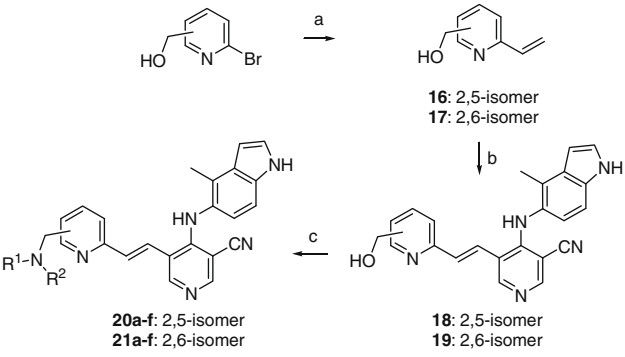
The corresponding 2-pyridine analogs of **3–5** were synthesized by the route shown in Scheme 1. Treatment of the 5-iodo-3-pyridinecarbonitrile **6** with 1-[(2-ethenyl-5-pyridinyl)methyl]-4-methyl-piperazine[17] in the presence of palladium acetate and tri-(*o*-tolyl)phosphine gave **7**. The 2,6-isomer **8** and the 2,4-isomer **9** were prepared via the corresponding isomeric intermediates. Analog **8** had an IC_{50} value of 1.1 nM for the inhibition of PKCθ (Table 1) with similar activity seen for **7** (1.9 nM). The 2,4-isomer **9** was the least active having an IC_{50} value of only 14 nM. Again, all three isomers were at least 10-fold selective over PKCδ. As shown in Table 1, the pyridine analogs had increased rat liver microsomal stability compared to the phenyl analogs. Increased aqueous solubility was also observed, with **7** and **8** having values of 26 and 58 μg/mL compared to values of 1.0 and 14 μg/mL for **3** and **4**, respectively. The increased solubility was correlated with the substantial decrease in clog *P* from 6.3 to 4.8 resulting from replacing the phenyl ring with a pyridine ring.

To facilitate variation of the amine solubilizing group of **3** and **4**, the route shown in Scheme 2 was developed. The vinyl pinacol borane **10** was prepared by reaction of 2-ethenyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and 4-bromobenzaldehyde as reported in the literature.[18,19] Suzuki reaction of **10** with **6** provided the aldehyde intermediate **12** which readily underwent reductive amination with a variety of amines to provide **14a–f**. Using a route analogous to that for the synthesis of **10**, the previously unreported **11** was prepared from 3-bromobenzaldehyde. Reaction of **11** with **6** and subsequent reductive amination of **13** provided **15a–f**.



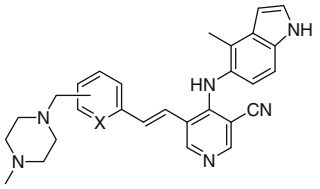
Scheme 2. Reagents and conditions: (a) 2-ethenyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $Pd(OAc)_2$, 1,10-phenanthroline, Et_3N, CH_3CN, 60 °C; (b) **6**, $Pd(PPh_3)_4$, DME, satd aq $NaHCO_3$, 100 °C; (c) R^1R^2NH, $Na(OAc)_3BH$, HOAc, THF, rt.

A different route was used to prepare analogs of **7** and **8** with additional water solubilizing amines. Reaction of 2-bromo-5-hydroxymethylpyridine with tributyl(vinyl)tin in the presence of



Scheme 3. Reagents and conditions: (a) tributyl(vinyl)tin, $Pd(PPh_3)_4$, toluene, reflux; (b) **6**, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, DMF, Et_3N, 95–120 °C; (c) (1) MsCl, Et_3N, THF, DMF, rt; (2) R^1R^2NH, rt.

Table 1
PKCθ and PKCδ inhibitory activity and rat liver microsomal stability of 5 vinylaryl-3-pyridinecarbonitriles



Compound number	Isomer	X	PKCθ IC_{50} (nM)[23]	PKCδ IC_{50} (nM)[23]	δ/θ	Rat liver microsomal stability half-life (min)
3	1,4	CH	3.6	200	58	6.5
4	1,3	CH	1.4	24	17	11
5	1,2	CH	9.9	96	10	9
7	2,5	N	1.9	68	36	17
8	2,6	N	1.1	29	25	25
9	2,4	N	14	240	18	18

tetrakis(triphenylphosphine)palladium(0) provided the 2-vinylpyridine **16**, which was coupled with **6** to yield **18** (Scheme 3). Conversion of **18** to the corresponding mesylate followed by displacement with a variety of amines gave the desired **20a**–**f**. A similar route was then used to obtain the 2,6 isomers of **20a**–**f**, namely **21a**–**f**.

The IC_{50} values for the inhibition of PKCθ and PKCδ along with the rat liver microsomal stability of the new analogs are shown in Table 2. With the exception of **20a**, **20b** and **20d**, all compounds had IC_{50} values of less than 10 nM for the inhibition of PKCθ. Only six compounds were less than 10-fold selective for PKCθ over PKCδ. The greatest disparity was seen with the compound half-lives in rat liver microsomes. While many analogs demonstrated poor stability, having half-lives of less than 10 min, several analogs had half-lives of greater than 30 min, including all four analogs with a 4-dimethylaminopiperidine solubilizing group. In summary, of the 30 compounds in Tables 1 and 2, only seven had IC_{50} values of 10 nM or less for the inhibition of PKCθ and were 10-fold or more selective for PKCθ over PKCδ with a half-life in rat liver microsomes of greater than 20 min. The seven compounds that survived this triage were evaluated in a cellular assay.

Stimulation of murine T cells with anti-CD3 and anti-CD28 induces the expression of IL-2 which is reduced in the presence of a PKCθ inhibitor. Table 3 shows that five of the seven compounds tested in this assay had IC_{50} values of 200 nM or less. All compounds had IC_{50} values of greater than 1 μM in a corresponding assay using T cells isolated from PKCθ deficient mice. The five compounds that met our initial cell activity criteria were then taken on into an assay looking at their ability to block the production of IL-2 in stimulated human whole blood.[20] The best inhibitory activity was seen with **8** and **15c** which had IC_{50} values of 500 and 450 nM, respectively. Both compounds had half-lives in C57 BL6 mouse liver microsomes of greater than 20 min. However when tested at 10 mg/kg (po) in a short term in vivo mouse model of cytokine production in response to anti-CD3, only **8** was efficacious in reducing IL-2 production.[21]

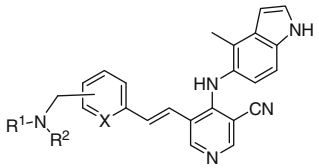
Table 3
Cell data and mouse liver microsomal stability for key 5-vinylaryl-3-pyridinecarbonitriles

Compound number	T cell IC_{50} (nM)[23]	HWB IC_{50} (nM)[20]	Mouse liver microsomal stability half-life (min)
8	34	500	29
14c	83	990	
15c	80	450	23
20c	150	1700	
20e	300		
21c	300		
21e	200	700	

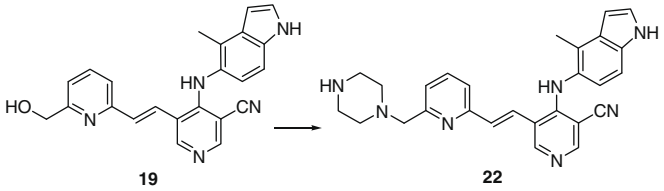
In pharmaceutical profiling assays **8** had good aqueous solubility (61 μg/mL) and moderate PAMPA permeability (0.39×10^{-6} cm/s). At a 3 μM substrate concentration, **8** provided 34% inhibition of CYP3A4 with less than 10% inhibition of CYP2C9 and CYP2D6 observed. When tested against other PKCs, **8** had IC_{50} values of 2.0 and 120 nM for the inhibition of PKCε and PKCη, two novel isoforms. Weaker activity was observed against both PKCβ, a classic isoform, and PKCζ, an atypical isoform (IC_{50} values of 700 and >100,000 nM, respectively). Moderate inhibition of several Src family kinases was seen, with **8** having IC_{50} values of 790, 540 and 130 nM for the inhibition of Lck, Lyn and Src, respectively. When tested against a panel of an additional 15 kinases, **8** inhibited PKA with an IC_{50} value of 750 nM, with IC_{50} values of greater than 3 μM observed against the other 14 kinases.

A oral PK study was performed in NZBWF1/J mice, a strain that spontaneously develops severe lupus.[22] A 25 mg/kg dose of **8** administered as a suspension in 2% Tween/0.5% methylcellulose

Table 2
PKCθ and PKCδ inhibitory activity and rat liver microsomal stability of additional 5-vinylaryl-3-pyridinecarbonitriles



Compound number	Isomer	X	NR^1R^2	PKCθ IC_{50} (nM)[23]	PKCδ IC_{50} (nM)[23]	δ/θ	Rat liver microsomal stability half-life (min)
14a	1,4	CH	Morpholine	4.0	180	5	7
14b	1,4	CH	Piperidine	4.1	51	12	10
14c	1,4	CH	4-Dimethylaminopiperidine	1.3	16	12	>30
14d	1,4	CH	4-Hydroxypiperidine	5.5	45	8	>30
14e	1,4	CH	*N*-(2-Hydroxyethyl)-piperazine	4.7	75	16	9
14f	1,4	CH	(2-Methoxyethyl)amino	5.2	45	9	23
15a	1,3	CH	Morpholine	1.2	300	240	4
15b	1,3	CH	Piperidine	4.9	85	17	18
15c	1,3	CH	4-Dimethylaminopiperidine	1.5	34	22	>30
15d	1,3	CH	4-Hydroxypiperidine	2.9	53	19	12
15e	1,3	CH	*N*-(2-Hydroxyethyl)-piperazine	1.5	41	27	13
15f	1,3	CH	(2-Methoxyethyl)amino	4.9	40	8	9
20a	2,5	N	Morpholine	56	770	14	12
20b	2,5	N	Piperidine	14	76	5	14
20c	2,5	N	4-Dimethylaminopiperidine	2.8	55	20	>30
20d	2,5	N	4-Hydroxypiperidine	31	150	5	>30
20e	2,5	N	*N*-(2-Hydroxyethyl)-piperazine	6.2	130	21	>30
20f	2,5	N	(2-Methoxyethyl)amino	3.2	36	11	8
21a	2,6	N	Morpholine	1.5	61	42	13
21b	2,6	N	Piperidine	4.3	61	14	10
21c	2,6	N	4-Dimethylaminopiperidine	0.84	18	21	>30
21d	2,6	N	4-Hydroxypiperidine	2.1	43	21	15
21e	2,6	N	*N*-(2-Hydroxyethyl)-piperazine	0.65	11	17	>30
21f	2,6	N	(2-Methoxyethyl)amino	4.2	90	22	6



Scheme 4. Reagents: (1) MsCl, DMF, THF, Et_3N; (2) *N*-Boc-piperazine; (3) TFA, CH_2Cl_2.

provided an C_{max} of 2.2 μM, an AUC of 17.8 h μM and a C_{avg} (AUC/24 h) of 740 nM. Both the C_{max} and the C_{avg} were higher than the HWB cell IC_{50} of 500 nM for this compound.

Stability studies in both human and monkey liver microsomes provided half-lives for **8** of greater than 30 min. The metabolism of **8** was determined in monkey and human liver microsomal incubations fortified with their respective liver cytosols, glutathione (GSH) and NADPH. The major metabolite was found to be the demethylated *N*-methyl piperazine analog **22**. Additional metabolites included oxidation of (1) the core pyridine ring, (2) the indole at C-4 and (3) the *N*-methylpiperazine. No GSH adducts were observed and there were no unique human metabolites.

The metabolite **22** was prepared as shown in Scheme 4. Conversion of the alcohol group of **19** to the corresponding mesylate followed by addition of *N*-Boc piperazine and subsequent deprotection with trifluoroacetic acid gave the desired product. Potent activity was observed against PKCθ, with **22** having an IC_{50} value of 0.32 nM. While **22** was 27-fold selective for PKCθ over PKCδ it had reduced activity in the T cell assay (IC_{50} = 460 nM) compared to **8**.

In summary, optimization of a series of 5-vinylaryl-3-pyridinecarbonitriles led to the identification of **8**, which had an IC_{50} value of 1.1 nM for the inhibition of PKCθ with 25-fold selectivity over PKCδ and good selectivity against other kinases. This compound potently blocked the production of IL-2 in both T cells (IC_{50} = 34 nM) and whole blood (IC_{50} = 500 nM). Unlike many of the earlier compounds in this series, **8** had good metabolic stability and solubility which resulted in this compound having acceptable plasma levels in NZBWF1/J mice.

Acknowledgments

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References and notes

1. Marsland, B. J.; Kopf, M. *Trends Immunol.* **2008**, *29*, 179.
2. Baier, G.; Wagner, J. *Curr. Opin. Cell Biol.* **2009**, *21*, 262.
3. Takeuchi, T.; Tsuzaka, K.; Kameda, H.; Amano, K. *Curr. Drug Targets Inflamm. Allergy* **2005**, *4*, 295.
4. Fujii, Y.; Fujii, K.; Tanaka, Y. *Autoimmun. Rev.* **2006**, *5*, 143.
5. Rahman, A.; Isenberg, D. A. *N. Eng. J. Med.* **2008**, *358*, 929.
6. Spitaler, M.; Cantrell, D. A. *Nat. Immunol.* **2004**, *5*, 785.
7. Mecklenbraeuker, I.; Saijo, K.; Zheng, N. Y.; Leitges, M.; Tarakhovsky, A. *Nature* **2002**, *416*, 860.
8. 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.
9. Boschelli, D. H. *Curr. Top. Med. Chem.* **2009**, *9*, 640.
10. Wagner, J.; von Matt, P.; Sedrani, R.; Albert, R.; Cooke, N.; Ehrhardt, C.; Geiser, M.; Rummel, G.; Stark, W.; Strauss, A.; Cowan-Jacob, S. W.; Beerli, C.; Weckbecker, G.; Evenou, J.-P.; Zenke, G.; Cottens, S. *J. Med. Chem.* **2009**, *52*, 6193.
11. Skvara, H.; Dawid, M.; Kleyn, E.; Wolff, B.; Meingassner, J. G.; Knight, H.; Dumortier, T.; Kopp, T.; Fallahi, N.; Stary, G.; Burkhart, C.; Grenet, O.; Wagner, J.; Hijazi, Y.; Morris, R. E.; McGeown, C.; Rordorf, C.; Griffiths, C. E. M.; Stingl, G.; Jung, T. *J. Clin. Invest.* **2008**, *118*, 3151.
12. Evenou, J.-P.; Wagner, J.; Zenke, G.; Brinkmann, V.; Wagner, K.; Kovarik, J.; Welzenbach, K. A.; Weitz-Schmidt, G.; Guntermann, C.; Towbin, H.; Cottens, S.; Kaminski, S.; Letschka, T.; Lutz-Nicoladoni, C.; Gruber, T.; Hermann-Kleiter, N.; Thuille, N.; Baier, G. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 792.
13. Cole, D. C.; Asselin, M.; Brennan, A.; Czerwinski, R.; Ellingboe, J.; Fitz, L.; Huang, X.; Joseph-McCarthy, D.; Greco, R.; Ho, M.; Deng, B.; Kiristis, M.; Lee, J.; Li, Y.; Morgan, P.; Stock, J.; Tsao, D.; Wissner, A.; Wei, S.-Q.; Yang, X.; Chaudhary, D. *J. Med. Chem.* **2008**, *51*, 5958.
14. Niu, C.; Boschelli, D. H.; Tumey, L. N.; Bhagirath, N.; Subrath, J.; Shim, J.; Wang, Y.; Wu, B.; Eid, C.; Lee, J.; Yang, X.; Brennan, A.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5829.
15. Tumey, L. N.; Bhagirath, N.; Brennan, A.; Brooijmans, N.; Lee, J.; Yang, X.; Boschelli, D. H. *Bioorg. Med. Chem.* **2009**, *17*, 7933.
16. Shim, J.; Eid, C.; Lee, J.; Liu, E.; Chaudhary, D.; Boschelli, D. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6575.
17. Tumey, L. N.; Boschelli, D. H.; Bhagirath, N.; Chen, J. J.; Floyd, M. B.; Li, Z.; Niu, C.; Shim, J.; Wang, Y.; Wang, Y. D.; Wu, B.; Eid, C. N. WO2009076602, 2009.
18. Stewart, S. K.; Whiting, A. *J. Organomet. Chem.* **1994**, *482*, 293.
19. Hunt, A. R.; Stewart, S. K.; Whiting, A. *Tetrahedron Lett.* **1993**, *34*, 3599.
20. *Protocol for human whole blood IL-2 assay*: Blood was collected from healthy subjects into heparinized Vacutainers. Blood was incubated with compounds in a 96-well round-bottom plate for 30 min at 37 °C with 5% CO_2. All compounds were prepared in RPMI, with final DMSO concentration of 0.2%. Soluble anti-CD3 (0.06 mg/ml) and anti-CD28 (0.3 mg/ml) antibodies were added after compound incubation. The plate was kept at 37 °C with 5% CO_2 for 24 h. Supernatant was collected for IL-2 ELISA (R&D) and data (n = 3) was analyzed using Softmax Pro software.
21. Chaudhary, D.; Bree, A., unpublished results.
22. Dubois, E. L.; Horowitz, R. E.; Demopoulos, H. B.; Teplitz, R. *JAMA* **1966**, *195*, 285.
23. For both the IMAP enzyme and the mouse T cell assay protocols see Ref. 13. IC_{50} values represent the mean of at least two determinations.