Bioorganic & Medicinal Chemistry Letters 19 (2009) 766–769

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Second generation 4-(4-methyl-1*H*-indol-5-ylamino)-2-phenylthieno[2,3-*b*]pyridine-5-carbonitrile PKCθ inhibitors

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ARTICLE INFO

Article history:
Received 3 November 2008
Revised 4 December 2008
Accepted 4 December 2008
Available online 10 December 2008

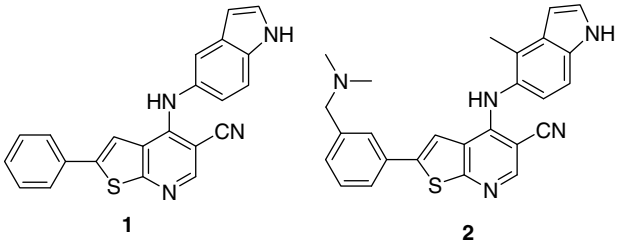
Keywords:
PKCθ
Thieno[2,3-*b*]pyridine-5-carbonitrile

ABSTRACT

Thieno[2,3-*b*]pyridine-5-carbonitrile **16** with a 4-methyl-5-indolylamine at C-4 and a 5-methoxy-2-(dimethylamino)-methylphenyl group at C-2 had an IC_{50} value of 16 nM for the inhibition of PKCθ. While moderate inhibition of PKCδ was also observed (IC_{50} = 130 nM), **16** had IC_{50} values of greater than 5 μM against Lyn and other members of the Src kinase family.

The members of the protein kinase C (PKC) family of serine threonine kinases have high sequence and structural homology but vary in both their tissue expression and activation requirements.[1] PKCθ, δ, ε, and η make up the subclass of novel isoforms, whose biochemical regulation requires calcium. The classic isoforms, α, β, and γ, require both calcium and diacylglycerol, while the atypical isoforms, ζ and λ, do not require either. PKCθ is important in the activation and survival of T cells[2,3] and inhibition of this kinase could be of therapeutic benefit in a variety of disease states including multiple sclerosis,[4,5] arthritis,[6] asthma,[7,8] inflammatory bowel disease,[9] and prevention of allograft rejection.[10] While three inhibitors of the classical PKCs, midostaurin,[11] enzastaurin,[12] and ruboxistaurin,[13] along with sotrastaurin, a PKCθ inhibitor that also inhibits the α and β isoforms,[14,15] are currently in clinical trials, no selective inhibitor of PKCθ has advanced to the clinic.

Small molecule inhibitors of PKCθ include 2,4-diaminopyrimidines,[16] 3-pyridinecarbonitriles,[17] and thieno[2,3-*b*]pyridine-5-carbonitriles[18,19] exemplified by **1**. We previously reported that structural modification of **1** by addition of a methyl group to C-4 of the indole ring and of a *meta* (dimethylamino)methyl group to the C-2 phenyl ring dramatically increased the PKCθ activity with **2** having an IC_{50} value of 7.5 nM, compared to an IC_{50} value of 460 nM for the parent compound.[18] Unfortunately, **2** was less than 4-fold selective for PKCθ over PKCδ, and also inhibited members of the Src family of kinases having IC_{50} values of 520 and 28 nM for the inhibition of Lyn and Lck, respectively. Inhibition of either PKCδ[20,21] or Lyn[22,23] is undesirable due to the role of these kinases in B-cell hyperresponsiveness.



In efforts to retain the potent activity against PKCθ and also reduce the off-target activity, we focused on varying the substituent at the C-2 position of **2**. The *para* and *ortho* isomers of **2**, namely **4** and **5**, were prepared by Suzuki reaction of **3** with the corresponding commercially available boronic acid or pinacol ester (Scheme 1). The C-2 thiophene analog **6** was obtained by first coupling **3** with 5-formyl-3-thiopheneboronic acid, followed by reductive amination with dimethylamine. A similar route was used to prepare the C-2 pyridine analog **7**. As shown in Table 1, **4**, the *para* isomer of **2**, lost activity against PKCθ and PKCδ, but had increased activity against Lyn. The *ortho* isomer **5** had greatly decreased activity against all three kinases, especially Lyn. The C-2 thiophene analog **6** had a similar kinase activity profile to that of **2**, while the C-2 pyridine analog **7** had a 10-fold decrease in PKCθ activity and also lost activity against PKCδ and Lyn.

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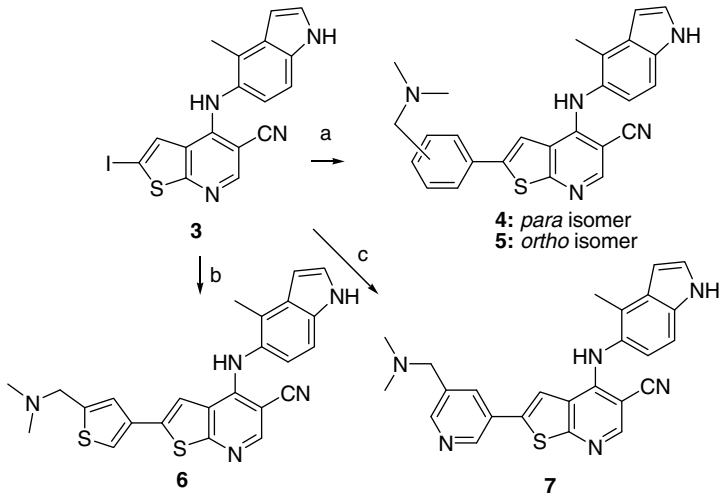
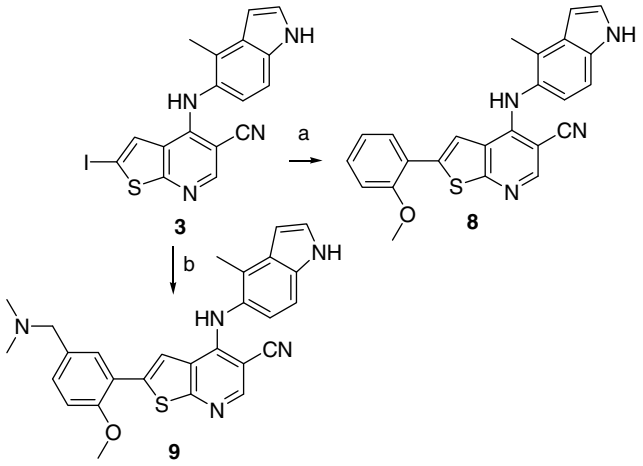
doi:10.1016/j.bmcl.2008.12.021



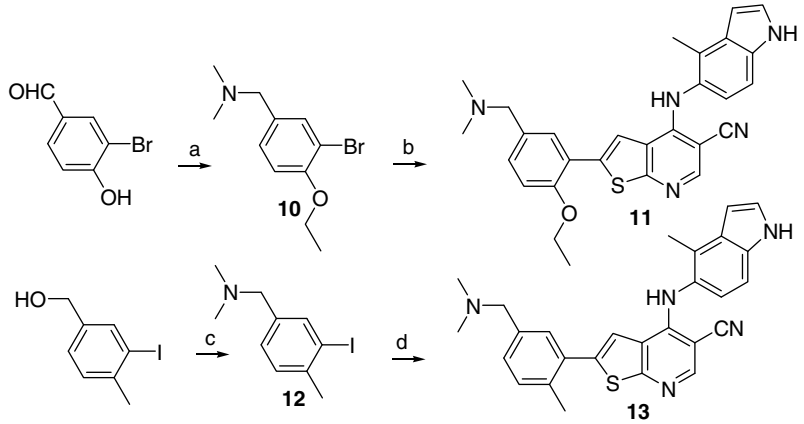
Scheme 1. Reagents: (a) for **4**: 4-(*N*,*N*-dimethylaminomethyl)phenyl-boronic acid pinacol ester hydrochloride, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$, for **5**: 2-(*N*,*N*-dimethylaminomethyl)phenylboronic acid, $(dppf)_2PdCl_2$-CH_2Cl_2, DME, aq $NaHCO_3$; (b) i—5-formyl-3-thiopheneboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$, ii—2 M Me_2NH in THF, $Na(OAc)_3BH$, CH_2Cl_2, DMF, HOAc; (c) i—5-formylpyridine-3-boronic acid pinacol ester $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$, ii—2 M Me_2NH in THF, $Na(OAc)_3BH$, CH_2Cl_2, DMF, HOAc.



Scheme 2. Reagents: (a) 2-methoxyphenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$,; (b) i—5-formyl-2-methoxyphenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$, ii—2 M Me_2NH in THF, $Na(OAc)_3BH$, CH_2Cl_2, DMF, HOAc.

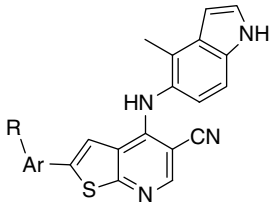
The C-2 phenyl group was retained for further SAR studies. Several mono substituted phenyl derivatives were prepared including the 2-methoxy analog **8** (Scheme 2) which was 10-fold selective for PKCθ over both PKCδ and Lyn. Combination of the C-2 phenyl substituents of **2** and **8** led to the C-2 2-methoxy, 5-(dimethylamino)methylphenyl analog **9**. Both **9** and **2** had similar activities against PKCθ and PKCδ, but **9** was a more potent inhibitor of both Lyn, and Lck having IC_{50} values of 23 and 6.0 nM, respectively.

To determine if the kinase profile of **9** could be enhanced by replacing the 2-methoxy group with other substituents, the corresponding 2-ethoxy and methyl analogs were prepared as shown in Scheme 3. Alkylation of 3-bromo-4-hydroxybenzaldehyde with ethyl iodine, followed by reductive amination with dimethylamine provided **10**. Bromide **10** was converted in situ to the boronic acid with triisopropoxyborane and *n*-butyl lithium, and then coupled with **3** under standard Suzuki conditions to provide **11**. Treatment of 3-iodo-4-methylbenzyl alcohol with *p*-toluenesulfonyl chloride followed by addition of dimethylamine provided **12**. Using the conditions employed for the preparation of **11** from aryl bromide **10**, the aryl iodine **12** was used to obtain **13**. As shown in Table 1, while **11**, the 2-ethoxy analog of **9**, retained activity against PKCθ, it exhibited a 3-fold decrease in activity against PKCδ and Lyn. De-

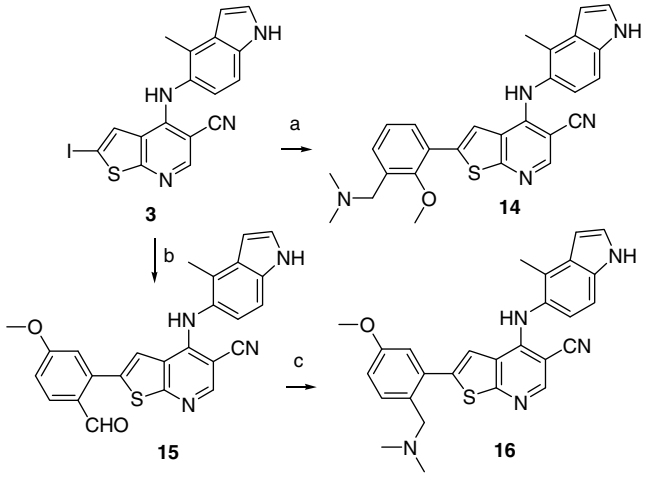


Scheme 3. Reagents: (a) i—K_2CO_3, EtI, acetone, ii—2 M Me_2NH in THF, $Na(OAc)_3BH$, CH_2Cl_2, HOAc; (b) i—$(^iPrO)_3B$, *n*-BuLi, THF, ii—**3**, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$; (c) i—TsCl, Et_3N, THF, ii—2 M Me_2NH in THF; (d) i—$(^iPrO)_3B$, *n*-BuLi, THF, ii—**3**, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$.

Table 1
PKCθ, PKCδ and Lyn inhibitory activity[24]



	Ar	R	PKCθ IC_{50} (nM)	PKCδ IC_{50} (nM)	Lyn IC_{50} (nM)
1[18]	Phenyl	H	52	370	3400
2[18]	Phenyl	3-CH_2-NMe_2	7.5	26	520
4	Phenyl	4-CH_2-NMe_2	14	88	100
5	Phenyl	2-CH_2-NMe_2	93	380	37,000
6	3-Thiophene	5-CH_2-NMe_2	16	51	410
7	3-Pyridine	5-CH_2-NMe_2	84	170	2200
8	Phenyl	2-OMe	23	250	310
9	Phenyl	2-OMe, 5-CH_2-NMe_2	8.7	24	23
11	Phenyl	2-OEt, 5-CH_2-NMe_2	9.2	82	74
13	Phenyl	2-Me, 5-CH_2-NMe_2	100	410	230
14	Phenyl	2-OMe, 3-CH_2-NMe_2	12	27	1700
16	Phenyl	5-OMe, 2-CH_2-NMe_2	16	130	13,000



Scheme 4. Reagents: (a) i—3-formyl-ii-methoxyphenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$, ii—2 M Me_2NH in THF, $Na(OAc)_3BH$, CH_2Cl_2, DMF, HOAc; (b) 2-formyl-5-methoxyphenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$; (c) 2 M Me_2NH in THF, $Na(OAc)_3BH$, CH_2Cl_2, DMF, HOAc.

creased activity, at least 10-fold against all three kinases, was seen with the 2-methyl analog **13**.

While we initially combined the groups on the C-2 phenyl ring of **2** and **8** to give the substitution pattern present in **9**, an isomer, namely **14**, also possesses the substituents present in **2** and **8**. As shown in Scheme 4, reaction of **3** with 3-formyl-2-methoxyphenylboronic acid followed by reductive amination with dimethylamine provided **14**. Compound **14** was a 12 nM inhibitor of PKCθ with only 2-fold selectivity over PKCδ, but with greater than 100-fold selectivity over Lyn. Intrigued by the reduced Lyn activity of **14**, we investigated other analogs of **5**, which was the weakest Lyn inhibitor in this series having an IC_{50} value of 37 μM. The initial target was **16**, which retains the *ortho*-(dimethylamino)methylphenyl group of **5**. Coupling of 2-formyl-5-methoxyphenylboronic acid with **3** provided aldehyde **15** with subsequent reductive amination with dimethylamine resulting in **16**. As hoped, **16** only weakly inhibited Lyn (IC_{50} = 13 μM) and in addition had an IC_{50} value of 16 nM for the inhibition of PKCθ with 8-fold selectivity over PKCδ.

When **16** was tested against other PKC family members while only weak inhibition of PKCβ, a classic isoform (IC_{50} = 22 μM) was observed, more potent inhibition of PKCη and PKCε was seen, with **16** having IC_{50} values against these two novel PKCs of 360 and 95 nM, respectively. Kinase profiling of **16** provided an IC_{50} value of 38 μM for Lck, and IC_{50}s of greater than 5 μM against other members of the Src kinase family, including Src, Hck and Fyn, and also VEGFR, PDGFR, MK2, and p38. While **16** had good permeability (1.04×10^{-6} cm/s as measured in a PAMPA assay) and acceptable solubility at pH 7.4 (12 μg/mL), this compound had very poor stability in mouse, rat, and human liver microsomes (½ lives of less than 10 min). Therefore, while we were able to eliminate the Lyn liability of this series, further structural modification is currently underway to reduce the PKCδ activity and increase the metabolic stability of these compounds.

Acknowledgments

We thank the Wyeth Chemical Technologies Department for compound characterization and the pharmaceutical profiling results, the Wyeth Screening Sciences group for the kinase selectivity panel results, and Drs. Janis Upeslacis and Tarek Mansour for their support.

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