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Synthesis and biological activities of new pyrrolocarbazole-imidazobenzimidazole conjugates

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ABSTRACT

New pyrrolocarbazole-imidazobenzimidazole conjugates were prepared and evaluated for their inhibitory potencies toward Pim-1 kinase, DNA binding and antiproliferative activities against human tumor cell lines. The results demonstrated that conjugation of pyrrolocarbazole Pim inhibitors with imidazobenzimidazole derivatives could enhance the antiproliferative potency of conjugates compared to the derived compounds.

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1. Introduction

Among new anticancer therapies, the development of protein kinase inhibitors has emerged as a very efficient strategy, with more than forty drugs that have reached the market to date. As part of our program dedicated to the design and synthesis of new heteroaromatic compounds with promising biological activities, we identified 1,10-dihydropyrrolo[2,3-*a*]carbazole-3-carbaldehyde as a potent and selective inhibitor of the Pim protein kinase family. This compound exhibited *in vitro* and *in vivo* anticancer activities, with a significant decrease of the volume and metastatic capacity of PC3 prostate cancer xenografts in mice (compound **A**, Fig. 1).^{1–3} *N*-Heterocyclic carbenes, well-known as ubiquitous ligands in catalysis,⁴ are very rarely used as reactants in synthesis.⁵ We have developed a new synthetic method to prepare imidazo[1,2-*a*]benzimidazole derivatives based on a copper-catalyzed *N*-heterocyclic carbene–nitrene cyclization (compounds **B**, Fig. 1).⁶ Imidazo[1,2-*a*]benzimidazole derivatives are known to be active toward different cellular targets and are therefore relevant in cancer therapy. Indeed, compounds containing the imidazo[1,2-*a*]benzimidazole moiety have been reported for their ability to modulate carcinogen metabolizing enzymes (*e.g.*, inhibition of cytochrome P-450 1A (CYP1A), induction of glutathione (GSH) and glutathione *S*-transferase (GST)).⁷ Furthermore, other reported imidazo[1,2-*a*]benzimidazoles exhibited inhibitory potencies toward various protein kinases, particularly nanomolar activities toward colony-stimulating factor 1 receptor (CSF1R), a

receptor tyrosine kinase that emerged as an attractive immunomodulatory drug target in cancer therapy.^{8,9}

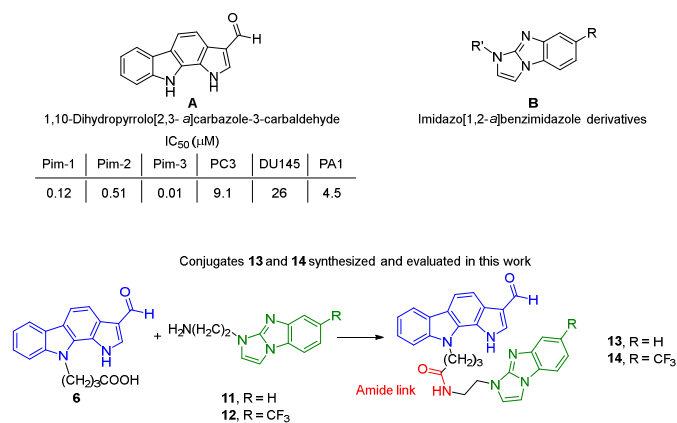


Figure 1. Chemical structures of lead 1,10-dihydropyrrolo[2,3-*a*]carbazole-3-carbaldehyde (**A**), imidazo[1,2-*a*]benzimidazole derivatives (**B**) and conjugates **13** and **14**. *In vitro* Pim 1–3 inhibitory potencies and cytotoxicities are shown for compound **A**.

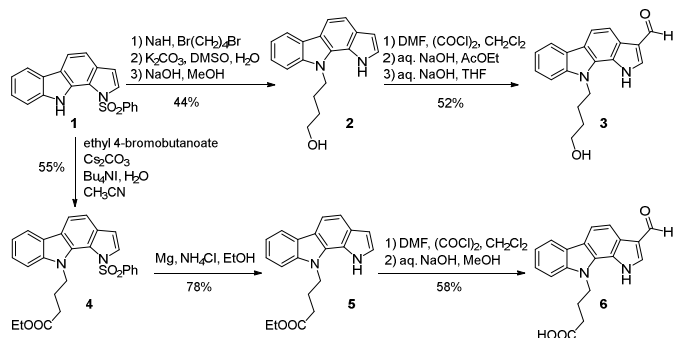
Drug resistance is a problem that frequently appears after a short period when protein kinase inhibitors are used in monotherapies. One way to circumvent this major drawback and enhance the anticancer therapy potency is the use of multi-target drugs directed toward various cellular structures.¹⁰ Therefore we synthesized conjugated compounds assembling both heteroaromatic scaffolds of **A** and **B** via an amide function that could be hydrolyzed by cellular peptidase (Fig. 1).

The synthesis of coupling partners 1,10-dihydropyrrolo[2,3-*a*]carbazole-3-carbaldehyde **6** and imidazo[1,2-*a*]benzimidazole derivatives **11** and **12**, as well as conjugates **13** and **14** will be described (Fig. 1). Then, in order to evaluate the impact of the conjugation on the biological activities of this new compound series, *in vitro* activities of compounds **A**, **3**, **6**, **11**, **12** and conjugates **13**, **14** will be evaluated toward Pim-1, DNA-binding and five human cancer cell lines (leukemia K562, colon adenocarcinoma HCT116, estrogen receptor positive breast cancer MCF-7, triple negative breast cancer MDA-MB-231 and SCOV ovarian cancer)

2. Results and Discussion

2.1. Chemistry

First, we attempted to prepare the carboxylic acid derivative **6** either from primary alcohols **2** or **3**. Compound **1**¹ was reacted with 1,4-dibromobutane in order to prepare the known *N*-substituted 4-bromobutyl intermediate.¹¹ Chromatography on silica gel was performed to remove 1,4-dibromobutane before substitution of the bromine atom by a hydroxyl group in DMSO/H₂O in the presence of K₂CO₃.¹² Partial cleavage of the benzenesulfonyl group was also observed at this step. Therefore, final deprotection of the pyrrolo[2,3-*a*]carbazole scaffold was performed with NaOH in MeOH/H₂O, leading to compound **2** in 44% overall yield. The next step was the formylation of the heteroaromatic part of **2** using standard conditions.¹³ However, the alcohol function was also partially formylated. Accordingly, an extra step was performed to hydrolyze the formate intermediate in THF/H₂O in the presence of NaOH¹⁴ to give compound **3** (Scheme 1). Unfortunately, despite many attempts using various oxidizing conditions, we were unable to obtain the expected carboxylic acid from **2** or **3**. However, as compound **3** constituted a new representative of this interesting pyrrolocarbazole series, its biological activity was also studied.

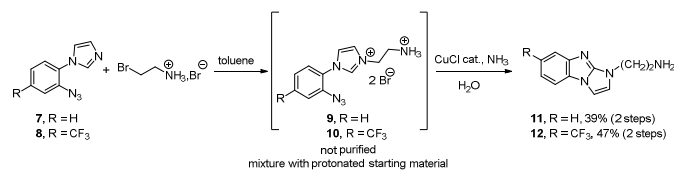


Scheme 1. Synthesis of the key intermediate **6**.

To succeed in preparing compound **6**, we used another synthetic pathway consisting first of alkylation of **1** with ethyl 4-bromobutanoate in the presence of cesium carbonate and tetrabutylammonium iodide.¹⁵ Next, deprotection of the *N*-10 position was performed in the presence of magnesium and ammonium chloride in ethanol according to Rosenberg and co-workers.¹⁶ Lastly, a formylation step followed by a basic hydrolysis afforded the carboxylic acid **6** (Scheme 1).

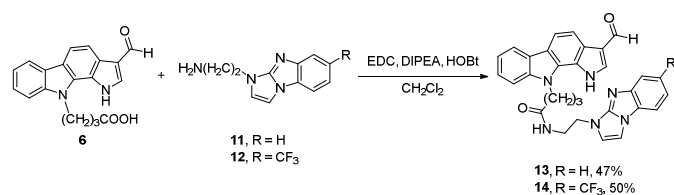
Imidazo[1,2-*a*]benzimidazole derivatives **11** and **12** were prepared in two steps from previously reported 1-(2-azidophenyl)-1*H*-imidazoles **7** and **8** (Scheme 2).⁶ The introduction of the amine chain was performed by alkylation with bromoethylammonium bromide.¹⁷ This procedure delivered an intractable mixture of *N*-(2-azidophenyl)imidazolium salts **9/10** and protonated starting materials. It was advantageous to perform the cyclization reaction on this crude mixture in the presence of

0.1 eq. copper(I) in aqueous ammonia. Indeed, the cyclization reaction required the formation of an intermediate copper-NHC complex¹⁸ which was possible only from **9/10** and not from protonated **7/8**. The latter were easily deprotonated during the last step. Separation of the final mixture relied on the marked difference of physicochemical properties between **7/8** and **11/12**.



Scheme 2. Synthesis of compounds **11** and **12**.

Having prepared all components, the final step was amide bond formation performed in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), hydroxybenzotriazole (HOBt) and *N,N*-diisopropylethylamine (DIPEA). Compound **13** and its trifluoromethylated analogue **14** were obtained in 47% and 50% yield, respectively (Scheme 3).



Scheme 3. Synthesis of conjugates **13** and **14**.

2.2. Biological evaluation

Compounds **A**, **3**, **6**, **11**, **12**, **13** and **14** were evaluated for their Pim-1 kinase inhibitory potencies. The results are expressed as % of residual kinase activities at 10 μM or 1 μM compound concentration. IC₅₀ values were determined only when the % of residual kinase activity was ≤ 25% at 1 μM. As shown in Table 1, the most active Pim-1 inhibitors were pyrrolocarbazoles **3**, **6** and conjugates **13**, **14** with (sub)micromolar potencies whereas imidazobenzimidazoles **11**, **12** did not exhibit any activity.

As planar compounds tend to intercalate in DNA, it is important to verify whether DNA can be an alternative target for these compounds. We tested compounds **A**, **3**, **6**, **11**, **12**, **13** and **14** for their ability to interact with DNA. Drug-DNA complex formation was studied using several approaches. During DNA titration (data not shown), even in the presence of the highest DNA concentration (500 μM base pairs) no significant change in intrinsic compound fluorescence was observed. Alternatively, binding to DNA was also investigated by displacement of the reference intercalating dye ethidium bromide (EtBr). We found that, at concentrations up to 100 μM, no displacement of EtBr was detectable.

Finally, the antiproliferative activities toward 5 human cancer cell lines (chronic myelogenous leukemia K562, colon adenocarcinoma HCT116, estrogen receptor positive breast carcinoma MCF-7, triple negative breast carcinoma MDA-MB-231 and SCOV ovarian carcinoma) were determined to assess the effect of conjugation. The most potent derivatives were conjugates **13** and **14** that demonstrated micromolar potencies toward all tested cell lines while others were either inactive (**6**, **11**, **12**) or cytotoxic (IC₅₀ value < 20 μM) only toward some cell lines (**A** toward MCF-7/SCOV3).

Table 1. Kinase inhibitory potencies and antiproliferative activities of reference compound **A** and new analogues **3**, **6**, **11**, **12**, **13** and **14**.

Cpd	clogP	Kinase inhibitory potency		Antiproliferative activity				
		Pim-1		K562	HCT116	MCF-7	MDA-MB-231	SCOV3
		10 μ M	1 μ M					
A	–	(0.12) ¹		> 50	33	18	>50	16
3	–	1.2 \pm 0.4	6 \pm 2	40	46	27	40	> 50
		(0.039)						
6	3.0	5 \pm 2	23 \pm 3	> 50	> 50	> 50	> 50	> 50
		(0.20)						
11	–	120 \pm 11	114 \pm 3	> 50	> 50	> 50	> 50	> 50
12	–	119 \pm 42	143 \pm 7	> 50	> 50	> 50	> 50	> 50
13	3.8	3 \pm 0	12 \pm 5	9	12	11	14	20
		(0.21)						
14	4.6	22 \pm 2	64 \pm 13	10	10	14	11	18

Residual kinase activities (% \pm SD) and cytotoxicity values (IC₅₀, μ M) in brackets. Assays were performed at the International Centre for Kinase Profiling (Dundee, UK).¹⁹ IC₅₀ values of compound **A** against Pim-1 has been previously reported.¹ clogP values calculated using DataWarrior software.²⁰

While compounds **6** and **13** exhibited comparable Pim-1 inhibitory potencies, the conjugate **13** was more active toward the cell lines tested. On another hand, **13** and its trifluoromethylated analogue **14** exhibited a cytotoxic potency in the same range, whereas **14** showed milder Pim-1 inhibition. These results demonstrated that Pim-1 inhibition does not correlate with the antiproliferative activities of the tested compounds. Nevertheless, conjugation of pyrrolocarbazole and imidazobenzimidazole moieties slightly enhanced the cytotoxicity compared to the derived compounds. This could be due to an increased intracellular penetration of conjugates **13**, **14** bearing an imidazobenzimidazole moiety compared to pyrrolocarbazole **6** (see calculated logP values, Table 1) and/or the involvement of other cellular target(s) important for the cytotoxicity of **13** and **14**.

3. Conclusion

New pyrrolocarbazoles (**3**, **6**), imidazobenzimidazoles (**11**, **12**) as well as pyrrolocarbazole-imidazobenzimidazole conjugates (**13**, **14**) have been prepared in reasonable to good yields. Compounds **3**, **6** and **13** exhibited sub-micromolar Pim-1 inhibitory potencies. In addition, conjugates **13** and **14** showed interesting antiproliferative activities in the micromolar range. However, Pim-1 inhibition does not seem to be directly correlated with the antiproliferative activities of these compounds. Nevertheless, the cytotoxicity could be enhanced by conjugation of pyrrolocarbazole and imidazobenzimidazole moieties.

Acknowledgments

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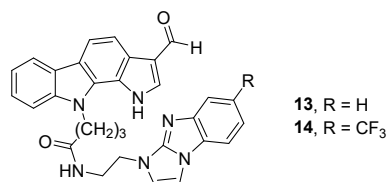
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Graphical Abstract

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	IC ₅₀ (μM)				
	K562	HCT116	MCF-7	MDA-MB-231	SCOV3
13	9	12	11	14	20
14	10	10	14	11	18