Bioorganic & Medicinal Chemistry Letters 21 (2011) 6687–6692

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

7-(4*H*-1,2,4-Triazol-3-yl)benzo[*c*][2,6]naphthyridines: A novel class of Pim kinase inhibitors with potent cell antiproliferative activity

Fabrice Pierre *, Eric Stefan, Anne-Sophie Nédellec, Marie-Claire Chevrel, Collin F. Regan, Adam Siddiqui-Jain, Diwata Macalino, Nicole Streiner, Denis Drygin, Mustapha Haddach, Sean E. O'Brien, Kenna Anderes, David M. Ryckman

Cylene Pharmaceuticals, 5820 Nancy Ridge Drive, Suite 200, San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 31 August 2011
Revised 14 September 2011
Accepted 15 September 2011
Available online 22 September 2011

Keywords:
Pim
Kinase inhibitor
Cancer
Leukemia
AML

ABSTRACT

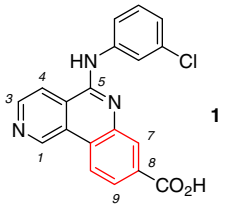
A novel class of pan-Pim kinase inhibitors was designed by modifying the CK2 inhibitor CX-4945. Introduction of a triazole or secondary amide functionality on the C-7 position and 2′-halogenoanilines on C-5 resulted in potent inhibitors of the Pim-1 and Pim-2 isoforms, with many analogs active at single digit nanomolar concentrations. The molecules inhibited the phosphorylation at Serine 112 of the apoptosis effector BAD, and had potent antiproliferative effects on the AML cell line MV-4-11 (IC_{50} <30 nM). This work delivers an excellent lead-optimization platform for Pim targeting anticancer therapies.

The Pim kinases (*P*roviral *I*ntegration site of moloney *M*urine leukemia virus)[1,2] constitute a family of oncogenic serine/threonine protein kinases overexpressed in a number of tumors of hematological and epithelial origin and often associated with strongly elevated MYC levels. The three isoforms Pim-1, Pim-2 and Pim-3 are constitutively active and largely regulated at the transcriptional and translational levels, mainly through the JAK/STAT signaling pathway. Pim kinases mediate cell survival through the direct phosphorylation of Bcl-2 antagonist of cell death (BAD) and other substrates.[3–5] In acute myeloid leukemia (AML) cell lines carrying the receptor tyrosine kinase FLT3-ITD mutation, Pim-1 has been shown to be one of the principal kinases mediating the anti-apoptotic function of FLT3-ITD signaling.[6,7]

A large body of work has proposed the pharmacological targeting of Pim kinases for anti-cancer therapy and several classes of inhibitors have already been described.[8–18] However, with a few notable exceptions,[19–23] most of these inhibitors potently inhibited only Pim-1 and there is increasing evidence that several protein isoforms should be concurrently modulated for optimal therapeutic efficiency. In particular, simultaneous targeting of Pim-1 and Pim-2 is necessary to overcome compensatory up-regulation mechanisms in protein tyrosine kinase-mediated leukemia.[24] The recent withdrawal from development of the first clinical stage Pim kinase inhibitor SGI-1776,[25] due to cardiac QTc prolongation,[26] stimulates the discovery of novel inhibitor chemotypes that inhibits multiple Pim isoforms and have fewer off-target toxicities.

A reasonable starting point for the design of Pim inhibitory agents is protein kinase CK2 inhibitors. There have been several reports of small molecules interacting with both proteins due to the structural similarity of their ATP binding site.[12,27] We recently described CX-4945 (**1**) (Fig. 1),[28,29] the first ATP-competitive inhibitor of CK2 to enter clinical trials for cancer. CX-4945 was also found to moderately inhibit Pim-1 in a biochemical kinase screen (IC_{50} = 0.046 μM), albeit inactive at 10 μM in a functional Pim cell-based assay.[29]

SAR and structural studies[28,30] revealed that the carboxylic acid moiety on C-8 was necessary for binding to CK2 and that its removal or displacement to the adjacent sites C-7 and C-9 suppressed inhibition of CK2. This observation led us to further



1

CK2 IC_{50} = 0.001 μM
Pim-1 IC_{50} = 0.046 μM

Figure 1. Structure of CX-4945 (**1**) and inhibitory activity against CK2 and Pim-1 in enzyme assays.[29]

* Corresponding author. Tel.: +1 858 875 5113; fax: +1 858 875 5101.
E-mail address: fpierre@cylenepharma.com (F. Pierre).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.09.059

explore the Pim SAR of the scaffold lower ring, intuitively speculating that the structural changes responsible for the loss of activity of the molecules against acidophilic CK2 might not be as detrimental for their ability to inhibit the Pim kinases. Herein, we report our preliminary exploration efforts and the ensuing discovery of novel Pim kinase inhibitors with potent cellular antiproliferative properties.

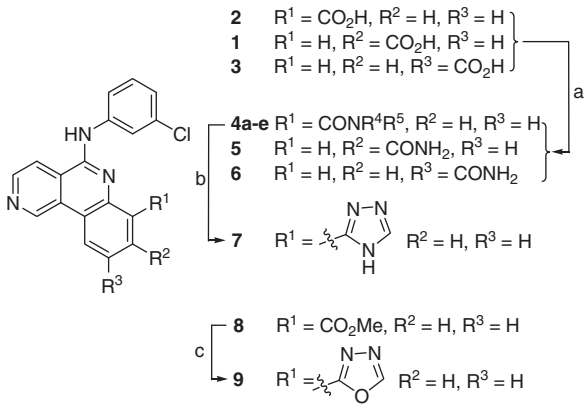
The synthesis of compounds **1–3, 8** (Scheme 1), **10** and **11** (Table 1) has been already described.[28] Carboxylic acids **1–3** were easily converted to carboxamides **4a**, **5** and **6** using amide coupling reagents and ammonium chloride as a source of nitrogen. Compounds **4b–e** bearing extended amides on R^1 were obtained in a similar manner. Carboxamide **4a** ($-NR^4R^5 = -NH_2$) was reacted with *N,N*-dimethylformamide dimethylacetal (DMF–DMA) and subsequently condensed with hydrazine in acetic acid to form triazole **7**. An oxadiazole ring could be introduced at the same position by reacting ester **8** with hydrazine and converting the resulting hydrazide to **9** using triethyl orthoformate.

The promising activity of triazole **7** (Table 1) prompted us to design chemistries allowing the straightforward introduction of amines on C-5 to study the SAR of that position (Scheme 2).

For that purpose, methyl 5-oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-7-carboxylate **12**[28] was converted to triazole **13** in three steps using chemistries described above. Reaction with phosphorus oxychloride afforded the corresponding crude 5-chlorobenzo[*c*][2,6]naphthyridine, which reacted with various amine reagents to form compounds **14a–p** and **15a–p**, that were isolated after purification by preparative HPLC. More amide analogs **17a–j**, diversely substituted on C-5 were prepared in three steps from **12** by first substituting the chlorine of **16** with various anilines and then converting the methyl ester to amides in two steps.

A number of disubstituted 2-halogenoanilines **18a–b** and **19a–c** (Scheme 3) bearing solubilizing groups in the *para* position were not commercially available and required synthesis using procedures derived from published documents.[31–34]

The compounds[35] described on Scheme 1 were tested in a radiometric enzymatic assay using recombinant human CK2 holoenzyme ($\alpha\alpha\beta\beta$) and recombinant human Pim-1 and Pim-2.[36] Preliminary SAR data (Table 1, compounds **1–3**, **4a**, **5**, **8**, **10–11**) showed that most of the structural changes on the C ring that reduced inhibitory activity against CK2 also affected Pim-1 and Pim-2 inhibition. However, the magnitude of change was significantly lower for the latter enzymes in compounds bearing on R^1 a carboxylate (**2**) or a carboxamide (**4a**) moiety. When compared with CX-4945 **1**, the structural modifications leading to **2** and **4a** resulted in an



Scheme 1. Reagents and conditions: (a) NH_4Cl or HNR^4R^5 (for structure of R^4 and R^5 see Table 1), NMP, EDCI, HOBt·H_2O, DIEA or NEt_3, 70 °C, 5–83%; (b) ($R^4 = R^5 = H$) $(MeO)_2CHNMe_2$ neat, 80 °C then $NH_2NH_2{\cdot}H_2O$, AcOH, 80 °C, 20%; (c) $NH_2NH_2{\cdot}H_2O$, MeOH, 60 °C then $(EtO)_3CH$, 120 °C, 20%.

increase in IC_{50} value for CK2 greater than 500-fold, while the IC_{50} values for Pim-1 (7- and 2-fold increase) and Pim-2 (up to 3-fold decrease) were comparatively less affected. A methyl amide in **4b** enhanced affinity for the Pim kinases to a greater degree (Pim-1 $IC_{50} = 0.055$ μM and Pim-2 $IC_{50} = 0.018$ μM) stimulating further exploration of that position of the scaffold. Tertiary amide **4c** was totally inactive against both isoforms, indicating the necessity for one H-bond donor to be present at that position for inhibition of the protein. Extension of the amides with bulkier groups (**4d** and **4e**) decreased activity.

4H-1,2,4-triazoles and 1,3,4-oxadiazoles are well known heterocyclic bioisosteres of amides.[37] Their straightforward chemical syntheses from carboxamides and esters led us to prepare compounds **7** and **9** from **4a** and **8**, respectively. 7-(4*H*-1,2,4-Triazol-3-yl)benzo[*c*][2,6]naphthyridine **7** was found to be a potent inhibitor of Pim-1 ($IC_{50} = 0.027$ μM) and Pim-2 ($IC_{50} = 0.018$ μM), 10-fold more active than 1,3,4-oxadiazole **9** (Pim-1 $IC_{50} = 0.228$ μM and Pim-2 $IC_{50} = 0.117$ μM), confirming the need for a H-bond donor in this area of the inhibitors.

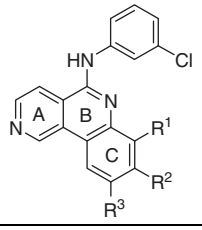
The chemistry described in the upper part of Scheme 2 allowed a convenient and facile examination of the SAR of 7-(4*H*-1,2,4-triazol-3-yl)benzo[*c*][2,6]naphthyridines analogs bearing various amine substituents on C-5. The inhibitory activities against Pim-1 and Pim-2 of the resulting compounds are reported in Tables 2 and 3. A series of analogs bearing diverse cycloalkyl amines (**14a–c**, Table 2) showed a reasonable double digit nanomolar activity, indicating a good potential for further optimization. An extended primary alkylamine (**14d**) or tertiary alkyl amine (**14e**) lowered activity.

Overall, aniline moieties on C-5 induced good activities against the Pim kinases (from 3 nM to 92 nM, **14f–p**, **7**, Table 2). A series of analogs mono-substituted at various positions revealed a noteworthy increase in potency against both isoforms of the enzyme when introducing on the aniline 2′-position a chlorine (**14k**), a fluorine (**14o**) or a methyl (**14p**) group. Analogs substituted in the *meta* and *para* position with the same substituents (**7**, **14j**,**m**,**n**) or others (**14g**,**i**) were generally less potent. Activity was lowered when a methoxy (**14h**) was in the *ortho* position, suggesting that substituents at this position were likely to interact with a small hydrophobic pocket. Finally, alkylation of the aniline amino group (**14l**) induced a modest reduction in activity.

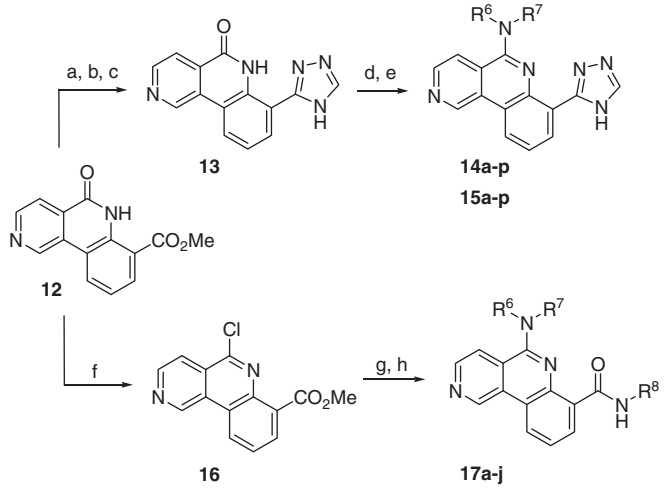
Following these results, a series of analogs disubstituted at the aniline and bearing 2′-chlorine (**15a–g**), 2′-methyl (**15h,i**) or 2′-fluorine (**15j–p**) moieties were prepared (Table 3). The data confirmed the potent activity of the 2′-chloroaniline analogs, five of them showing single digit nanomolar inhibitions of at least one isoform. A preliminary exploration showed that the aniline moiety could sustain large solubilizing groups in 4′-position of the aniline and retain potent activity (**15e**, **15f**). Certain substituents, such as alkylsulfone (**15c**), trifluoromethoxy (**15d**) or fluorine (**15a**) decreased activity at the same position. Finally 3′-methoxy analog **15g** displayed good activity, indicating another possible position for the introduction of solubilizing groups. 2′-Methyl and 2′-fluorine analogs (**15h,i**, **15j–p**) were also very potent, notably compound **15j**, bearing two fluorine atoms in the *ortho* positions of the aniline and potently inhibiting Pim-1 ($IC_{50} = 0.002$ μM) and Pim-2 ($IC_{50} = 0.001$ μM). Large solubilizing groups could also be placed on the 2′-fluoro-anilines resulting in a minimum loss of activity (**15n–p**).

The robust activity of 2′-halogenoaniline inhibitors encouraged the synthesis and testing of more amide compounds bearing these moieties (**17a–j**, Table 4). Although the compounds were generally as effective as their triazole counterpart in inhibiting the Pim-2 isoform, they were slightly less potent against Pim-1. On average, amides were fivefold selective for Pim-2 as measured by Pim-1/Pim-2 IC_{50} ratios (from 2- to 9-fold). Extending the amide function

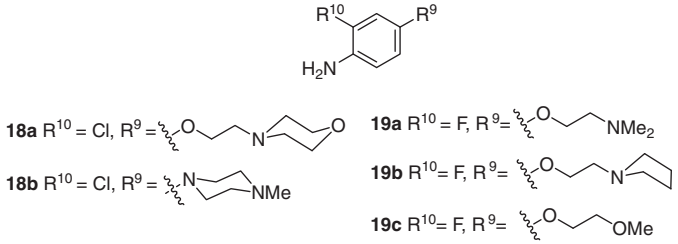
Table 1
CK2, Pim-1, Pim-2 inhibitory activity of various substituted 5-(3-chlorophenylamino) benzo[*c*][2,6]naphthyridines



Compd	R^1	R^2	R^3	CK2 IC_{50} (μM)	Pim-1 IC_{50} (μM)	Pim-2 IC_{50} (μM)
1	H	CO_2H	H	0.001	0.048	0.186
10	H	CO_2Me	H	>0.5	>2.5	>2.5
5	H	$CONH_2$	H	0.417	0.798	1.25
11	H	H	H	>0.5	>2.5	>2.5
3	H	H	CO_2H	0.350	0.577	0.294
6	H	H	$CONH_2$	>5	>2.5	>2.5
8	CO_2Me	H	H	>0.5	2.22	0.894
2	CO_2H	H	H	>0.5	0.342	0.094
4a	$CONH_2$	H	H	>0.5	0.100	0.054
4b	CONHMe	H	H	>0.5	0.055	0.018
4c	$CONMe_2$	H	H	>0.5	>2.5	>2.5
4d	CONH(*c*-Pr)	H	H	>0.5	0.107	0.074
4e	$CONH(CH_2)_2OMe$	H	H	>0.5	0.114	0.158
7	C-(4*H*-1,2,4-Triazol-3-yl)	H	H	>0.5	0.027	0.018
9	C-(1,3,4-Oxadiazole)	H	H	>0.5	0.228	0.117



Scheme 2. Reagents and conditions: (a) NaOH, H_2O, EtOH, reflux, 93%; (b) NH_4Cl, NMP, EDCI, HOBt·H_2O, DIEA, 80 °C, 96%; (c) $(MeO)_2CHNMe_2$ neat, 80 °C then $NH_2NH_2·H_2O$, AcOH, 80 °C, 92%; (d) $POCl_3$, ACN, 100 °C, >100% (crude); (e) HNR^6R^7 (for structure of R^6 and R^7 see Tables 2 and 3), NMP, μwave 120–160 °C, preparative HPLC, 2–93%; (f) $POCl_3$, reflux, 92%; (g) HNR^6R^7 (for structure of R^6 and R^7 see Table 4), NMP, 80 °C or μwave 140 °C, 85–93% then NaOH, H_2O, EtOH, 80 °C, 56–98%; (h) NH_4Cl or R_8NH_2 (for structure of R^8 see Table 4), dioxane, EDCI, HOBt·H_2O, DIEA, 80 °C, 39–100%.



Scheme 3. Structure of anilines **18a–b** and **19a–c**.

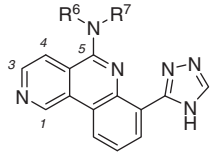
with bulkier alkyl groups (**17d–f**), did not induce any substantial loss of activity suggesting that further derivatization at this position was possible.

Representative triazole **14k** was chosen for a biochemical screen against 107 kinases to assess its selectivity profile (Performed at Millipore, Dundee, UK; see Supplementary data). Using a concentration of 0.5 μM (500-fold greater than the IC_{50} of Pim-1), only 10 of the 107 kinases were inhibited by more than 90%. Pim-1 (99% inhibition at 0.5 μM) and Pim-2 (98%) were within the top 5 of the kinases affected, while Pim-3 was inhibited at a lower level (88%).

At the concentration tested, **14k** exerted potent inhibition of Flt3 (95%). Because Flt-3 regulates the Pim kinases in leukemia,[7,6] its level of inhibition is important information for the interpretation of cellular experiments intended to study Pim targeted drugs. Compound **14k**, eight additional representative triazoles **14f,o**, **15b,e,l–o** and the amide **17b** were therefore subjected to Flt3 IC_{50} determination (Table 5). Additionally, Pim-3 IC_{50} values were measured as well as Pim-1 and Pim-2 under the same Millipore conditions.[38] The molecules generally affected Flt3 with double digit nanomolar IC_{50} values, with some degree of enzyme selectivity (IC_{50} Flt3/Pim-1 >20) observed for triazoles **15b** and **14k**. The data confirmed our measures of potent inhibition of Pim-1 and Pim-2, and revealed that the molecules inhibited the Pim-3 isoform with IC_{50} values generally higher by one to two orders of magnitude than for the two other isoforms. The most potent Pim-3 inhibitor **15o** had an IC_{50} of 35 nM. The Pim-3 isoform has been shown to be aberrantly expressed in pancreatic, gastric and colon cancer,[39–41] therefore appropriate optimization of Pim-3 inhibition could lead to new therapies for these malignancies.

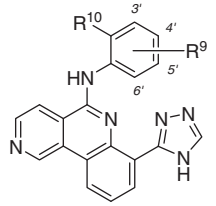
Several potent molecules (Pim-1 or Pim-2 IC_{50} <5 nM) were evaluated for their ability to inhibit the phosphorylation of the apoptosis effector BAD at Ser112[6,5] in MV-4-11,[42] a leukemia cell line carrying the FLT3-ITD activating mutation (Table 6). All of the

Table 2
Pim-1 and Pim-2 inhibitory activity of various substituted 7-(4*H*-1,2,4-triazol-3-yl)benzo[*c*][2,6]naphthyridines



Compd	–NR^6R^7	Pim-1 IC_{50} (μM)	Pim-2 IC_{50} (μM)
14a	–HN(*c*-Pr)	0.013	0.008
14b	–HN(*c*-Bu)	0.025	0.020
14c	–HN(*c*-Pen)	0.039	0.026
14d	$-HN(CH_2)_2OH$	0.052	0.055
14e	Pyrrolidin-1-yl	0.194	0.174
14f	–HN-Phenyl	0.017	0.010
14g	–HN(3′-MeO-Phenyl)	0.023	0.026
14h	–HN(2′-MeO-Phenyl)	0.041	0.092
14i	–HN(3′-Acetylenyl-phenyl)	0.028	0.010
7	–HN(3′-Cl-Phenyl)	0.027	0.018
14j	–HN(4′-Cl-Phenyl)	0.028	0.021
14k	–HN(2′-Cl-Phenyl)	0.005	0.006
14l	–(Me)N(2′-Cl-Phenyl)	0.021	0.015
14m	–HN(3′-F-Phenyl)	0.074	0.059
14n	–HN(4′-F-Phenyl)	0.018	0.016
14o	–HN(2′-F-Phenyl)	0.005	0.003
14p	–HN(2′-Me-Phenyl)	0.007	0.006

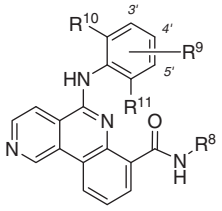
Table 3
Pim-1 and Pim-2 inhibitory activity of 7-(4*H*-1,2,4-triazol-3-yl)benzo[*c*][2,6]naphthyridines bearing various disubstituted anilines



Compd	R^{10}	R^9	Pim-1 IC_{50} (μM)	Pim-2 IC_{50} (μM)
14k	Cl	H	0.005	0.006
15a	Cl	4′-F	0.026	0.031
15b	Cl	4′-OH	0.005	0.002
15c	Cl	$4'-SO_2Me$	0.026	0.044
15d	Cl	$4'-OCF_3$	0.045	0.113
15e	Cl	4′-(2-Morpholinoethoxy)	0.002	0.007
15f	Cl	4′-(4-Methylpiperazin-1-yl)	0.002	0.012
15g	Cl	3′-OMe	0.008	0.001
14p	Me	H	0.007	0.006
15h	Me	4′-F	0.007	0.008
15i	Me	5′-F	0.006	0.008
14o	F	H	0.005	0.003
15j	F	6′-F	0.002	0.001
15k	F	6′-OMe	0.013	0.012
15l	F	4′-F	0.016	0.006
15m	F	5′Cl	0.010	0.010
15n	F	$4'-O(CH_2)_2OMe$	0.007	0.010
15o	F	4′-(2-(Pyrrolidin-1-yl)ethoxy)	0.004	0.012
15p	F	$4'-O(CH_2)_2NMe_2$	0.007	0.020

compounds tested were potent in this cellular assay with half of the molecules tested displaying IC_{50} values below 100 nM. It is noteworthy that Flt3 autophosphorylation was mildly affected in the same cell line upon the same drug exposure time (4 h), indicating

Table 4
Pim-1 and Pim-2 inhibitory activity of various substituted 5-(phenylamino)-benzo[c][2,6]naphthyridine-7-carboxamides



Compd	R^{11}	R^{10}	R^9	R^8	Pim-1 IC_{50} (μM)	Pim-2 IC_{50} (μM)
17a	H	Cl	H	H	0.016	0.008
17b	H	Cl	H	Me	0.009	0.001
17c	H	Cl	3′-OMe	Me	0.010	0.005
17d	H	Cl	H	Et	0.008	0.002
17e	H	Cl	H	*i*-Pr	0.016	0.002
17f	H	Cl	H	*sec*-Bu	0.021	0.005
17g	H	F	H	H	0.041	0.010
17h	H	F	H	Me	0.024	0.004
17i	F	F	H	H	0.025	0.004
17j	F	F	H	Me	0.013	0.002

Table 5
Inhibitory activity of selected molecules in Flt3, Pim-3, Pim-1 and Pim-2 Millipore enzyme assays (see note[38])

Compd	Flt3 IC_{50} (μM)	Pim-3 IC_{50} (μM)	Pim-1 IC_{50} (μM)	Pim-2 IC_{50} (μM)
14k	0.104	0.086	0.001	0.006
14f	0.103	0.343	0.022	0.015
14o	0.070	0.057	0.005	0.005
15b	0.039	0.875	0.002	0.001
15e	0.017	0.12	0.002	0.014
15l	0.095	0.154	0.010	0.015
15m	0.046	0.459	0.008	0.012
15n	0.044	0.235	0.011	0.030
15o	0.061	0.035	0.005	0.028
17b	0.023	0.367	0.016	0.005

that within this time frame, the dephosphorylation of BAD was likely due to a direct inhibition of Pim enzymatic activity. All tested molecules potently inhibited MV-4-11 cellular proliferation with IC_{50} values $\leqslant$150 nM, many being lower than 30 nM, showing the efficiency of targeting the Pim kinases in this cell phenotype. Other cancer cell lines were affected by the compounds at higher submicromolar or micromolar levels, indicating their potential therapeutic use against solid tumors in addition to hematological malignancies.

In summary, we have discovered a novel class of potent benzo[*c*][2,6]naphthyridines Pim kinase inhibitors by relocating and modifying functional groups of the potent CK2 inhibitor CX-4945. Introduction of a triazole or secondary amide functionality on the C-7 position of the scaffold dramatically lowered affinity for CK2, and 2′-chloro or 2′-fluoro anilines on C-5 resulted in single digit nanomolar Pim kinase inhibitors. The molecules exerted a powerful in vitro antiproliferative activity on solid and hematological cancer cell lines. In the most sensitive leukemia cell lines (MV-4-11), dephosphorylation of BAD at Ser112 was observed, providing a preliminary link between the cellular antiproliferative properties of the molecules and their enzyme inhibitory activity.

Preliminary SAR suggests a number of possible areas of exploration for further optimization. We have shown that the inhibitors could bear large solubilizing groups on the aniline moieties, and the favorable activity of aliphatic amines analogs predicts that a

Table 6
Activity of the most potent Pim inhibitors (Pim-1 or Pim-2 IC_{50} <5 nM) in various cellular assays[42]

Compd	Inhibition of phosphorylation MV-4-11 EC_{50} (μM)[a]		Cell viability EC_{50} (μM)				
	BAD S112	Flt3 T591	MV-4-11	K-562	PC3	MDA-MB-231	MIA PaCa-2
14k	0.03	4.8	<0.030	1.03	1.24	0.29	0.30
14o	0.14	>10	<0.030	0.27	0.73	1.60	1.15
15b	0.09	1.5	<0.030	0.24	0.15	2.38	0.58
15e	0.05	6.0	0.050	1.90	>30	>10	0.05
15f	0.03	>10	<0.030	0.65	3.66	2.94	1.50
15g	1.58	ND	0.093	1.47	3.68	>10	6.81
15j	0.53	5.3	0.032	0.22	2.05	3.14	0.83
15o	0.04	>10	<0.030	0.44	9.26	0.79	0.53
17b	1.02	2.6	<0.030	0.54	1.03	1.78	1.24
17c	0.10	4.4	<0.030	0.24	0.42	1.90	0.97
17d	0.21	ND	<0.030	2.47	2.42	2.12	2.96
17e	0.89	ND	0.224	6.38	>10	7.47	8.06
17f	0.98	ND	0.14	8.11	>10	7.87	8.90
17h	0.20	8.9	<0.030	0.10	0.47	1.00	1.05
17i	0.10	ND	0.036	0.29	0.15	0.69	0.44
17j	0.76	ND	0.030	0.15	0.27	0.59	0.36

[a] Cells were treated with the molecules for 4 h.

larger pool of potent C-5 substituted analogs remain to be discovered. Other areas of modifications include the amide functionality and the scaffold A-ring, whose interaction in CX-4945 with the hinge region of CK2 has proven critical.[30] Additional optimization of the selectivity and drug properties of these tricyclic molecules will be the matter of future reports.

Supplementary data

Supplementary data (the complete kinase selectivity profile of **14k** (107 kinases)) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.059.

References and notes

1. Nawijn, M. C.; Alendar, A.; Berns, A. *Nat. Rev. Cancer* **2010**, *11*, 23.
2. Amaravadi, R.; Thompson, C. B. *J.Clin. Invest.* **2005**, *115*, 2618.
3. Aho, T. L.; Sandholm, J.; Peltota, K. J.; Mankonen, H. P.; Lilly, M.; Koskinen, P. J. *FEBS Lett.* **2004**, *571*, 43.
4. Macdonald, A.; Campbell, D. G.; Toth, R.; McLauchlan, H.; Hastie, C. J.; Arthur, J. S. *BMC Cell Biol.* **2006**, *7*, 1.
5. Yan, B.; Zemskova, M.; Holder, S.; Chin, V.; Kraft, A.; Koskinen, P. J.; Lilly, M. *J. Biol. Chem.* **2003**, *278*, 45358.
6. Kim, K. T.; Levis, M.; Small, D. *Br. J. Haematol.* **2006**, *134*, 500.
7. Kim, K. T.; Baird, K.; Ahn, J. Y.; Meltzer, P.; Lilly, M.; Levis, M.; Small, D. *Blood* **2005**, *105*, 1759.
8. Morwick, T. *Expert Opin. Ther. Pat.* **2010**, *2010*, 193.
9. Ren, J. X.; Li, L. L.; Zheng, R. L.; Xie, H. Z.; Cao, Z. X.; Feng, S.; Pan, Y. L.; Chen, X.; Wei, Y. Q.; Yang, S. Y. *J. Chem. Inf. Model.* **2011**, *51*, 1364.
10. Xiang, Y.; Hirth, B.; Asmussen, G.; Biemann, H. P.; Bishop, K. A.; Good, A.; Fitzgerald, M.; Gladysheva, T.; Jain, A.; Jancsics, K.; Liu, J.; Metz, M.; Papoulis, A.; Skerlj, R.; Stepp, J. D.; Wei, R. R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3050.
11. Blanco-Aparicio, C.; Collazo, A. M.; Oyarzabal, J.; Leal, J. F.; Albaran, M. I.; Lima, F. R.; Pequeno, B.; Ajenjo, N.; Becerra, M.; Alfonso, P.; Reymundo, M. I.; Palacios, I.; Mateos, G.; Quinones, H.; Corrionero, A.; Carnero, A.; Pevarello, P.; Lopez, A. R.; Fominaya, J.; Pastor, J.; Bischoff, J. R. *Cancer Lett.* **2010**, *300*, 145.
12. Lopez-Ramos, M.; Prudent, R.; Moucadel, V.; Sautel, C. F.; Barette, C.; Lafanechere, L.; Mouawad, L.; Grierson, D.; Schmidt, F.; Florent, J. C.; Filippakopoulos, P.; Bullock, A. N.; Knapp, S.; Reiser, J. B.; Cochet, C. *Faseb J.* **2010**, *24*, 3171.
13. Pierce, A. C.; Jacobs, M.; Stuver-Moody, C. *J. Med. Chem.* **2008**, *52*, 1972.
14. Pogacic, V.; Bullock, A. N.; Fedorov, O.; Filippakopoulos, P.; Gasser, C.; Biondi, S.; Meyer-Monard, S.; Knapp, S.; Schwaller, J. *Cancer Res.* **2007**, *67*, 6916.
15. Grey, R.; Pierce, A. C.; Bemis, G. W.; Jacobs, M. D.; Moody, C. S.; Jajoo, R.; Mohal, N.; Green, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3019.
16. Bullock, A. N.; Debreczeni, J. E.; Fedorov, O. Y.; Nelson, A.; Marsden, B. D.; Knapp, S. *J. Med. Chem.* **2005**, *48*, 7604.
17. Holder, S.; Zemskova, M.; Zhang, C.; Tabrizizad, M.; Bremer, R.; Neidigh, J. W.; Lilly, M. B. *Mol. Cancer Ther.* **2007**, *6*, 163.
18. Cheney, I. W.; Yan, S.; Appleby, T.; Walker, H.; Vo, T.; Yao, N.; Hamatake, R.; Hong, Z.; Wu, J. Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1679.
19. Xia, Z.; Knaak, C.; Ma, J.; Beharry, Z. M.; McInnes, C.; Wang, W.; Kraft, A. S.; Smith, C. D. *J. Med. Chem.* **2009**, *52*, 74.
20. Tao, Z. F.; Hasvold, L. A.; Leverson, J. D.; Han, E. K.; Guan, R.; Johnson, E. F.; Stoll, V. S.; Stewart, K. D.; Stamper, G.; Soni, N.; Bouska, J. J.; Luo, Y.; Sowin, T. J.; Lin, N. H.; Giranda, V. S.; Rosenberg, S. H.; Penning, T. D. *J. Med. Chem.* **2009**, *52*, 6621.
21. Qian, K.; Wang, L.; Cywin, C. L.; Farmer, B. T.; Hickey, E.; Homon, C.; Jakes, S.; Kashem, M. A.; Lee, G.; Leonard, S.; Li, J.; Magboo, R.; Mao, W.; Pack, E.; Peng, C.; Prokopowicz, A.; Welzel, M.; Wolak, J.; Morwick, T. *J. Med. Chem.* **2009**, *52*, 1814.
22. Akue-Gedu, R.; Nauton, L.; Thery, V.; Bain, J.; Cohen, P.; Anizon, F.; Moreau, P. *Bioorg. Med. Chem.* **2010**, *18*, 6865.
23. Tong, Y.; Stewart, K. D.; Thomas, S.; Przytulinska, M.; Johnson, E. F.; Klinghofer, V.; Leverson, J.; McCall, O.; Soni, N. B.; Luo, Y.; Lin, N. H.; Sowin, T. J.; Giranda, V. L.; Penning, T. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5206.
24. Adam, M.; Pogacic, V.; Bendit, M.; Chappuis, R.; Nawijn, M. C.; Duyster, J.; Fox, C. J.; Thompson, C. B.; Cools, J.; Schwaller, J. *Cancer Res.* **2006**, *66*, 3828.
25. Chen, L. S.; Redkar, S.; Bearss, D.; Wierda, W. G.; Gandhi, V. *Blood* **2009**, *114*, 4150.
26. Phase I of SGI-1776 was discontinued in Nov. 2010 (http://www.supergen.com/investors/pressrelease.html?pressReleaseId=905).
27. Pagano, M. A.; Bain, J.; Kazimierczuk, Z.; Sarno, S.; Ruzzene, M.; Di Maira, G.; Elliott, M.; Orzeszko, A.; Cozza, G.; Meggio, F.; Pinna, L. A. *Biochem. J.* **2008**, *415*, 353.
28. Pierre, F.; Chua, P. C.; O'Brien, S. E.; Siddiqui-Jain, A.; Bourbon, P.; Haddach, M.; Michaux, J.; Nagasawa, J.; Schwaebe, M. K.; Stefan, E.; Vialettes, A.; Whitten, J. P.; Chen, T. K.; Darjania, L.; Stansfield, R.; Anderes, K.; Bliesath, J.; Drygin, D.; Ho, C.; Omori, M.; Proffitt, C.; Streiner, N.; Trent, K.; Rice, W. G.; Ryckman, D. M. *J. Med. Chem.* **2011**, *54*, 635.
29. Siddiqui-Jain, A.; Drygin, D.; Streiner, N.; Chua, P. C.; Pierre, F.; O'Brien, S. E.; Bliesath, J.; Omori, M.; Ho, C.; Proffitt, C.; Schwaebe, M. K.; Ryckman, D. M.; Rice, W. G.; Anderes, K. *Cancer Res.* **2010**, *70*, 10288.
30. Battistutta; Cozza, G.; Pierre, F.; Papinutto, E.; Lolli, G.; Sarno, S.; O'Brien, S. E.; Siddiqui-Jain, A.; Haddach, M.; Anderes, K.; Ryckman, D. M.; Meggio, F.; Pinna, L. A.*Biochemistry* **2011**, *50*, 8478.
31. Galan, A. A.; CHen, J.; Du, H.; Forsyth, T.; Huynh, T. P.; Johnson, H. W. B.; Kearney, P.; Leahy, J. W.; Lee, M. S.; Mann, G.; Ridgway, B. H.; Takeuchi, C. S.; Zhou, P. Patent application WO2008/042282A2, 2008, 142.
32. Andrews, D. M.; Jones, C. D.; Simpson, I.; Ward, R. A. Patent application WO2009/024824A1, 2009, 87.
33. Bold, G.; Furet, P.; Guagnano, V. Patent application WO2007/71752, 2007, 84.
34. Jones, R. M.; Lehmann, J.; Wong, A. S. T.; Hurst, D.; Y.J., S. Patent application US2006/1555128 A1, 2006, 52.
35. All final compounds were characterized by LCMS and found to be ⩾95% pure, except for compounds **15a**, **14l** and **3** (90–93% purity). Selected compounds were characterized by NMR.
36. CK2, Pim-1 and Pim-2 inhibitions were measured in radiometric assays using human recombinant CK2 (ααββ-holoenzyme) at [ATP] = 15 μM (substrate RRRDDDSDDD), human recombinant Pim-1 at [ATP] = 30 μM (substrate RSRHSSYPAGT) and human recombinant Pim-2 at [ATP] = 5 μM (substrate RSRHSSYPAGT). The IC_{50} values were derived from eight concentrations of test inhibitors.
37. Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147.
38. Millipore assays used a distinct substrate for Pim-1 (KKRNRTLTV) and different concentrations of ATP: Pim-1, [ATP] = 90 μM; Pim-2, [ATP] = 15 μM. The radiometric assay for Pim-3 used RSRHSSYPAGT as a substrate in the presence of [ATP] = 155 μM. The Flt3 assay used EAIYAAPFAKKK and [ATP] = 200 μM. The IC_{50} values were derived from ten concentrations of test inhibitors.
39. Li, Y. Y.; Popivanova, B. K.; Nagai, Y.; Ishikura, H.; Fujii, C.; Mukaida, N. *Cancer Res.* **2006**, *66*, 6741.

40. Zheng, H. C. ; Tsuneyama, K.; Takahashi, H.; Miwa, S.; Sugiyama, T.; Popivanova, B. K.; Fujii, C.; Nomoto, K.; Mukaida, N.; Takano, Y. *J. Cancer Res. Clin. Oncol.* **2008**, *134*, 481.
41. Popivanova, B. K.; Li, Y. Y.; Zheng, H.; Omura, K.; Fujii, C.; Tsuneyama, K.; Mukaida, N. *Cancer Sci.* **2007**, *98*, 321.
42. The AML cells MV-4-11 were treated with 8 different concentrations of the tested compounds for 4 h. Samples were lysed under non-denaturing conditions and levels of Phospho-Bad (Ser112) and Phospho-FLT3 (Tyr591) were quantified using a commercial ELISA kit from Cell Signaling, according to the manufacturer's protocols. Cell proliferation inhibition was determined by Alamar Blue assay, exposing various cell lines to the tested compounds for 4 days.