Bioorganic & Medicinal Chemistry Letters 18 (2008) 4420–4423

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

2-Alkenylthieno[2,3-*b*]pyridine-5-carbonitriles: Potent and selective inhibitors of PKCθ

L. Nathan Tumey [a,*], Diane H. Boschelli [a], Julie Lee [b], Divya Chaudhary [b]

[a] *Wyeth Research, Chemical and Screening Sciences, 401 N. Middletown Road, Pearl River, NY 10965, USA*
[b] *Wyeth Research, Inflammation, 200 Cambridge Park Drive, Cambridge, MA 12140, USA*

ARTICLE INFO

Article history:
Received 8 May 2008
Revised 12 June 2008
Accepted 12 June 2008
Available online 18 June 2008

Keywords:
Kinase
PKC
PKCθ
PKCδ
Indole
Inflammation
T cell
Thienopyridine
Cyano
Nitrile

ABSTRACT

A series of 2-alkenyl thieno[2,3-*b*]pyridine inhibitors of PKCθ were synthesized as potential inflammatory modulators. This series led to the discovery of 2-alkenyl amides, which are exceptionally potent and selective inhibitors of PKCθ. Compound **8** has an IC_{50} of 3.8 nM against PKCθ and shows excellent selectivity over a variety of PKC isoforms.

T cell receptor (TCR) activation is an essential step in the mounting of an effective immune response. The binding of an antigen/major histocompatibility complex (MHC) to the TCR leads to the formation of a supramolecular activation cluster (SMAC) which, in conjunction with stimulation of CD28, begins a phosphorylation cascade that ultimately results in transcriptional activation of interleukin-2 (IL-2).[1] Increased levels of IL-2, in turn, promote T cell proliferation and differentiation, contributing to an inflammatory response.[2]

Protein kinase C theta (PKCθ) is a serine/threonine kinase expressed primarily in lymphocytes and mast cells. PKCθ knockout mice have been reported to have significant defects in their response to T cell stimulation. PKCθ plays an essential role in the TCR-mediated activation of transcription factors which, in turn, upregulate IL-2 gene expression.[3] Specifically, PKCθ knockout mice have shown diminished responses in various T cell mediated disease models including the type II collagen-induced arthritis (CIA) model,[4] the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis,[5,6] and the ovalbumin challenge (OVA) model of asthma.[7,8] Interestingly, in spite of their defective T cell activation pathway, PKCθ knockout mice have been shown to have normal Th1 cell response in the lung and normal viral clearance.[7,9] For these reasons, the inhibition of PKCθ has recently become an attractive target for treatment of autoimmune and inflammatory diseases.[10–13]

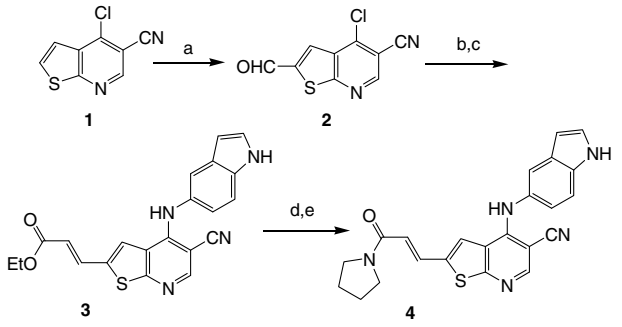
We recently reported a series of 2-phenylthieno[2,3-*b*]pyridine-5-carbonitrile inhibitors of PKCθ.[13] The previous work[13,14] suggested that the presence of a solubilizing amine tail and/or a hydrogen-bond acceptor on the phenyl ring significantly improved the inhibitory activity against PKCθ. We reasoned that the phenyl group may serve primarily as a 'linker' connecting the thieno[2,3-*b*]pyridine core to the important hydrogen-bond accepting residue. With this in mind, we studied variations of this linker. The present work focuses on attachment of various alkenyl amides that connect the core to the requisite polar side chain.

The initial synthesis of this class of compounds is illustrated in Scheme 1. The previously reported thieno[2,3-*b*]pyridine **1**[15] was deprotonated with LDA at −78 °C and subsequently quenched with DMF to give aldehyde **2**. Wittig coupling with the appropriate phosphorus ylid followed by introduction of the aminoindole headpiece by nucleophilic aromatic substitution gave ester **3**. Saponification followed by amidation gave the desired pyrrolidine amide **4**.

Compound **4** proved to be a potent inhibitor of PKCθ with an IC_{50} of 130 nM, but more importantly showed approximately 140-fold selectivity over PKCδ. Selectivity for PKCθ is desirable considering reports that PKCδ deficiency in mice results in B cell

* Corresponding author. Tel.: +1 845 602 1876; fax: +1 845 602 5561.
E-mail address: nathantumey@yahoo.com (L. Nathan Tumey).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.040



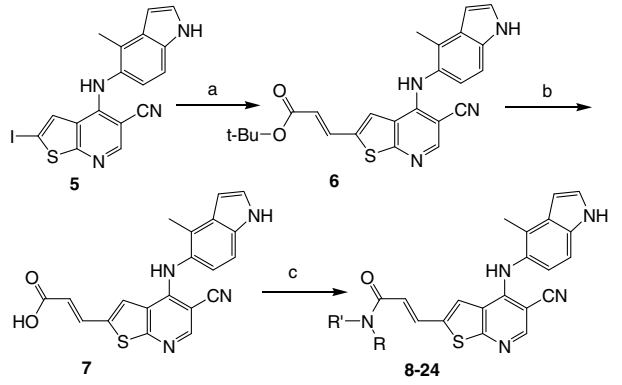
Scheme 1. Reagents: (a) LDA, −78 °C followed by DMF; (b) $EtO_2CCH{=}PPh_3$, THF; (c) 5-aminoindole, EtOH, reflux; (d) NaOH, EtOH, 80 °C; (e) pyrrolidine, EDCI, DMF.

hyperproliferation.[16,17] Due to the very high active-site sequence identity with PKCθ, selectivity over PKCδ has been difficult to achieve in the related 2-phenylthieno[2,3-*b*]pyridine series.[13]

Due to its potent activity and good selectivity, we desired a more versatile synthesis of alkenyl amides related to compound **4**. Therefore, a new synthesis was devised (Scheme 2) that shortened the prior sequence by one step and avoided the need for cryogenic lithiation. Based on our previous studies with the related 2-phenylthieno[2,3-*b*]pyridine series,[13] we decided to incorporate the more active 4-methyl-5-aminoindole headpiece in place of the 5-aminoindole headpiece in **4**. The new synthetic sequence utilized phosphite promoted Heck coupling[18] of **5**[13] with *tert*-butyl acrylate to give compound **6**. Acidic hydrolysis followed by carbodiimide coupling gave the various acrylamides shown in Table 1.

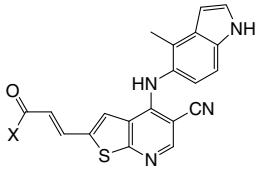
In agreement with our previous findings, incorporation of a 4-Me group on the indole significantly improved inhibitory activity against PKCθ. We found that compound **8** was approximately 30-fold more active against PKCθ than compound **4** (3.8 nM vs 130 nM, respectively). More importantly, compound **8** retains and even slightly improves upon the PKCδ/PKCθ ratio that was observed for compound **4** (340-fold vs 140-fold, respectively).

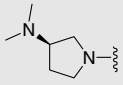
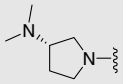
Addition of a hydroxyl or tertiary amine solubilizing tail to the pyrrolidine ring (**9**–**11**) resulted in a loss of over one log unit of activity against PKCθ (shown in Table 1). Furthermore, expansion of the five-membered ring to a six-membered ring (**12**–**15**) also resulted in a 10- to 15-fold loss in PKCθ activity. Interestingly, most of these examples proved to be more potent against PKCδ than the parent compound (**8**). This resulted in dramatic losses of selectivity for most of these analogs. On the other hand, small dialkyl amides such as **17** and **18** retained both the activity and selectivity of the parent compound. Monosubstituted amides (**19**–**21**), however, were approximately 4- to 15-fold less potent against PKCθ than the parent compound.



Scheme 2. Reagents: (a) *t*-Bu acrylate, $P(OMe)_3$, $Pd(OAc)_2$, DMF, Et_3N, 80 °C, 2 h; (b) 5% TFA/DCM; (c) RR′NH, EDCI, DMF.

Table 1
PKCθ and PKCδ activities of various alkenyl amide substituted thieno[2,3-*b*]pyridines



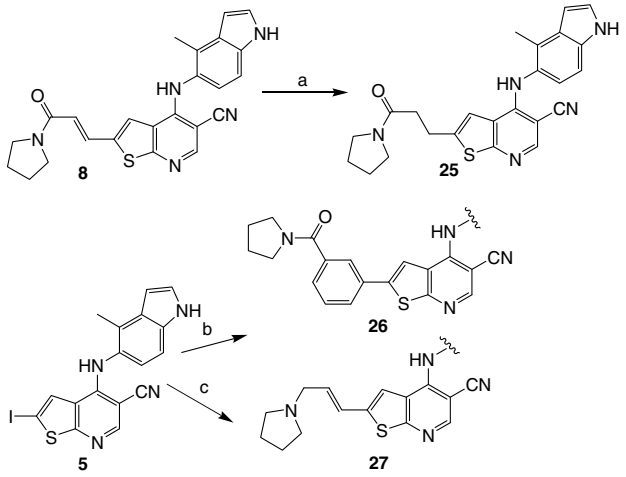
Compound	X	PKCθ IC_{50}[a] (nM)	PKCδ IC_{50}[a] (nM) (PKCδ/PKCθ)
8	Pyrrolidine	3.8	1300 (340×)
9	(±) 3-Hydroxy pyrrolidine	41	450 (11×)
10		180	430 (2×)
11		170	340 (2×)
12	Piperidine	48	450 (9×)
13	Morpholine	53	4100 (78×)
14	Piperazine	88	200 (2×)
15	N–Me–piperazine	43	73 (2×)
16	H_2N–	12	67 (6×)
17	Me_2N–	1.6	2100 (1300×)
18	Et_2N–	2.0	250 (120×)
19	EtNH–	15	500 (34×)
20	PhNH–	110	500 (4×)
21	MeO–$(CH_2)_2$–NH–	48	1800 (38×)
22	Me_2N–CH_2–CH_2–N(Me)–	150	790 (5×)
23	Me_2N–NH–	100	420 (4×)
24		370	330 (1×)

[a] Values are means of two or more experiments.

In spite of the excellent activity and selectivity observed for compounds **8**, **17**, and **18**, these compounds suffered from very poor aqueous solubility (data not shown). However, the incorporation of amine solubilizing groups (**10–11, 14–15, and 22**) resulted in a significant loss of PKCθ inhibitory activity and/or selectivity against PKCδ. Movement of the basic moiety closer to the carbonyl via acyl hydrazines (**23**, **24**) also resulted in compounds with poor PKCθ inhibition.

Having extensively explored the amino moiety (Table 1), we next made variations of the carbonyl and alkene portion of amide **8** (Scheme 3). Alkene **8** could be catalytically reduced with hydrogen to give propanamide **25**. Suzuki coupling of **5** with 3-(pyrrolidine-1-carbonyl)phenylboronic acid gave **26**. Likewise, Suzuki coupling of **5** with (*E*)-3-chloroprop-1-enylboronic acid in the presence of pyrrolidine gave allyl amine **27**. Interestingly, this Suzuki reaction requires the presence of the secondary amine. In the absence of an amine nucleophile, **5** was recovered unchanged. It is believed that nucleophilic displacement of the allyl chloride frees the palladium catalyst from the π-allyl complex that it forms with (*E*)-3-chloroprop-1-enylboronic acid, thereby allowing the Suzuki reaction to take place to give **27**. This reaction has proven to be rather general in scope and further examples will be reported elsewhere (unpublished observations).

In addition to exploring the α,β-unsaturated carbonyl linker, we were also interested in the effect of a methyl group on the thiophene core. The 3-methylthieno[2,3-*b*]pyridine skeleton was made as illustrated in Scheme 4. The 2-aminothiophene **28** was formylated with DMF–DMA and treated with *tert*-butylcyanoacetate to give enamine **29**. Upon heating to 250 °C in diphenyl ether, compound **29** underwent a thermal elimination of isobutylene followed by



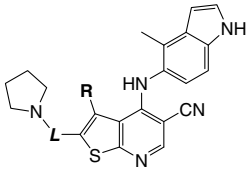
Scheme 3. Reagents: (a) H_2, Pd/C, EtOH/toluene; (b) 3-(pyrrolidine-1-carbonyl)phenylboronic acid, $Pd(PPh_3)_4$, $NaHCO_3$, dioxane, reflux; (c) (*E*)-3-chloroprop-1-enylboronic acid, pyrrolidine, Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, DMF, 130 °C (μW).

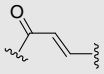
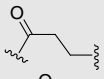
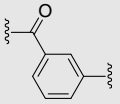
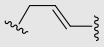
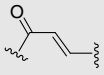
decarboxylation and concomitant cyclization to form thieno[2,3-*b*]pyridone **30**. Iodination followed by treatment with $POCl_3$ gave intermediate **31**. This compound was converted in 4 steps to **33** by the previously described route.

As shown in Table 2, propanamide **25** lost nearly two log units of inhibitory activity against PKCθ as compared to the parent compound **8**. This suggested a significant structural role for the rigidity provided by the alkene. Replacement of the alkene with a phenyl (**26**) resulted in loss of both potency and selectivity. Replacement of the carbonyl with a methylene (**27**) resulted in a loss of approximately one log unit of activity suggesting that the carbonyl plays a role in the binding to PKCθ. However, the significant improvement of PKCδ inhibitory activity observed for this compound suggested that the carbonyl is key for selectivity against this PKC isoform. Addition of a methyl group to C-3 of the core thieno[2,3-*b*]pyridine seemed to have little effect on potency and selectivity. Compound **33** retained the excellent potency of parent compound **8** (3.5 nM vs 3.8 nM) and the selectivity of the parent compound (110× vs 340×).

While extensive SAR was determined for compound **8**, no significant improvements were identified in potency (Tables 1 and 2) or in pharmaceutical profile (data not shown). We therefore subjected compound **8** to further biological characterization in order to determine if it was a suitable candidate for advancement. We found that compound **8** inhibited the anti-CD3/anti-CD28 promoted release of IL-2 from wild-type murine T cells, with an IC_{50} of 170 nM.[12] As was expected based on the rather selective inhibition of PKCθ, the compound had a significantly reduced effect on IL-2 release upon stimulation of T cells that were isolated from PKCθ knockout mice (IC_{50} > 1000 nM). Compound **8** had a $T_{1/2}$ greater than 30 min in rat liver microsomes and had limited inhibition of various cytochrome P450 isozymes at 3 μM (<50% inhibition of 2D6 and 2C9 and ~60% inhibition of 3A4).

Table 2
PKCθ and PKCδ activities of various pyrrolidinyl-thieno[2,3-*b*]pyridines

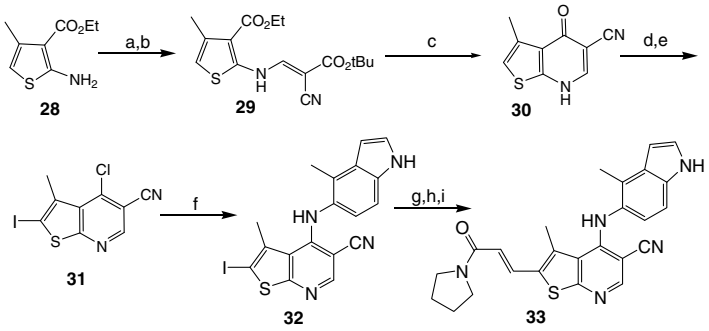


Compound	L	R	PKCθ IC_{50}[a] (nM)	PKCδ IC_{50}[a] (nM) (PKCδ/PKCθ)
8		H	3.8	1300 (340×)
25		H	250	1800 (7×)
26		H	58	180 (3×)
27		H	22	100 (5×)
33		Me	3.5	380 (110×)

[a] Values are means of two or more experiments.

Table 3 illustrates the kinase selectivity profile for this compound. Compound **8** is exceptionally selective over other PKC isoforms. However, significant inhibitory activity was observed against Src family kinases. Due to their role in T cell activation, the inhibition of certain Src family kinases such as Lck and Fyn may actually be beneficial in a PKCθ targeting therapeutic.[19] However, inhibition of other Src family kinases such as Lyn may lead to impaired B-cell response.[20] The in vitro selectivity necessary for safe clinical use is unknown at this time.

In spite of the promising overall profile of compound **8**, its poor aqueous solubility placed great limitations on our ability to obtain meaningful pharmacokinetic (PK) results (data not shown). Additional work will be reported at a later time regarding efforts to improve upon the PK profile and Src family selectivity of this family of compounds.



Scheme 4. (a) DMF–DMA, 100° C; (b) *tert*-butyl cyanoacetate, *t*-BuOH, rt; (c) PhOPh, reflux, 2 h; (d) $(CF_3CO_2)_2PhI$, I_2, $CHCl_3$; (e) $POCl_3$, 100 °C; (f) 5-amino-4-methylindole, EtOH, reflux; (g) *t*-Bu acrylate, $P(OMe)_3$, $Pd(OAc)_2$, DMF, Et_3N, 80 °C, 2 h; (h) 5% TFA/DCM; (i) pyrrolidine, EDCI, DMF.

Table 3
Kinase selectivity panel for compound **8**

Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
PKCθ (target)	3.8	MK2	>50,000
PKCα	>50,000	VEGFR2	>50,000
PKCβ	>100,000	ERK2	>50,000
PKCδ	1300	P38α	>50,000
PKCε	3000	IKKα	>50,000
PKCη	51,000	IKKβ	>50,000
PKCζ	>100,000	MET	>50,000
RSK1	>50,000	PDGFRα	>50,000
Aurora B	>50,000	ITK	>20,000
CDK1/cyclin B	>50,000	SRC	330
CHK1	>50,000	FYN	25
PKA	>50,000	HCK	26
ROCK1	>50,000	LCK	46
CK1γ1	>50,000	LYN	28

In summary, we have described the synthesis and SAR of a series of 2-alkenylthieno[2,3-*b*]pyridines as PKCθ inhibitors. Incorporation of the alkenylamide moiety resulted in the identification of several low nM compounds with >100-fold selectivity over the closely related PKCδ isoform. One of these compounds (**8**) was more fully characterized biologically and found to have good selectivity over other PKC isoforms and a reasonable pharmaceutical profile. Compound **8** was shown to potently inhibit the activation of wild-type murine T cells. Comparison of this activity with that against T cells lacking PKCθ strongly suggests that this compound is exerting its activity by blockade of the PKCθ pathway.

Acknowledgments

We thank the Wyeth Chemical Technologies department for spectroscopic characterization and pharmaceutical profiling, the Discovery Synthetic Chemistry team for scaleup of 4-methyl-5-aminoindole, Agnes Brennan for cellular testing, the kinase profiling team for selectivity testing, and Drs. Tarek Mansour and Janis Upeslacis for their support.

References and notes

1. Thebault, S. *Prog. Immunol. Res.* **2005**, 1.
2. Sedwick, C. E.; Altman, A. *Mol. Immunol.* **2004**, *41*, 675.
3. Hayashi, K.; Altman, A. *Pharmacol. Res.* **2007**, *55*, 537.
4. Healy, A. M.; Izmailova, E.; Fitzgerald, M.; Walker, R.; Hattersley, M.; Silva, M.; Siebert, E.; Terkelsen, J.; Picarella, D.; Pickard, M. D.; LeClair, B.; Chandra, S.; Jaffee, B. *J. Immunol.* **2006**, *177*, 1886.
5. Salek-Ardakani, S.; So, T.; Halteman, B. S.; Altman, A.; Croft, M. *J. Immunol.* **2005**, *175*, 7635.
6. Tan, S.-L.; Zhao, J.; Bi, C.; Chen, X. Y. C.; Hepburn, D. L.; Wang, J.; Sedgwick, J. D.; Chintalacharuvu, S. R.; Na, S. *J. Immunol.* **2006**, *176*, 2872.
7. Marsland, B. J.; Soos, T. J.; Spaeth, G.; Littman, D. R.; Kopf, M. *J. Exp. Med.* **2004**, *200*, 181.
8. Salek-Ardakani, S.; So, T.; Halteman, B. S.; Altman, A.; Croft, M. *J. Immunol.* **2004**, *173*, 6440.
9. Berg-Brown, N. N.; Gronski, M. A.; Jones, R. G.; Elford, A. R.; Deenick, E. K.; Odermatt, B.; Littman, D. R.; Ohashi, P. S. *J. Exp. Med.* **2004**, *199*, 743.
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. Prien, O.; Bader, B.; Zuegel, U.; Ince, S. J.; Huwe, C.; Schuck, K.; Eis, K.; Luecking, U.; Jautelat, R.; Guenther, J.; Husemann, M. WO 2007025540.
12. Cole, D. C.; Asselin, M.; Brennan, A.; Czerwinski, R.; Ellingboe, J. W.; Fitz, L.; Greco, R.; Huang, X.; Joseph-McCarthy, D.; Kirisits, M.; Lee, J.; Li, Y.; Morgan, P.; Stock, J. R.; Tsao, D. H. H.; Wissner, A.; Yang, X.; Chaudhary, D. *J. Med. Chem.*, in press.
13. 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.
14. Boschelli, D. H.; Cole, D. C.; Asselin, M.; Barrios Sosa, A. C.; Wu, B.; Tumey, L. N. WO2007038519.
15. Boschelli, D. H.; Wu, B.; Barrios Sosa, A. C.; Durutlic, H.; Ye, F.; Raifeld, Y.; Golas, J. M.; Boschelli, F. *J. Med. Chem.* **2004**, *47*, 6666.
16. Mecklenbrauker, I.; Saijo, K.; Zheng, N. Y.; Leitges, M. *Nature* **2002**, 860.
17. 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.
18. Kwok, T. J.; Virgilio, J. A. *Org. Process Res. Dev.* **2005**, *9*, 694.
19. Palacios, E. H.; Weiss, A. *Oncogene* **2004**, *23*, 7990.
20. Nishizumi, H.; Taniuchi, I.; Yamanashi, Y.; Kitamura, D.; Ilic, D.; Mori, S.; Watanabe, T.; Yamamoto, T. *Immunity* **1995**, *3*, 549.