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Kinase inhibitory potencies and in vitro antiproliferative activities of N-10 substituted pyrrolo[2,3-*a*]carbazole derivatives

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ABSTRACT

Development of potent and selective Pim kinase inhibitors has recently emerged as an important field for the design of new anti-cancer drugs. We report the synthesis of new N-10-substituted pyrrolo[2,3-*a*]carbazole derivatives and their evaluation as Pim kinase inhibitors. Moreover, in vitro antiproliferative activity of these compounds was evaluated toward a human fibroblast primary culture and three human solid cancer cell lines (PA1, PC3 and DU145). Compounds **3**, **7** and **10** showed inhibitory potencies toward Pim-1 and Pim-3 in the nanomolar range. Additionally, dimethylamino analog **10** also demonstrated interesting sub-micromolar antiproliferative activities toward the cell lines tested.

The oncogenic Ser/Thr protein kinases Pim (Provirus Insertion site of Moloney murine leukemia virus), comprising three highly homologous isoforms Pim-1, Pim-2 and Pim-3, are part of the CAMK (calcium/calmodulin-dependent protein kinase) family. Pim kinases control various proteins involved in significant biological processes such as cell cycle progression and apoptosis. As Pim kinases are constitutively active, their functions are principally regulated at the expression level, especially by the JAK/STAT signaling pathway. Over-expression of Pim kinases have been noticed in human leukemia and lymphoma, as well as in various cases of solid tumors such as prostate, pancreatic, oral, hepatic and colon cancers. Pim kinases are implicated in many signaling pathways contributing to tumorigenesis. For example, it has been shown that Pim-2 over-expression favors hematopoietic cell line survival in a growth-factor independent manner. Moreover, in hematological cells, production of cytokines and various survival factors is improved by Pim-1 stimulation of several transcription factors like c-Myb, NFATc1 and the RUNX family proteins. Additionally, the three Pim isoforms are able to phosphorylate the pro-apoptotic protein Bad leading to its inactivation and apoptosis inhibition.[1–4] Recently, Pim kinases have been described as potent stimulators of cancer cell migration and invasion.[5] All these data demonstrate the major importance of Pim kinases as signaling proteins that could be intended for the identification of new antitumour drugs.[6,7]

Recently, the synthesis and biological activities of pyrrolo[2,3-*a*]carbazole-3-carbaldehyde **A** were reported.[8] Compound **A** (Fig. 1) exhibited potent and selective inhibitory potencies toward Pim kinase family members when tested against a panel of 66 protein kinases. Thus, in order to identify new Pim kinase inhibitors, a large structure–activity relationship study was performed within this pyrrolocarbazole family.[8–10] Highly potent inhibitors were identified, mostly targeting Pim-1 and Pim-3 isoforms. Unfortunately, possibly due to limitations in the solubility of this relatively hydrophobic scaffold, despite potent kinase inhibitions, most of pyrrolocarbazole derivatives have shown only moderate in vitro antiproliferative activities.

The study of compound **A** crystal structure in complex with Pim-1 revealed an unique H-bond established between the formyl group at the 3-position of the pyrrolo[2,3-*a*]carbazole scaffold and Lys67 side chain of the ATP binding site. Moreover, it was found that none of the pyrrolocarbazole nitrogen atoms was involved in the interaction between **A** and the Pim-1 ATP-binding site. Thus, in order to improve the hydrophily/hydrophoby balance of this series, the N-10 position was selected for the introduction of an aminoalkylated side chain, assuming that as the formyl group present at the 3-position of the pyrrolo[2,3-*a*]carbazole moiety is part of a

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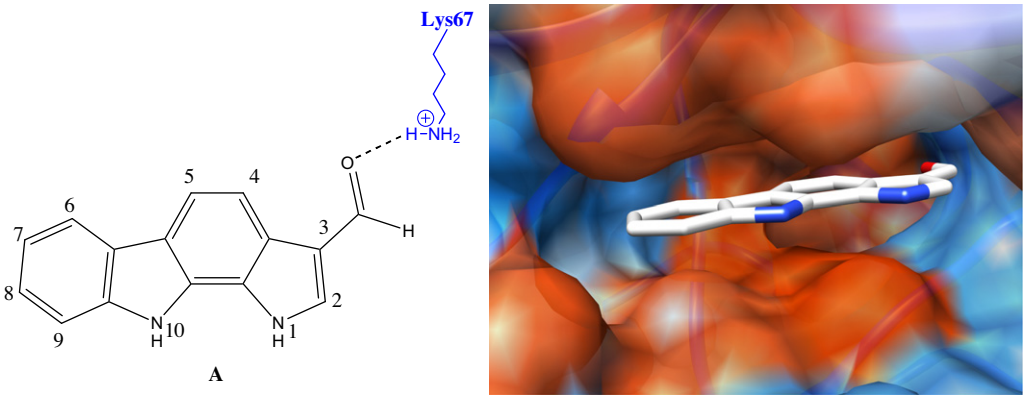


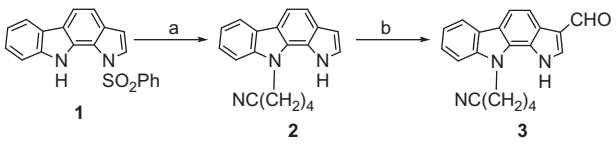
Figure 1. Binding of compound **A** to Pim-1 ATP binding site (surfaces are colored in orange-red (hydrophobic) and in blue (hydrophilic)) [**8**, PDB 3JPV]. H-bond indicated in dashed line.

vinylogous formamide system, the formyl carbonyl function should be less prompt to react with primary or secondary amino groups.[8] Therefore, in this Letter, we report our efforts to synthesize new N-10 aminoalkylated substituted pyrrolo[2,3-*a*]carbazole derivatives and their ability to inhibit Pim kinases. Moreover, in vitro antiproliferative activity of these compounds was evaluated by a fluorometric assay (resazurin reduction test) toward a human fibroblast primary culture and three human solid cancer cell lines (PA1, PC3 and DU145). The drug likeness of new compounds was also estimated and compared to the one of the reference **A**.

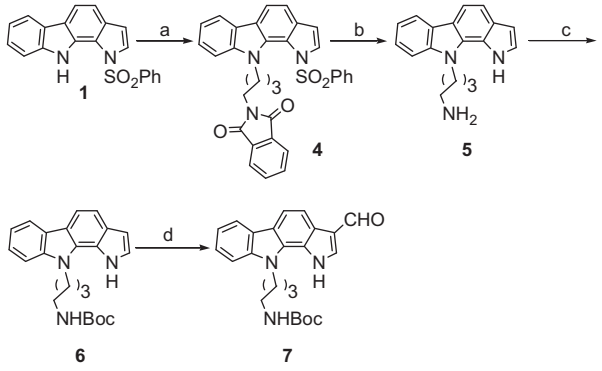
In order to synthesize the target molecules, our initial approach included the introduction of aminoalkyl side chains from the corresponding nitrile analog (Scheme 1). Thus, compound **1**[8] was first alkylated with 5-bromopentanenitrile in the presence of potassium *tert*-butoxide. Subsequent benzenesulfonyl group deprotection using NaOH in H_2O/MeOH solution led to compound **2** in 86% yield. The Vilsmeier–Haack formylation step was performed under microwave irradiation as already described[8,9] to give compound **3** in 64% yield. Unfortunately, our attempts to reduce the nitrile group under mild hydrogenation conditions (HCO_2NH_4, Pd/C or HCO_2NH_4, PtO_2 in MeOH) failed to afford the desired reduced product.

An alternative pathway was to prepare the amine function from a phthalimide intermediate (Scheme 2). Therefore, compound **1** was allowed to react with *N*-(4-bromobutyl)phthalimide in the presence of *t*BuOK leading to the corresponding alkylated derivative **4**. Prior to the formylation step, the benzenesulfonyl group was removed and the protecting phthalimide group was switched to a Boc group after hydrazinolysis to give **6** in 88% yield from compound **4** (3 steps). After formylation using oxalyl chloride/DMF in MeOH, compound **7** was obtained in 55% yield. Unfortunately, we did not manage to isolate the deprotected analog.

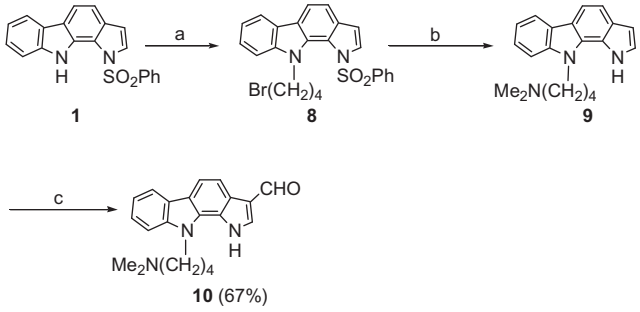
Based on these results, we directed our synthetic efforts toward the preparation of a tertiary amino analog. Dimethylamino derivative **10** was synthesized in four steps from compound **1** (Scheme 3). Brominated derivative **8** was prepared by reaction of **1** with 1,4-dibromobutane in the presence of NaH.[10] Tertiary amine **10** was successfully obtained in 67% yield by treatment of **8** with dimethylamine, deprotection and formylation.



Scheme 1. Synthesis of nitrile derivative **3**. Reagents and conditions: (a) (i) 5-bromopentanenitrile, *t*BuOK, DMF, (ii) NaOH, MeOH, 86%; (b) $POCl_3$, DMF, MW, 64%.



Scheme 2. Synthesis of compound **7**. Reagents and conditions: (a) *N*-(4-bromobutyl)phthalimide, *t*BuOK, 60%; (b) (i) H_2N–NH_2, H_2O, EtOH, (ii) NaOH, 94%; (c) Boc_2O, $NaHCO_3$, H_2O, THF, 94%; (d) (i) $(COCl)_2$, DMF, CH_2Cl_2, (ii) NaOH, 55%.



Scheme 3. Preparation of compound **10**. Reagents and conditions: (a) 1,4-dibromobutane, NaH, 84%; (b) (i) $Me_2NH{\cdot}HCl$, K_2CO_3, KI, THF, (ii) NaOH, MeOH, 57%; (c) (i) $(COCl)_2$, DMF, CH_2Cl_2, (ii) NaOH, 67%.

In vitro kinase inhibitory potencies of compounds **3**, **7** and **10** were evaluated toward the three Pim kinase isoforms in duplicate as already described in the literature by Cohen's group.[11] The percentages of residual activity, when the compounds were tested at 10 μM and 1 μM toward Pim kinases (Pim-1, Pim-2 and Pim-3), are reported in Table 1. IC_{50} values were determined when the percentages of residual activity were inferior to 25% when the compounds were tested at 1 μM.

Compounds **3** and **10** have shown similar inhibitory profile toward the three Pim kinases. Compared to reference compound **A**, both of them are more active against Pim-1 with IC_{50} in the nanomolar range (46 nM for **3** and 75 nM for **10**). Regarding Pim-3, compounds **3** and **10** were slightly less active than reference **A**. In contrast, no potent activity was measured toward Pim-2 for

Table 1
Kinase inhibitory potencies: % of residual kinase activity at 10 and 1 μM (IC_{50} in μM in brackets when determined) and antiproliferative activities of compounds **A**, **3**, **7** and **10** (IC_{50} in μM)

Compd	Kinase inhibition—% of residual kinase activity						Antiproliferative activity (IC_{50} in μM)			
	Pim-1		Pim-2		Pim-3		Fibro	PA1	PC3	DU145
	10 μM	1 μM	10 μM	1 μM	10 μM	1 μM				
A	2 ± 0.4 (0.12 ± 0.01)	nd	7 ± 1 (0.51 ± 0.23)	nd	1 ± 5 (0.01 ± 0.00)	nd	21 ± 1	4.5 ± 0.4	9.5 ± 0.5	26 ± 2
3	1 ± 0.1 (0.046 ± 0.003)	3 ± 0.8	40 ± 3 (nd)	32 ± 7	2 ± 0.5 (0.041 ± 0.009)	3 ± 1	14.5 ± 0.8	8 ± 2	16 ± 2	20 ± 1
7	4 ± 1 (0.47 ± 0.05)	23 ± 1	54 ± 3 (nd)	97 ± 1	3 ± 1 0.20 ± 0.01	16 ± 5	17.3 ± 0.9	14.9 ± 0.5	13.1 ± 0.6	12.2 ± 0.3
10	10 ± 1 (0.075 ± 0.015)	24 ± 4	22 ± 1 (nd)	74 ± 15	1 ± 0 (0.08 ± 0.00)	7 ± 0	0.63 ± 0.02	0.486 ± 0.003	0.65 ± 0.02	0.96 ± 0.06

nd: not determined.

these two compounds. *t*Butoxycarbamate derivative **7** was the less active of the series with IC_{50} values of 0.47 μM and 0.20 μM toward Pim-1 and Pim-3, respectively. The results obtained demonstrated that Pim-1 inhibitory potency of this series could be improved by the substitution of the N-10 position with a cyanobutyl or a dimethylaminobutyl chain.

In vitro antiproliferative activities of compounds **3**, **7** and **10** were evaluated toward a human fibroblast primary culture and three human solid cancer cell lines: PA1 (ovarian carcinoma), PC3 and DU145 (prostatic carcinoma). The antiproliferative effect of the tested drug was assessed by the resazurin reduction test.[12] As shown Table 1, compounds **3** and **7** have demonstrated antiproliferative activities in the range of 8–20 μM without significant variation as compared to reference **A**. In contrast, the dimethylamino derivative **10** exhibited interesting sub-micromolar antiproliferative activities toward all the cell line tested.

Finally, the physico-chemical properties (*c*Log*P*, solubility, drug likeness, drug score) of compounds **A**, **3**, **7** and **10** were evaluated using Osiris property explorer (Table 2).[13,14] Compounds log*P* values depict their partition coefficient between *n*-octanol and water, measuring their hydrophylicity. Usually compounds with log*P* values inferior to 5.0 are supposed to present good absorption and permeation properties. This was the case for compounds **A**, **3**, **7** and **10** with calculated log*P* values ranging from 3.19 to 4.62. Log*S* value is directly related to compounds aqueous solubility. More than 80% of the marketed drugs have estimated log*S* values superior to −4. In this series, only dimethylamino derivative **10** has met this criteria with an estimated log*S* value of −3.86 (compared to −4.49 for **A**). Finally, the drug likeness and drug scores, that evaluate the overall potential of a compound to become a drug, were calculated for each compound. As shown Table 2, the drug likeness scores of compounds **3** and **7** were not very good with values of −16.4 and −57, respectively. Compound **10** has shown the best drug likeness (−0.04) and drug score (0.45) of the series, compared to −3.57 and 0.3 for reference compound **A**. The Osiris molecular properties were also calculated for doxorubicin, a well-known anti-cancer agent, used for the treatment of solid tumors. As indicated in Table 2, the global estimated drug score of compound **10** is in the same range as the one of doxorubicin.

Table 2
Osiris molecular properties predictions for compounds **A**, **3**, **7**, **10** and doxorubin

Compd	MW	*c*Log*P*	Log*S*	DL	DS
A	234	3.19	−4.49	−3.57	0.3
3	315	4.32	−5.12	−16.4	0.14
7	405	4.62	−5.57	−57	0.19
10	333	3.56	−3.82	−0.04	0.45
Doxorubicin	543	0.48	−4.51	7.19	0.55

MW: molecular weight in g/mol; *S*: solubility; DL: Drug likeness; DS: drug score.

Overall, compound **10**, that has shown improved Pim-1 kinase inhibitory potency as well as enhanced in vitro antiproliferative activity and favorable calculated drug-like properties, is particularly attractive for the development of this series.

In conclusion, the synthesis of three new N-10-substituted pyrro[2,3-*a*]carbazole derivatives was successfully achieved. The results obtained have shown that the improvement of the drug likeness by introducing a *N*,*N*-dimethylaminobutyl chain at the N-10 position of the pyrrolocarbazole scaffold (compound **10**) could improve both Pim-1 inhibition and antiproliferative activities toward the cancer cell line tested.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.098.

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