Bioorganic & Medicinal Chemistry Letters 22 (2012) 190–193

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel and selective spiroindoline-based inhibitors of sky kinase

Noel A. Powell *, Jeffrey T. Kohrt, Kevin J. Filipski, Michael Kaufman †, Derek Sheehan, Jeremy E. Edmunds ‡, Amy Delaney, Yuli Wang, Francis Bourbonais, Doh-Yeel Lee, Frank Schwende, Fang Sun, Pat McConnell, Cornel Catana, Huifen Chen, Jeff Ohren, Lisa A. Perrin

Pfizer Global Research & Development, Michigan Laboratories, Ann Arbor, MI 48105, USA

ARTICLE INFO

Article history:
Received 1 October 2011
Revised 8 November 2011
Accepted 9 November 2011
Available online 16 November 2011

Keywords:
Kinase inhibitor
Platelet aggregation
Crystal structure

ABSTRACT

We report the discovery of a novel series of spiroindoline-based inhibitors of Sky kinase that bind in the ATP-binding site and exhibit high levels of kinome selectivity through filling the Ala571-subpocket. These inhibitors exhibit moderate oral bioavailability in the rat due to low absorption across the gut wall.

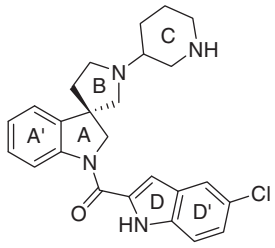
Coronary heart disease is the leading cause of mortality and morbidity in the United States.[1] There are an estimated 40 million people in the US at risk for thrombotic events due to atherosclerosis or prior history of cardiovascular disease or stroke. The current front-line treatment for arterial thrombosis is clopidogrel (Plavix®), which has significant limitations in terms of efficacy without aspirin, and is also a pro-drug that must be metabolized by liver enzymes to present a pharmacodynamic effect. About one quarter to one third of patients do not adequately metabolize clopidogrel and therefore are resistant to its effect.[2] In addition, the active drug binds irreversibly to platelets, resulting in a high risk for increased bleeding. Thus, there is a need for safer, more effective anti-platelet agents useful to a broad patient population.

Gas6 (growth arrest specific gene 6) is a member of the Vitamin K dependent family of proteins which have all been shown to have a role in thrombosis and hemostasis.[3] Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis.[4,5] Mechanistically, Gas6 appears to play a critical role as a platelet response amplifier, enhancing platelet aggregation and granule secretion to known endogenous agonists. Gas6 is a ligand for three tyrosine kinase receptors designated Sky (Tyro3/Rse), Axl, and Mer that are expressed on human and mouse platelets,[5] and induces intracellular signaling through the PI3K and Akt pathways.[6] Gas6-neutralizing antibodies inhibit platelet aggregation, without increased bleeding in mice.[5] In addition, Gas6 knockout mice do not have any significant difference in bleeding time compared to wild type animals.[4] Gas6 receptor knockout ($Sky^{-/-}$, $Axl^{-/-}$, or $Mer^{-/-}$) mice also exhibit protection against thromboembolism with normal bleeding times.[7] Previous studies from our laboratories have demonstrated that Sky specific antibodies inhibit human platelet degranulation and aggregation to the same extent as Gas6 inhibition, and result in comparable efficacy to clopidogrel treatment in a mouse model of thrombosis with no significant increase in bleeding time.[5] Thus, inhibition of Sky potentially represents the first antithrombotic therapy that is not associated with bleeding side effects.

When this project started, there were no literature reports of selective small-molecule Sky inhibitors. Since then, several reports of pan-inhibitors of the broad HGFR superfamily (Sky, Mer, Axl, HGFR/Met) have emerged.[8] To find chemical leads for a selective inhibitor of Sky kinase, a UHT screen of the Pfizer compound library was performed. We identified **1** as a structurally interesting lead with low micromolar activity (IC_{50} = 1.04 μM). As a literature review failed to identify any reports of spiroindoline-2-carboxyindoles with reported kinase activity, we chose to pursue the spiroindoline template for further SAR studies due to the potential for selectivity against other members of the HGFR family and the broader kinome.

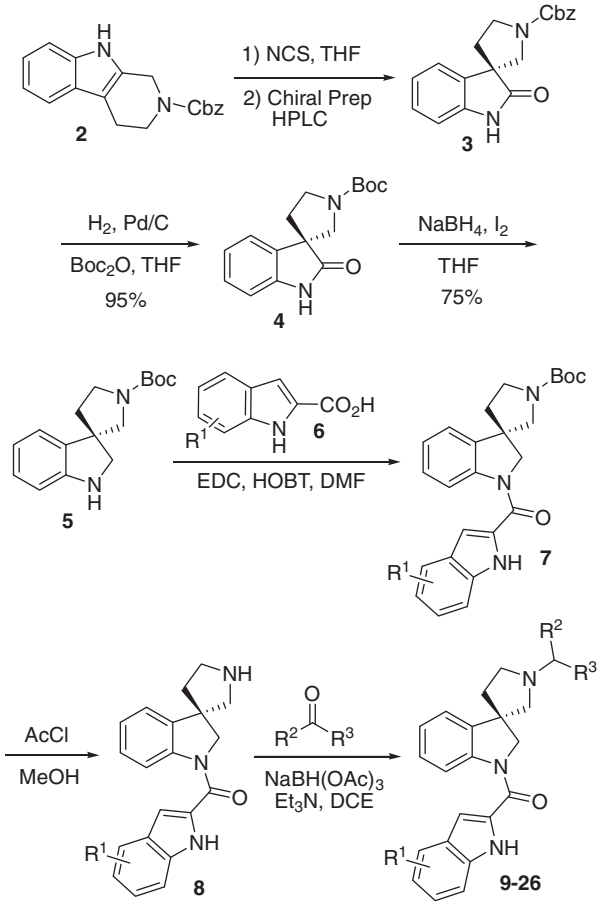
* Corresponding author at present address: Proteostasis Therapeutics, 200 Technology Square, Suite 402, Cambridge, MA 02139, USA. Tel.: +1 617 225 7837x2142.
E-mail address: napowell@comcast.net (N.A. Powell).
† Present address: Deciphera Pharmaceuticals, LLC, 643 Massachusetts, Suite 200, Lawrence, KS 66044, USA.
‡ Present address: Abbott Labs, 381 Plantation Street, Worchester, MA 01605, USA.

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.11.036



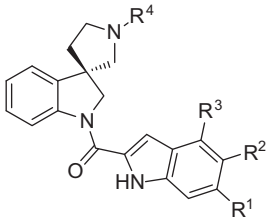
1
Sky IC_{50} = 1.04 μM

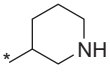
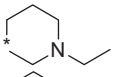
Analogs were prepared as described in Scheme 1. Treatment of the Cbz-protected 3,4-dihydro-1*H*-pyrido[3,4-*b*]indole **2** with NCS lead to rearrangement to the racemic Cbz-protected 2-oxo-1,2-dihydro-1*H*-spiro[indole-3,3′pyrrolidine] **3**.[9] Separation of the enantiomers by chiral preparatory HPLC afforded the *S* and *R* enantiomers in high chiral purity (*R* enantiomer not shown). Hydrogenation over Pd/C in the presence of Boc_2O enabled exchange of the Cbz protecting group for a Boc group to afford **4** in high yield. Reduction of the lactam bond afforded the Boc-protected 1,2-dihydro-1*H*-spiro[indole-3,3′pyrrolidine] **5**, which could be coupled with 2-indolecarboxylic acids under standard conditions to afford **7**. Deprotection of the Boc group under mild acidic conditions afforded **8**, which underwent smooth reductive amination with a variety of aldehydes and ketones to afford analogs **9**–**26**, which were isolated as mixtures of diastereomers at the C ring stereocenter.



Scheme 1. Synthesis of spiroindoline-2-carboxyindole analogs.

Table 1
SAR of spiroindoline series



ID	R^1	R^2	R^3	R^4	Sky IC_{50}[a] (μM)
1	H	Cl	H		1.04
9	H	Cl	H		0.495
10	H	Cl	H		8.66
11	H	F	H		0.476
12	H	F	H		1.81
13	H	F	H		2.88
14	H	F	H		4.41
15	H	F	H		4.93
16	H	F	H		27.9
17	H	F	H		37.8
18	H	CH_3	H		0.811
19	H	OCH_3	H		0.501
20	OCH_3	H	H		0.843
21	$C(O)NH_3$	H	H		2.20
22	CN	H	H		1.13
23	H	H	Cl		0.560
24	F	H	H		0.598
25	H	H	H		0.246
26	F	H	H		0.189

* Point of attachment.
[a] IC_{50} values obtained in duplicate using an ELISA format assay.[10]

We initially investigated the SAR around the C ring (Table 1). All analogs were made with the *S* configuration at the spiroindoline center as the *R* enantiomer was >10-fold less active against Sky (data not shown). The C ring proved to be very sensitive to manipulation. Contraction of the piperidine ring to a pyrrolidine ring resulted in a two-fold improvement in potency (**9**, IC_{50} = 0.495 μM). Moving the N atom in the C ring to

the 4-position to remove the chiral center resulted in an eight-fold loss of potency (**10**, IC_{50} = 8.66 μM). Replacing the 5-Cl atom on the indole ring with a F atom resulted in a two-fold potency improvement (**11**, IC_{50} = 0.476 μM), subsequently further analogs were made with the 5-F indole substituent. The pyrrolidine C ring size proved optimal, as a four-membered ring (**12**, IC_{50} = 1.81 μM) or an *N*-methyl-4-piperidine ring (**13**, IC_{50} = 2.88 μM) were less active. A 1-carbon extension of the 3-piperidine ring also resulted in a loss of activity (**14**, IC_{50} = 4.41 μM). The secondary NH of the 3-piperidine ring was also vital for activity, as alkylation with an ethyl group (**15**, IC_{50} = 4.93 μM), or replacement of the piperidine ring with a cyclohexyl ring (**16**, IC_{50} = 27.9 μM) resulted in a loss of activity. In addition, aromatic replacements were also not tolerated (**17**, IC_{50} = 37.8 μM).

Having determined that a 3-piperidine or 3-pyrrolidine C ring was vital for Sky inhibition activity, we turned to explore the SAR of the indole D/D′ rings. Electron donating methyl or methoxy groups at the R^2 position resulted in similar potency to the electron withdrawing Cl and F substituents (**18**, IC_{50} = 0.811 μM and **19**, IC_{50} = 0.501 μM, respectively). An electron donating methoxy at the R^1 position had a negligible affect on Sky inhibition activity (**20**, IC_{50} = 0.843 μM), while electron withdrawing primary carboxyamide and cyano groups had a deleterious affect on activity (**21**, IC_{50} = 2.20 μM and **22**, IC_{50} = 1.13 μM). Moving the halogen atom to the R^1 or R^4 position gave similar inhibitory activity to **9** (**23** and **24**, IC_{50} = 0.56 and 0.598 μM, respectively). Surprisingly, the largest boost in potency was observed with the addition of a methyl group at the α-N position in the 3-piperidine C ring (**25**, IC_{50} = 0.246 μM and **26**, IC_{50} = 0.189 μM). All of the compounds in the spiroindoline series exhibited limited activity in the functional platelet aggregation assays (**9** and **26** inhibited platelet aggregation with IC_{50} = 13 and 20.7 μM, respectively).

Compounds **9** and **24** in the spiroindoline-series exhibited modest in vitro ADME data (Table 2). As expected from the presence of two basic nitrogen atoms, both **9** and **24** possessed low *c*log*P* values. As a consequence, **9** exhibited low cellular permeability in an artificial membrane PAMPA assay. Both **9** and **24** exhibited modest aqueous solubility and excellent microsomal stability. However, both were also potent inhibitors of CYP2D6. Modest oral bioavailability in rats was also observed for **9** and **24** (Table 3). As modest plasma concentrations were observed following oral dose, the oral bioavailability of **9** (*F* = 14%) was presumably limited by absorption across the gut wall and first pass metabolism. Interestingly, moving the halogen atom by one position on the indole ring in **24** resulted in improved clearance (Cl = 5 mL/min/kg) and lower volume of distribution. However, the oral bioavailability of **24** (*F* = 11%) was still presumably limited by absorption across the gut wall.

The structural novelty of the spiroindoline-2-carboxyindole template as a kinase inhibitor suggested the possibility of a novel binding mode. However, **1** was shown to be an ATP-competitive inhibitor. Docking studies suggested several possible binding modes in the ATP active site of Sky, which were resolved by obtaining a 2 Å X-ray crystal structure of **11** bound in the ATP active site of the murine Sky kinase domaine (Fig. 1).[11] Analysis of the X-ray data indicated that the spiroindoline core was oriented in the ATP site with the phenyl A′ ring ~4 Å from the methyl sidechain of the Ala571 residue that lies beneath the Leu593 gatekeeper residue. The N of the pyrrolidine B ring formed a salt bridge with the Asp663 carboxylic acid sidechain, while the N of the piperidine C ring formed a similar charged interaction with the backbone carbonyl of Arg649. The 2-carboxyindole D/D′ ring bound in a *syn*-conformation and made HBD and HBA interactions with the Met596 residue in the hinge region.

Table 2
In vitro ADME profile of selected compounds[a]

Analog	*c*log*P*	Solubility[b] (μM)	Permeability ($\times 10^{-6}$ cm/s)	HLM $t_{1/2}$ (min)	CYP2D6 IC_{50}[c] (μM)
9	2.92	5.2	0.03[d]	60	0.014
24	2.35	7.8	NT	52	0.655
26	3.58	11.7	5.13[e]	44	94% @ 3 μM

[a] Values are means of duplicate experiments, (NT = not tested).
[b] Aqueous solubility measured at pH 6.5.
[c] CYP2D6 substrate = dextromethorphan.
[d] PAMPA artificial membrane assay.
[e] Caco-2 permeability assay.

Table 3
In vivo rat PK profile of selected compounds[a]

Analog	$t_{1/2}$ (h)	Cl (mL/min/kg)	Vd_{ss} (L/kg)	*F* (%)
9	3.1	60	13	14
24	4.0	5	1.6	11

[a] Values are means of duplicate experiments. Sprague-Dawley rats were dosed as a suspension in 5% PEG-200/95% (0.5% w:w) methyl-cellulose.

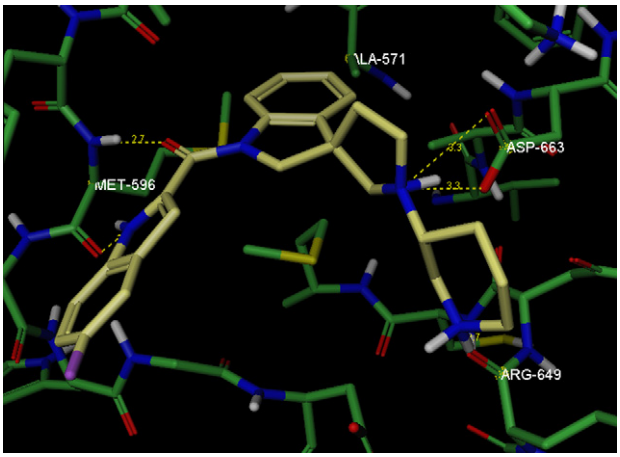
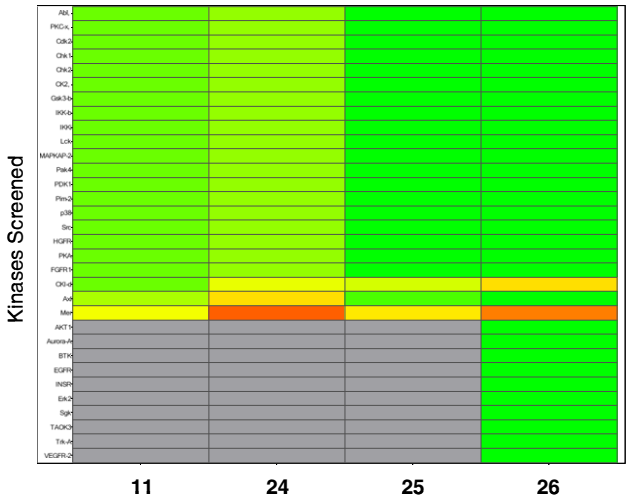


Figure 1. 2 Å X-ray crystal structure of **11** (yellow stick) bound in the ATP active site of murine Sky kinase domain (pdb ID code: 3QUP).[11] Dotted lines denote hydrogen bonds formed with protein residues.

The Ala571 residue presents a unique subpocket in the Sky ATP-binding site, as an analysis of the amino acid sequences of 140 human protein tyrosine kinases indicates that all of the other kinases possess a sterically larger amino acid sidechain at this position. Consequently, compounds that bind in the ATP-binding site of Sky and occupy the Ala571-subpocket were hypothesized to demonstrate kinase selectivity. The spiroindoline series confirmed this hypothesis and demonstrated excellent selectivity for Sky versus 20–30 other kinases in an in vitro screening panel designed to broadly represent the kinome (Table 4).[12] Compounds **11**, **24**, and **25** were essentially inactive in 19 kinases with modest inhibition activity in CKI-δ (18–63-fold). Compound **26** was inactive in an additional 10 kinases with slightly increased CKI-δ activity (nine-fold). Sky is a member of the HGFR family of tyrosine kinases, which consists of HGFR/Met, Sky, Axl, and Mer. All of the compounds tested displayed no activity against HGFR, very modest inhibition of Axl, and 4- to 14-fold selectivity against Mer.

In summary, we have discovered a novel series of Sky inhibitors based on a spiroindoline-2-carboxyindole template. These inhibitors display modest Sky potency with only slight modifications in the C and D/D′ rings tolerated. Addition of hydrophobic groups in the C ring afforded the most potent compounds. This series exhibited poor inhibitory activity in functional platelet aggregation

Table 4
Heat map of kinase fold-selectivity for selected compounds[a]



[a] Fold selectivity (kinase IC_{50}/Sky IC_{50}) colored by green = >100-fold, yellow = 10–100-fold, red = <10-fold.

assays, possibly due to high plasma protein binding or the low cellular permeability. While good microsomal stability was observed in in vitro assays, all compounds exhibited potent CYP2D6 inhibition. The moderate oral bioavailability in the rat of **9** and **24** appeared to be largely limited by absorption across the gut wall and/or high in vivo clearance. X-ray crystallography revealed a unique binding mode in the ATP-binding pocket with the central A′ phenyl ring occupying the Ala571-subpocket. Consequently, these compounds exhibited excellent selectivity for Sky against the broader kinome. Good selectivity was also observed against other members of the HGFR subfamily.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.036.

References and notes

1. American Heart Association. Heart Disease and Stroke Statistics—2007 Update.
2. Cattaneo, M. *Arterioscler. Thromb. Vasc. Biol.* **2004**, *24*, 1980.
3. Saller, F.; Burnier, L.; Schapira, M.; Angelillo-Scherrer, A. *Blood Cells Mol. Dis.* **2006**, *36*, 373.
4. Angelillo-Scherrer, A.; de Frutos, P. G.; Aparicio, C.; Melis, E.; Savi, P.; Lupu, F.; Arnout, J.; Dewerchin, M.; Hoylaerts, M. F.; Herbert, J.-M.; Collen, D.; Dahlbäck, B.; Carmeliet, P. *Nat. Med.* **2001**, *7*, 215.
5. Gould, W. R.; Baxi, S. M.; Schroeder, R.; Peng, Y. W.; Leadley, R. J.; Peterson, J. T.; Perrin, L. A. *J. Thromb. Haemost.* **2005**, *3*, 733.
6. Nagata, K.; Ohashi, K.; Takano, T.; Arita, H.; Zong, C.; Hanafusa, H.; Mizuno, K. *J. Biol. Chem.* **1996**, *271*, 30022.
7. Angelillo-Scherrer, A.; Burnier, L.; Flores, N.; Savi, P.; DeMol, M.; Schaeffer, P.; Herbert, J.-M.; Lemke, G.; Goff, S. P.; Matsushima, G. K.; Earp, H. S.; Vesin, C.; Hoylaerts, M. F.; Plaisance, S.; Collen, D.; Conway, E. M.; Wehrle-Haller, B.; Carmeliet, P. *J. Clin. Invest.* **2005**, *115*, 237.
8. Schroeder, G. M.; An, Y.; Cai, Z.-W.; Chen, X.-T.; Clark, C.; Cornelius, L. A. M.; Dai, J.; Gullo-Brown, J.; Gupta, A.; Henley, B.; Hunt, J. T.; Jeyaseelan, R.; Kamath, A.; Kim, K.; Lippy, J.; Lombardo, L. J.; Manne, V.; Oppenheimer, S.; Sack, J. S.; Schmidt, R. J.; Shen, G.; Stefanski, K.; Tokarski, J. S.; Trainor, G. L.; Wautlet, B. S.; Wei, D.; Williams, D. K.; Zhang, Y.; Zhang, Y.; Fargnoli, J.; Borzilleri, R. M. *J. Med. Chem.* **2009**, *52*, 1251. and references cited therein.
9. Kauffman, G. S.; Li, C.; Lippa, B. S.; Morris, J.; Pan, G. WO Patent Application 2006/090261.
10. IC_{50} data was generated by measuring the inhibition of the phosphorylation of Src substrate by a murine truncated Sky protein in an ELISA based assay.
11. Coordinates for the Sky/**11** complex (pdb ID code: 3QUP) have been deposited at www.rcsb.org.
12. IC_{50} data for individual kinases in the broad screening panel can be found in the Supplementary data.