Bioorganic & Medicinal Chemistry Letters 22 (2012) 3327–3331

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel potent dual inhibitors of CK2 and Pim kinases with antiproliferative activity against cancer cells

Fabrice Pierre *, Collin F. Regan, Marie-Claire Chevrel, Adam Siddiqui-Jain, Diwata Macalino, Nicole Streiner, Denis Drygin, Mustapha Haddach, Sean E. O'Brien, William G. Rice, David M. Ryckman

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

ARTICLE INFO

Article history:
Received 24 January 2012
Revised 25 February 2012
Accepted 28 February 2012
Available online 6 March 2012

Keywords:
CK2
Pim
Multikinase inhibitors
Cancer
Kinase inhibitor

ABSTRACT

A novel family of potent dual inhibitors of CK2 and the Pim kinases was discovered by modifying the scaffolds of tricyclic Pim inhibitors. Several analogs were active at single digit nanomolar IC_{50} values against CK2 and the Pim isoforms Pim-1 and Pim-2. The molecules displayed antiproliferative activity in various cell phenotypes in the low micromolar and submicromolar range, providing an excellent starting point for further drug discovery optimization.

A significant proportion of kinase inhibitors approved for cancer therapy are multikinase targeting agents able to simultaneously modulate several biological processes of the disease.[1] The pharmacologic benefits of multi-targeting include the blockage of redundant compensatory pathways and the reduction of resistance development through the modulation of several cancer hallmarks.[2] Although many of these drugs were not deliberately created for their multi-targeting profile, a more rational design of molecules with predefined profiles has emerged.[3]

A group of enzymes suitable for multi-targeting comprises protein kinase CK2[4–6] (from the misnomer casein kinase 2) and the Pim kinases[7,8] (Proviral Integration site of moloney Murine leukemia virus). These constitutively active serine/threonine kinases are overexpressed in a number of tumors and are known to mediate important hallmarks of cancer such as apoptosis resistance and cell growth. Moreover, both enzymes share a relatively similar ATP binding site, allowing small molecules to inhibit both proteins in enzyme assays.[9] The rational design of dual CK2/Pim inhibitors for cancer therapy has been theorized in detail in a pioneering paper by Lopez-Ramos et al.[10] The best analog reported in that article had IC_{50} values for CK2 and Pim-1 of 63 and 20 nM, respectively, but was inactive in cells, as a likely result of poor cell penetration.

We have recently described CX-4945 **1** (Fig. 1),[11–13] the first ATP-competitive inhibitor of CK2 to enter clinical trials for cancer. CX-4945 was also a moderate inhibitor of Pim-1 and Pim-2 isoforms in enzyme assays (IC_{50} values of 0.048 and 0.186 μM, respectively), albeit inactive against Pim in a functional cell-based assay. Structural modification of **1** led to the discovery of 7-(4*H*-1,2,4-triazol-3-yl)benzo[*c*][2,6]naphthyridine **2**,[14] a single digit nanomolar inhibitor of Pim-1 and Pim-2, able to modulate cellular phosphorylation of the Pim substrate Bad at serine 112, but inactive against CK2.

As part of our continuing effort to develop this series of molecules, we considered modifying the left pyridine ring of **2** to diversify our pool of active molecules and optimize their drug like properties. A series of more compact scaffolds bearing five-membered ring replacements of the pyridine was designed and derivatized. These modifications maintained inhibition of the Pim kinases, but also resulted in the restoration of potent CK2 inhibition. In this paper, we describe the preliminary SAR and characterization of the resulting dual inhibitors of CK2 and the Pim kinases.

A variety of scaffolds bearing sulfur or nitrogen five-membered rings were prepared. The domino Suzuki coupling/intramolecular amide formation used for the sulfur containing molecules is based on the chemistry used to prepare similar molecules.[11,14] Methyl 2-amino-3-bromobenzoate **4** (Scheme 1, prepared according to Refs. 11 or 15) was converted to key-boronic ester **5** by a Miyaura borylation. Compound **5** was formed alongside a significant amount of the reduced product methyl 2-aminobenzoate, which could be easily separated from less polar **5** by flash chromatography on silica gel.[16]

Boronic ester **5** was coupled under palladium catalysis with various 2-bromo esters **3a–e** to form the core molecules **6a–e**. Reaction with $POCl_3$ led to compounds **7a–e**, which were converted in

* Corresponding author. Tel.: +1 858 622 0177; fax: +1 858 875 5101.
E-mail addresses: fpierre@cylenepharma.com, fpierre22@hotmail.com (F. Pierre).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.02.099

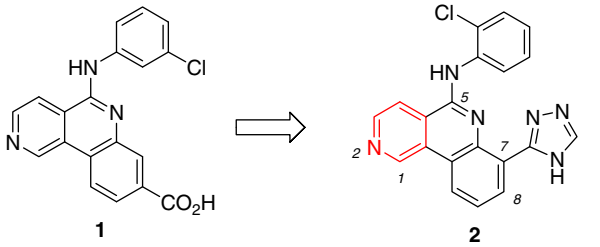
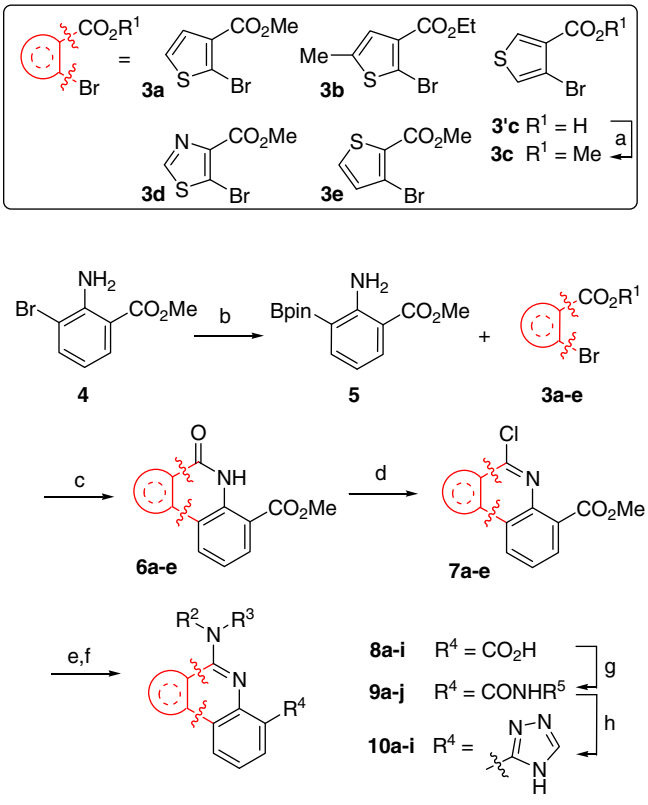


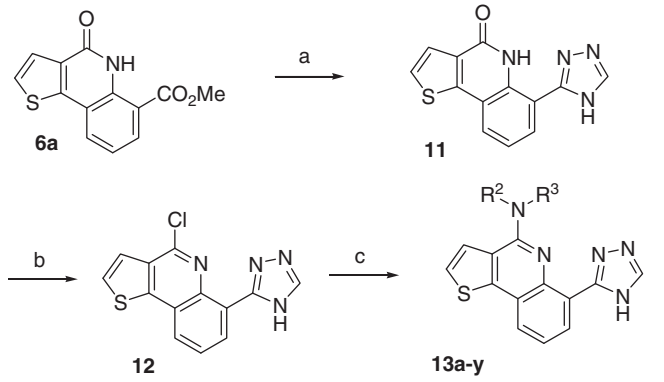
Figure 1. Structure of CX-4945 **1**[11] and representative 7-(4*H*-1,2,4-triazol-3-yl)benzo[*c*][2,6]naphthyridine **2** inhibitor of the Pim kinases.[14]

various acids **8a–i** through a two-step procedure. After amide coupling leading to **9a–j**, carboxamides **9a–i** were converted to triazoles **10a–i** by a two-step process utilizing DMF–DMA and hydrazine.

The chemistry depicted in Scheme 2 was designed to study the SAR of C-4 substituted 6-(4*H*-1,2,4-triazol-3-yl)thieno[3,2-*c*]quinolines. The ester **6a** was converted to a triazole **11** in three steps, and the central pyridone ring was transformed to a chloropyridine by reacting with $POCl_3$. The resulting crude **12** was reacted with various substituted amines leading to compounds **13a–y**, isolated after purification by chromatography.

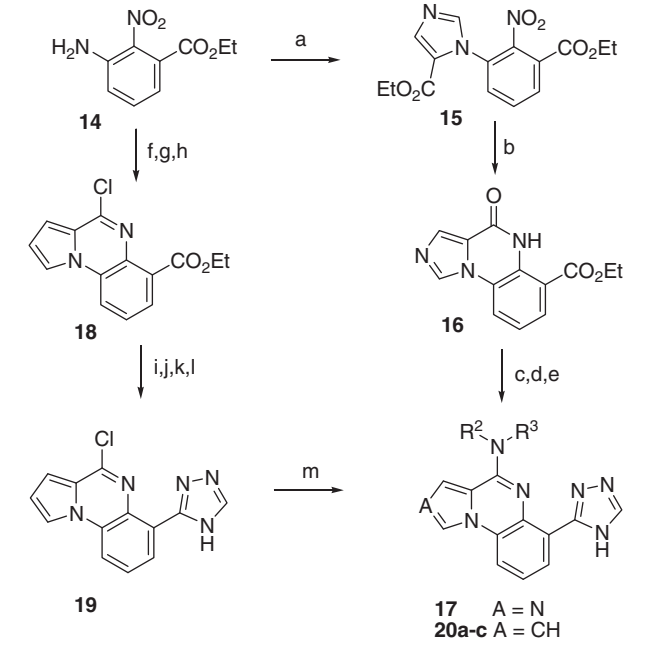


Scheme 1. Reagents and conditions: (a) $(COCl)_2$, DMF cat., CH_2Cl_2 then MeOH, 63%; (b) PinBBPin, KOAc, $PdCl_2(dppf).CH_2Cl_2$, toluene, 100 °C, 45%; (c) Cs_2CO_3, $PdCl_2(dppf)\cdot CH_2Cl_2$, dioxane/water (20:1, v/v) 100 °C, 57–83%; (d) $POCl_3$, NEt_3, ACN, 100 °C, 48–95%; (e) HNR^2R^3 (for structure of R^2 and R^3 see Tables 1 and 3), NMP, μwave or conventional heating 120–140 °C; (f) NaOH, H_2O, EtOH 60–80 °C, 22–91% over two steps; (g) NH_4Cl or $MeNH_2$·HCl (R^5 = Me, compound **9j**), HOBt·H_2O, DIEA, EDCI, NMP or dioxane, 70–80 °C, 28–82%; (h) (R^5 = H) $(MeO)_2CHNMe_2$ neat, 80 °C then $NH_2NH_2\cdot H_2O$, AcOH, 80 °C, 32–67%.



Scheme 2. Reagents and conditions: (a) NaOH, H_2O, EtOH, 80 °C, 92%; NH_4Cl HOBt·H_2O, DIEA, EDCI, NMP, 80 °C, 97%; $(MeO)_2CHNMe_2$ neat, 80 °C then $NH_2NH_2\cdot H_2O$, AcOH, 80 °C, 89%; (b) $POCl_3$, NEt_3, ACN, 100 °C 88%; (c) HNR^2R^3 (for structure of R^2 and R^3, see Table 2), NMP, μwave 120–160 °C, preparative HPLC or TLC, 4–71%.

The buildup of scaffolds bearing nitrogenous five-membered rings started from ethyl 3-amino-2-nitrobenzoate **14**, (Scheme 3) prepared according to a published procedure.[17,18] The amino function of **14** was expanded to an ethyl 1*H*-imidazole-5-carboxylate in a process involving the reaction of TosMIC with the condensation product of ethyl glyoxylate and **14** (using a procedure described in Ref. 19). Iron reduction of the nitro group at 100 °C and intramolecular addition of the aniline function on the ester resulted in the formation of ethyl 4-oxo-4,5-dihydroimidazo[1,5-*a*]quinoxaline-6-carboxylate **16**. Chemical transformations similar to the previous descriptions provided triazole analog **17**.



Scheme 3. Reagents and conditions: (a) EtO_2CCHO, MeOH, Dean–Stark reflux, then TosMIC, K_2CO_3, 50 °C, 15%; (b) HOAc, Fe, 100 °C, 27%; (c) (i) $POCl_3$, NEt_3, ACN, 100 °C, 99%, (ii) HNR^2R^3, NMP, μwave 140 °C, 50%, (iii) NaOH, H_2O, EtOH, 80 °C, 60%; (d) NH_4Cl, HOBt·H_2O, DIEA, EDCI, dioxane, 80 °C, 52%; (e) $(MeO)_2CHNMe_2$ neat, 80 °C then $NH_2NH_2\cdot H_2O$, AcOH, 80 °C, 26%; (f) 2,5-dimethoxytetrahydrofuran, 120 °C, 88%; (g) EtOH, Pd/C, H_2 (40 psi), 91%; (h) triphosgene, toluene, 120 °C, 73%; (i) NaOH, H_2O, 80 °C, 91%; (j) NH_4Cl, HOBt·H_2O, DIEA, EDCI, dioxane, 80 °C; (k) $(MeO)_2CHNMe_2$ neat, 80 °C then $NH_2NH_2\cdot H_2O$, AcOH, 80 °C, 89% over two steps; (l) $POCl_3$, NEt_3, ACN, 100 °C, 84%; (m) HNR^2R^3 (for structure of R^2 and R^3, see Table 1), NMP, μwave 120–140 °C, preparative HPLC, 6–30%.

The aniline function of ethyl 3-amino-2-nitrobenzoate **14** could form a pyrrole by a Paal–Knorr condensation with 2,5-dimethoxytetrahydrofuran. Hydrogenation of the resulting intermediate and reaction with triphosgene led to the isolation of cyclized intermediate ethyl 4-chloropyrrolo[1,2-*a*]quinoxaline-6-carboxylate **18**. After simultaneous hydrolysis of the ester and substitution of the chlorine by a hydroxyl group in the presence of sodium hydroxide, intermediate **19** was built using chemistries similar to those depicted in Scheme 2. Compounds **20a–c** were prepared by substituting the chlorine of **19** with various anilines.

All compounds[20] were tested in a radiometric enzymatic assay using recombinant human Pim-1, Pim-2 or CK2 holoenzyme ($\alpha\alpha\beta\beta$)[21] The structure–activity relationship (SAR) studies depicted in Table 1 investigated variations of the left ring of various scaffolds substituted on the upper position by aniline (compounds **10a**, **10d**, **10f** and **20a**) or by 2′-halogeno anilines (**10b–c**, **10e**, **10g–h**, **20b–c** and **17**), which were previously shown to increase potency against the Pim kinases.[14] Our first choice of thiophene analogs **10a–c** provided potent inhibitors of Pim-1, Pim-2 and CK2. The analogs substituted by a chlorine (**10b**) or a fluorine (**10c**) inhibited the 3 enzymes with single digit nanomolar IC_{50} values. Moving the sulfur to various positions (**10d–g**) resulted in a slight decrease of affinity for the three enzymes, regardless of the substitutions. A similar trend was observed when adding a nitrogen atom within the five-membered ring of the molecule. When comparing thiazole **10h** with thiophene analog **10b**, IC_{50} values for the three enzymes tested increased three to four fold. Finally, less lipophilic nitrogen-bearing heterocycles in **20a–c** and **17** decreased activity further to double digit nanomolar values, with the exception of **20c** which inhibited the Pim-1 isoform with an IC_{50} value equal to 0.008 μM.

The potent activity of the 6-(4*H*-1,2,4-triazol-3-yl)thieno[3,2-*c*]quinolines **10b** and **10c** against the three enzymes prompted us to further explore this chemical series, and in particular the SAR of analogs diversely substituted at C-4 by amines (Table 2), using the chemistry described in Scheme 2. Alkyl secondary (**13a**) or tertiary (**13b**) amines as well as benzyl (**13c**) or heteroaryl (**13d–e**) amines reduced the activity against the three enzymes. When compared to **10b**, their ability to inhibit Pim-1 and Pim-2 ranged from 0.004 to 0.091 μM while for CK2 the IC_{50} values were in a significantly higher range (0.048–0.216 μM).

As observed in similar molecules,[11,14] aniline moieties at C-4 demonstrated the greatest activity against the three enzymes. Unsubstituted aniline analog **10a** was already very potent, having IC_{50} values close to 2′-chloro aniline **10b** and 3′-chloroaniline **13f**. Moving the chlorine atom to the *para* position of the aniline (**13g**) lowered the affinity for the three targets. Other disubstituted analogs bearing a chlorine atom in the 2′-position (**13h–k**) were slightly less potent against at least one of the enzymes. Compounds bearing a fluorine atom on the phenyl ring were all very potent (**10c**, **13l–m**) and changing the position of the fluorine atom induced minimal changes in activity. The 2′-position appeared however to be the most favorable for a fluorine atom, as confirmed by the highest potency of *ortho*-fluoro disubstituted molecules **13n** and **13o**, in comparison with **13p** and **13q**. Interestingly, among the methoxy analogs (**13r–t**), the *para*-methoxy aniline **13t** was the most potent molecule, displaying IC_{50} values below 6 nM for the three enzymes. However, extension of the alkyl chain (**13u**) decreased the potency of the drug. Finally, among several aniline bearing various groups in the *meta* position (**13v–x**), the 3-acetylenyl-phenyl **13x** was the most potent modulator of the three enzymes.

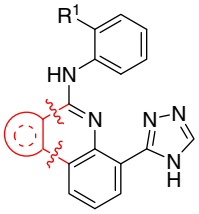
Overall, the SAR of the C-4 position indicated that the three tested proteins could accommodate anilines bearing small hydrophobic moieties at variable positions. A preliminary exploration of other sites of the scaffold is reported in Table 3. While a carboxylate on R^3 significantly decreased inhibitory activity against the three targets, carboxamides in **9a** and **9j** maintained single digit nanomolar inhibition of the Pim-1 isoform. Adding a methyl on R^1 on the thiophene of fluoro aniline analog **10c** gave **10i**, which preserved the potent inhibition of Pim-2 and Pim-1 and induced a slight loss of CK2 modulation. This suggests that further exploration of this position with larger moieties and solubilizing groups is possible and may lead to improved drug properties.

In our previous paper,[14] we showed that several analogs of the potent Pim inhibitor **2** also inhibited the kinase Flt-3 with IC_{50} values ranging from 0.017 to 0.104 μM, a property that is also observed with CK2 inhibitor CX-4945 **1** (Flt-3 IC_{50} = 0.035 μM[11]). Selected triazoles **10b, e, g, 20b** and methyl amide **9j** were tested against Flt-3[22] in an enzymatic assay (Table 4). The molecules showed no activity at concentration below 5 μM, suggesting that the present dual CK2/Pim inhibitors may have a kinase selectivity profile different than **2** and its close analogs.[14]

The most potent dual Pim/CK2 inhibitors (Pim-1, Pim-2 and CK2 IC_{50}<10 nM) and representative analogs of other scaffolds depicted in Table 1 were tested for their antiproliferative activity against a number of cancer cell lines (Table 5).[23] All compounds were generally active in several cell lines with EC_{50} values in the low micromolar range. In the most sensitive leukemia cells MV-4-11, several compounds were active at submicromolar concentration, with IC_{50} values as low as 100 nM. This cell line carries the Flt-3 ITD activating mutation, and Pim-1 and Pim-2 are known to be the principal kinases mediating the anti-apoptotic function of Flt-3-ITD signaling,[24,25] explaining the high sensitivity of this cell line to Pim inhibitors.

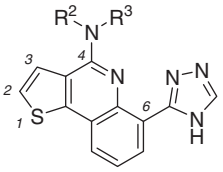
In summary, we have discovered a novel chemotype of potent dual inhibitors of CK2 and the Pim kinases, of which several analogs were active with single digit nanomolar IC_{50} values against the three targets. The molecules displayed antiproliferative activity in various cancer cells in the low micromolar and submicromolar range, a result that brings a significant improvement over previously published dual inhibitors of these enzymes.

Table 1
Pim-1, Pim-2 and CK2 inhibitory activity of various tricyclic analogs



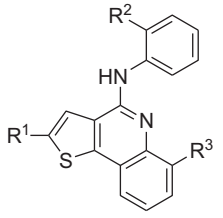
Left ring	Compd	R^1	Pim-1 IC_{50} (μM)	Pim-2 IC_{50} (μM)	CK2 IC_{50} (μM)
	**10a**	H	0.004	0.015	0.010
	10b	Cl	0.003	0.002	0.009
	10c	F	0.002	0.002	0.004
	**10d**	H	0.027	0.017	0.010
	10e	Cl	0.007	0.008	0.020
	**10f**	H	0.016	0.014	0.030
	10g	Cl	0.004	0.006	0.021
	**10h**	Cl	0.008	0.009	0.039
	**20a**	H	0.014	0.030	0.101
	20b	Cl	0.012	0.041	0.089
	20c	F	0.008	0.020	0.021
	**17**	F	0.038	0.043	0.035

Table 2
Pim-1, Pim-2 and CK2 inhibitory activity of various C-4 substituted 6-(4*H*-1,2,4-triazol-3-yl)thieno[3,2-*c*]quinolines



Compd	$-NR^2R^3$	Pim-1 IC_{50} (μM)	Pim-2 IC_{50} (μM)	CK2 IC_{50} (μM)
13a	–NH*c*-Pr	0.015	0.025	0.062
13b	–Morpholino	0.035	0.010	0.128
13c	–NHBn	0.004	0.052	0.048
13d	–NH(3′-pyridyl)	0.017	0.022	0.076
13e	–NH(4′-pyridyl)	0.091	0.068	0.216
10a	–NHphenyl	0.004	0.015	0.010
10b	–NH(2′-Cl-phenyl)	0.003	0.002	0.009
13f	–NH(3′-Cl-phenyl)	0.006	0.006	0.015
13g	–NH(4′-Cl-phenyl)	0.014	0.086	0.033
13h	–NH(2′-Cl, 4′-OH-phenyl)	0.014	0.004	0.010
13i	–NH(2′-Cl, 4′-F-phenyl)	0.009	0.009	0.052
13j	–NH(2′Cl, 3′-OMe-phenyl)	0.005	0.021	0.017
13k	–NH(2′-Cl, 4′-OCF_3-phenyl)	0.064	0.076	0.075
10c	–NH(2′-F-phenyl)	0.002	0.002	0.004
13l	–NH(3′-F-phenyl)	0.003	0.005	0.007
13m	–NH(4′-F-phenyl)	0.004	0.007	0.007
13n	–NH(2′-F, 4′-F-phenyl)	0.002	0.002	0.011
13o	–NH(2′-F,2′-MeO-phenyl)	0.002	0.014	0.009
13p	–NH(3′-F, 4′-F-phenyl)	0.009	0.015	0.077
13q	–NH(3′-F, 5′-F-phenyl)	0.009	0.024	0.151
13r	–NH(2′-OMe-phenyl)	0.007	0.049	0.084
13s	–NH(3′-OMe-phenyl)	0.011	0.028	0.011
13t	–NH(4′-OMe-phenyl)	0.004	0.006	0.005
13u	–NH(4′-$O(CH_2)_2NMe_2$-phenyl)	0.016	0.034	0.136
13v	–NH(3′-CF_3-phenyl)	0.016	0.052	0.024
13w	–NH(3′-CN-phenyl)	0.010	0.012	0.126
13x	–NH(3′-acetylenyl-phenyl)	0.006	0.005	0.002
13y	–NH(4′-$CONH_2$-phenyl)	0.017	0.017	0.139

Table 3
Pim-1, Pim-2 and CK2 inhibitory activity of various substituted *N*-phenylthieno[3,2-*c*]quinolin-4-amines



Compd	R^1	R^2	R^3	Pim-1 IC_{50} (μM)	Pim-2 IC_{50} (μM)	CK2 IC_{50} (μM)
8a	H	Cl	CO_2H	0.069	0.121	0.139
9a	H	Cl	$CONH_2$	0.005	0.012	0.022
9j	H	Cl	CONHMe	0.005	0.028	0.048
10b	H	Cl	C-(4*H*-1,2,4-triazol-3-yl)	0.003	0.002	0.009
10c	H	F	C-(4*H*-1,2,4-triazol-3-yl)	0.002	0.002	0.004
10i	Me	F	C-(4*H*-1,2,4-triazol-3-yl)	0.015	0.006	0.026

Table 4
Flt-3 inhibitory activity of various thieno[3,2-*c*]quinolines

Compd	**10g**	**10e**	**20b**	**10b**	**9j**
Flt-3 IC_{50} (μM)	>10	>10	>10	>10	5.3

This work delivers interesting antiproliferative molecules for the study of the dual inhibition of CK2 and the Pim kinases in cancer, and provides a useful starting point for lead optimization. A fundamental aspect of their future characterization remains the assessment of their kinase selectivity profile, as well as the correlation of their kinase inhibitory activity with antiproliferative properties. However, the lack of significant inhibitory activity against Flt-3 (Table 4), one of the most frequently inhibited kinases,[26] suggests a reasonable selectivity profile for this new class of molecules.

Finally, crystallographic studies will also be essential to assess the structural features responsible for the intriguing increase in CK2 inhibition induced by the modification of the left ring of triazole **2**, an observation implying the possibility of a binding mode

Table 5
Antiproliferative activity of potent inhibitors (Pim-1, Pim-2 and CK2 IC_{50} <10 nM) and of representative analogs of various scaffold depicted in Table 1

Compd	Cell viability EC_{50} (μM)				
	BxPC3	SUM-149PT	K-562	MDA-MB453	MV-4-11
10b	11.7	4.4	3.6	2.6	0.5
10c	>30	9.5	3.6	7.1	0.6
13x	24.6	9.6	6.7	9.3	5.4
13m	>30	>30	>10	6.6	>10
13l	9.1	8.1	>10	2.6	4.6
13t	28.0	8.2	7.2	7.4	6.5
13n	>30	>30	4.8	4.6	1.7
10a	>30	7.0	6.1	7.8	2.0
10e	4.5	2.6	>10	4.9	0.6
10g	16.1	2.6	>10	1.3	3.2
20c	>30	22.8	8.2	28.4	3.7
10h	2.8	12.6	>10	2.0	4.7
17	15.0	5.4	5.4	3.1	0.1

slightly distinct from that of CX-4945 **1**. The optimization and structural biology exploration of these tricyclic molecules will be the matter of further research.

References and notes

1. Li, R.; Stafford, J. A. *Kinase Inhibitor Drugs*; John Wiley & Sons: New York, 2009.
2. Hanahan, D.; Weinberg, R. A. *Cell* **2011**, *144*, 646.
3. Morphy, R. *J. Med. Chem.* **2010**, *53*, 1413.
4. Sarno, S.; Pinna, L. A. *Mol. Biosyst.* **2008**, *4*, 889.
5. Guerra, B.; Issinger, O. G. *Curr. Med. Chem.* **1870**, *2008*, 15.
6. Trembley, J. H.; Chen, Z.; Unger, G.; Slaton, J.; Kren, B. T.; Van Waes, C.; Ahmed, K. *Biofactors* **2010**, *36*, 187.
7. Nawijn, M. C.; Alendar, A.; Berns, A. *Nat. Rev. Cancer* **2010**, *11*, 23.
8. Amaravadi, R.; Thompson, C. B. *J. Clin. Invest.* **2005**, *115*, 2618.
9. 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.
10. 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.
11. 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.
12. 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.
13. Battistutta, R.; 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.
14. Pierre, F.; Stefan, E.; Nédellec, A.-S.; Chevrel, M.-C.; Regan, C. F.; Siddiqui-Jain, A.; Macalino, D.; Streiner, N.; Drygin, D.; Haddach, M.; O'Brien, S. E.; Anderes, K.; Ryckman, D. M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6687.
15. Jacobs, R.T.; Folmer, J.J.; Simpson, T.R.; Chaudhari B.; Frazee, W.J.; Davenport, T.W.; Sundarababu, G. U.S. patent 6,399,603 B1, 2002.
16. Boronic ester **5**: R_f = 0.5, methyl 2-aminobenzoate: R_f = 0.30, SiO_2, 20% EtOAc in hexanes. Compound **5** was isolated in 45% yield by flash chromatography on silica gel using a 1.5–2.5% gradient of AcOEt in hexanes.
17. Staab, H. A.; Zipplies, M. F.; Mueller, T.; Storch, M.; Krieger, C. *Chem. Ber.* **1994**, *127*, 1667.
18. Yamasaki, N.; Imoto, T.; Murai, Y.; Hiramura, T.; Oku, T.; Sawada, K. U.S. patent 6,166,219, 2000.
19. Chen, B.-C.; Sundeen, J.E.; Bednarz, M.S.; Chen, P. PCT patent application WO2004/078714 A2, 2004.
20. All final compounds were characterized by LC–MS and found to be ⩾95% pure. Selected compounds were characterized by NMR.
21. 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
22. The Flt-3 enzymatic assay was carried out at Millipore, Billerica, MA, using their standard protocol.
23. Cell proliferation inhibition was determined by Alamar Blue assay, exposing various cell lines to the tested compounds for 48 days.
24. Kim, K. T.; Levis, M.; Small, D. *Br. J. Haematol.* **2006**, *134*, 500.
25. Kim, K. T.; Baird, K.; Ahn, J. Y.; Meltzer, P.; Lilly, M.; Levis, M.; Small, D. *Blood* **2005**, *105*, 1759.
26. Anastassiadis, T.; Deacon, S. W.; Devarajan, K.; Ma, H.; Peterson, J. R. *Nat. Biotechnol.* **2011**, *29*, 1039.