Bioorganic & Medicinal Chemistry Letters 19 (2009) 2461–2463

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and PKCθ inhibitory activity of a series of 4-indolylamino-5-phenyl-3-pyridinecarbonitriles

Russell G. Dushin [a,*], Thomas Nittoli [a], Charles Ingalls [a], Diane H. Boschelli [a], Derek C. Cole [a], Allan Wissner [a], Julie Lee [b], Xiaoke Yang [b], Paul Morgan [b], Agnes Brennan [b], Divya Chaudhary [b]

[a] Wyeth Research, Chemical Sciences, 401 N. Middletown Road, Pearl River, NY 10965, USA
[b] Wyeth Research, Inflammation, 200 Cambridge Park Drive, Cambridge, MA 02140, USA

ARTICLE INFO

Article history:
Received 17 February 2009
Revised 11 March 2009
Accepted 12 March 2009
Available online 18 March 2009

Keywords:
PKC
4-Amino-3-pyridinecarbonitrile
Inflammation
Kinase

ABSTRACT

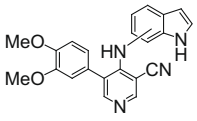
A series of 4-indolylamino-5-phenyl-3-pyridinecarbonitrile inhibitors of PKCθ were synthesized as potential anti-inflammatory agents. The effects of specific substitution on the 5-phenyl moiety and variations of the positional isomers of the 4-indolylamino substituent were explored. This study led to the discovery of compound 12d, which had an IC_{50} value of 18 nM for the inhibition of PKCθ.

© 2009 Elsevier Ltd. All rights reserved.

The protein kinase Cs (PKCs) comprise a group of serine-threonine kinases that share sequence and structural homology but differ in their activation requirements and tissue distribution.[1] Conventional PKCs (α, β, and γ) are regulated by calcium and diacyl glycerol (DAG), while novel isoforms (δ, ε, η, and θ) are regulated by DAG, and atypical isoforms (ζ and λ) are insensitive to either calcium or DAG.

PKCθ, a novel isoform, is primarily expressed in T-cells and thymocytes, and plays an important role in the activation and survival of T-cells by mediating T-cell receptor (TCR) signalling.[2,3] PKCθ-deficient or knocked out (KO) mice have demonstrated significantly reduced responses in T-cell mediated disease models of arthritis,[4] multiple sclerosis,[5,6] inflammatory bowel disease,[7] and asthma,[8,9] thus indicating the potential value of a PKCθ inhibitor for the treatment of these diseases.

Recently, a number of small molecules have been reported to be ATP-competitive inhibitors of PKCθ, including 2,4-diaminopyrimidines,[10] 4-arylamino-pyridine-3-carbonitriles[11] (e.g., **1a–b**, Fig. 1), and related 4-(indol-5-ylamino)thieno[2,3-*b*]pyridine-5-carbonitriles[12–14] Herein we report on the synthesis and biological activities of an expanded series of 4-indolylamino-5-phenyl-3-pyridinecarbonitriles in which we have varied the alkoxy substituents on the phenyl ring in the context of three isomeric aminoindolyl 'headpieces'.



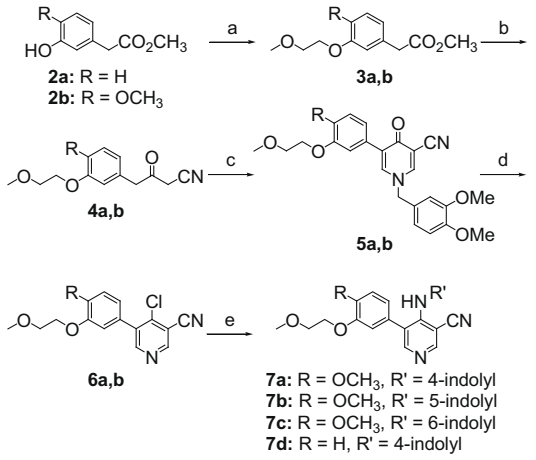
1a: 5-indolyl
1b: 6-indolyl
1c: 4-indolyl

Figure 1. Structures of compounds **1a–c**.

Compound **1c** was prepared according to the procedures described for **1a** and **1b**,[11] however efficient syntheses of our initially targeted molecules (**7a–d**) required slight modification of our previously reported route (Scheme 1). Thus, alkylation of the free hydroxyl groups of substituted phenylacetic acid esters **2a,b** with 2-bromoethyl methyl ether provided substituted phenylacetic acid esters **3a,b**, which were uneventfully converted to the corresponding phenyl-3-oxo-butyro-nitriles **4a,b** by cryogenic treatment with the lithium anion of acetonitrile. Conversions of **4a,b** to the respective pyridines by stepwise treatment with DMF–DMA followed by ammonium acetate, as performed previously,[11] only resulted in low yields of relatively impure products. This was attributed in part to the increased solubility of the intermediates and final products that in prior instances were collected from the reaction medium simply by filtration. As a reliable alternative to this procedure, oxo-butyro-nitriles **4a,b** were treated with DMF–DMA as before, but then the resulting crude bis-enamides were reacted with veratrylamine in refluxing toluene, thus producing the N-alkylated

* Corresponding author. Tel.: +1 845 602 3430; fax: +1 845 602 5561.
E-mail address: dushinr@wyeth.com (R.G. Dushin).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.053



Scheme 1. Reagents and conditions: (a) $CH_3OCH_2CH_2Br$, Cs_2CO_3, $Bu_4N^+I^-$, acetone; (b) CH_3CN, *n*-BuLi, −78 °C, THF; (c) (1) DMF–DMA, reflux, DMF; (2) Veratrylamine, toluene, reflux; (d) $POCl_3$, LiCl, reflux; (e) 4-, 5-, or 6-aminoindole, $Pd_2(dba)_3$, DAVEPHOS[15], K_3PO_4, DME, reflux.

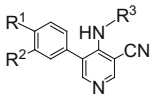
pyridones **5a,b**, which were isolated and purified by silica gel chromatography. Treatment of **5a,b** with phosphorous oxychloride in the presence of lithium chloride gave the 4-chloropyridines **6a,b** directly and without the need for a separate nitrogen-deprotection step. Subsequent couplings of 4-, 5-, or 6-aminoindole with 4-chloropyridines **6a,b** were accomplished under mild conditions by employing a palladium catalyzed aryl amination to give the targeted compounds **7a–d**.

PKCθ inhibitory activities for compounds **1c** and **7a–c** revealed that the 4-indolylamino-analogs **1c** and **7a** were considerably more potent (roughly five-fold) than the corresponding 5-indolylamino compounds, **1a** and **7b**, which were in turn more potent (roughly 20- to 30-fold) than the corresponding 6-indolylamino-compounds, **1b** and **7c** (Table 1). It is noteworthy that this general trend was observed previously in the related 4-(indolylamino)thieno[2,3-*b*]pyridine-5-carbonitriles.[12] Additionally, within each isomeric indolylamino series, replacement of the *meta*-methoxy substituents on the 5-phenyl moiety with *meta*-methoxyethoxy substituents uniformly resulted in a three- to five-fold loss of activity (cf. IC_{50} values of compounds **1c** and **7a**, **1a** and **7b**, **1b** and **7c**). However, while **7a** was roughly five-fold less potent as a PKCθ inhibitor than **1c,** compound **7d**, in which the *para*-methoxy substituent in **7a** was removed, was only 2.5-fold less potent than compound **1c** (IC_{50} values of 13, 63, and 32 nM for **1c**, **7a**, and **7d**, respectively). Thus, it appears that there is limited tolerance for steric bulk in the vicinity of the dimethoxy-substituents of bound **1a–c**, but that the losses in activity seen in dialkoxy-substituted compounds containing larger *meta*-substituents can be partially offset by removal of the *para*-substituent. With this observation in mind we set out to prepare analogs of **7d** that contained *meta*-aminoalkoxy substituents on the 5-phenyl moiety in the hopes that incorporation of amine functionality might confer improvements in the physicochemical properties of the series, particularly solubility, which was generally poor to moderate with **1a–c** and **7a–d** (data not shown).

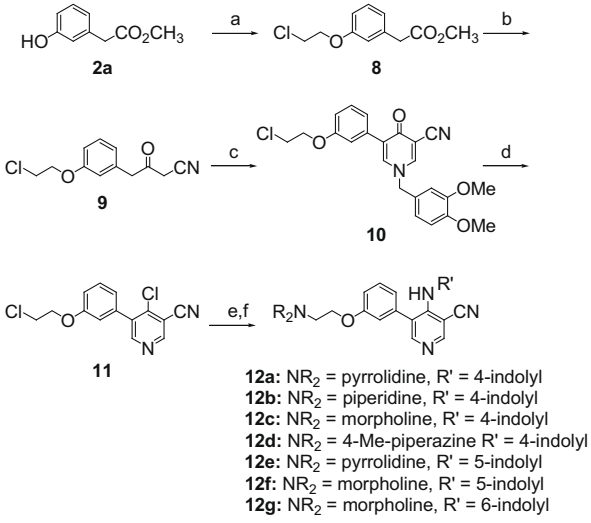
Aminoalkoxy containing derivatives **12a–g** were prepared as outlined in Scheme 2 using the same general approach used to prepare **7a–d**. Thus, alkylation of the free phenol in **2a** with 2-chloroethyl-*p*-toluenesulfonate provided methyl-[3(2-chloroethoxy) phenyl]acetate (**8**), which was converted to the substituted phenyl-3-oxo-butyro-nitrile derivative **9** by cryogenic treatment with the lithium anion of acetonitrile. Conversion to the functionalized pyridone **10** was accomplished by treatment of **9** with DMF–DMA, followed by reaction of the resulting crude bis-enamide with veratrylamine in refluxing toluene. Protected pyridone **10** was then converted to the 4-chloropyridine **11** by treatment with phosphorous oxychloride and lithium chloride. Application of Buchwald–Hartwig aminations on **11** using 4-, 5-, or 6-aminoindole gave the corresponding isomeric aminoindolyl-substituted 5-phenyl-3-pyridinecarbonitrile intermediates containing the chloroethoxy substituent on the phenyl ring. Lastly, amine displacements of the chlorine on the chloroethoxy side chains of these three intermediates by treatment with cyclic secondary amines in refluxing ethanol gave the desired derivatives **12a–g**.

As shown in Table 1, all four of the 4-indolylamino-substituted analogs of **7d** bearing aminoalkoxy functionality on the phenyl substituent (compounds **12a–d**) were strong inhibitors of PKCθ. Only compound **12b**, with a *N*-piperidinyl-2-ethoxy group on the phenyl ring, was less potent than **7d**, and even then by only a factor of two. As was observed previously, the 4-indolylamino-substituted compounds were uniformly more potent than the corresponding 5-indolylamino-substituted isomers (**12a** vs **12e**, **12c** vs

Table 1
PKCθ IC_{50} values for compounds **1a**–**c**, **7a**–**d**, and **12a**–**g**[16]



Compound	R^1	R^2	R^3	PKC-θ IC_{50} (nM)
1a	OMe	OMe	5-Indolyl	70[11]
1b	OMe	OMe	6-Indolyl	2200[11]
1c	OMe	OMe	4-Indolyl	13
7a	OMe	OCH_2CH_2OMe	4-Indolyl	63
7b	OMe	OCH_2CH_2OMe	5-Indolyl	320
7c	OMe	OCH_2CH_2OMe	6-Indolyl	6300
7d	H	OCH_2CH_2OMe	4-Indolyl	32
12a	H	OCH_2CH_2–*N*-pyrrolidine	4-Indolyl	29
12b	H	OCH_2CH_2–*N*-piperidine	4-Indolyl	60
12c	H	OCH_2CH_2–*N*-morpholine	4-Indolyl	17
12d	H	OCH_2CH_2–*N*-(4-Me-piperazine)	4-Indolyl	18
12e	H	OCH_2CH_2–*N*-pyrrolidine	5-Indolyl	190
12f	H	OCH_2CH_2–*N*-morpholine	5-Indolyl	200
12g	H	OCH_2CH_2–*N*-morpholine	6-Indolyl	9500



Scheme 2. Reagents and conditions: (a) $TsOCH_2CH_2Cl$, Cs_2CO_3, $Bu_4N^+I^-$, acetone; (b) CH_3CN, *n*-BuLi, −78 °C, THF; (c) (1) DMF–DMA, reflux, DMF (2) Veratrylamine, toluene, reflux; (d) $POCl_3$, LiCl, reflux; (e) 4-, 5-, or 6-aminoindole, $Pd_2(dba)_3$, DAVEPHOS[15], K_3PO_4, DME, reflux; (f) R_2NH, EtOH, reflux.

12f), and the 5-indolylamino-substituted compound bearing an *N*-morpholino-2-ethoxyphenyl substituent (compound **12f)** was more potent than its 6-indolylamino isomer (**12g**).

Compound **12d**, an 18 nM PKCθ inhibitor that displayed excellent aqueous solubility (>100 μg/mL at pH 7.4), was selected for further evaluation against an expanded panel of kinases from the PKC family. This compound inhibited PKCδ, PKCε, and PKCη, which all belong to the novel PKC class, with IC_{50} values of 37, 39, and 940 nM, respectively. In assays measuring inhibition of PKCβ, a conventional PKC, **12d** was a 2.0 μM inhibitor, and it had an IC_{50} value of >100 against PKCζ, an atypical PKC. Thus, although **12d** was only two-fold selective against PKCδ and PKCε, it displayed excellent selectivity against members of other classes of PKCs. Inhibitory activity against PKCδ remains a concern, however, due to the known and undesirable role of PKCδ in stimulating B-cell hyperresponsiveness in mice.[17,18]

Inhibitory properties of compound **12d** were also measured against the Src family kinases Lyn and Lck as well as against Akt. Compound **12d** was found to be inactive against Lyn and Akt at the highest concentration tested (IC_{50} values >100 μM) and was only moderately active against Lck (IC_{50} value of 24 μM).

Lastly, compound **12d** was evaluated in a functional cellular assay that measures IL-2 production from T-cells derived from wild-type (WT) and PKCθ KO mice following T-cell activation by anti-CD3 and anti-CD28.[11] Compound **12d** inhibited IL-2 production from WT cells with an IC_{50} value of 854 nM, but had a markedly reduced effect (IC_{50} value of >15 μM) on cells derived from PKCθ KO mice. This >20-fold difference in activity is consistent with a mechanism that involves PKCθ selective inhibition.

In summary, we have described the synthesis and biological evaluation of a series of 4-indolylamino-3-pyridinecarbonitriles as PKCθ inhibitors that expand upon compounds discovered in our earlier hit-to-lead studies.[11] This effort has culminated in the identification of compound **12d**, a potent inhibitor of PKCθ that displayed modest selectivity over closely related members of the PKC family of kinases, and has shown activity in a cellular assay that measures the functional activity of PKCθ. The on-going efforts to further improve the potency, selectivity, and physicochemical properties of this series of compounds will be reported in due course.

Acknowledgements

We thank the Wyeth Chemical Technologies department for compound characterizations and solubility measurements, and Drs. John Ellingboe, Janis Upeslacis, and Tarek Mansour for their support.

References and notes

1. Spitaler, M.; Cantrell, D. A. *Nat. Immunol.* **2004**, *5*, 785.
2. Baier, G.; Telford, D.; Giampa, L.; Coggeshall, K. M.; Baier-Bitterlich, G.; Isakov, N.; Altman, A. *J. Biol. Chem.* **1993**, *268*, 4997.
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. Nagahama, K.; Ogawa, A.; Shirane, K.; Shimomura, Y.; Sugimoto, K.; Mizoguchi, A. *Gastroenterology* **2008**, *134*, 459.
8. Marsland, B. J.; Soos, T. J.; Späth, G.; Littman, D. R.; Kopf, M. *J. Exp. Med.* **2004**, *200*, 181.
9. Salek-Ardakani, S.; So, T.; Halteman, B. S.; Atlman, A.; Croft, M. *J. Immunol.* **2004**, *173*, 6440.
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. Cole, D. C.; Asselin, M.; Brennan, A.; Czerwinski, R.; Ellingboe, J. W.; Fitz, L.; Greco, R.; Huang, X.; Joseph-McCarthy, D.; Kelly, M. F.; Kiristis, 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.
12. 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.
13. Tumey, L. N.; Boschelli, D. H.; Lee, J.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4420.
14. Wu, B.; Boschelli, D. H.; Lee, J.; Yang, X.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 766.
15. The IUPAC name for DAVEPHOS is 2-(2-dicyclohexylphosphanylphenyl)-*N*,*N*-dimethylaniline.
16. See Ref. 11 for assay protocols. IC_{50} values represent the mean of at least two determinations.
17. Mecklenbräuker, I.; Saijo, K.; Zheng, N.-Y.; Leitges, M.; Tarakhovsky, A. *Nature* **2002**, *416*, 860.
18. 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.