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Preliminary Communications

New N-1,N-10-bridged pyrrolo[2,3-*a*]carbazole-3-carbaldehydes: Synthesis and biological activities

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ABSTRACT

The synthesis of new pyrrolocarbazoles substituted at N-1/N-10 positions is described. All the compounds tested demonstrated moderate to high Pim-1/Pim-3 kinase inhibitory potency. The most active inhibitors identified in this series (**3**, **17**) have an alkyl chain bridging the N-1 and N-10 positions. These compounds (**3**, **17**) exhibited apoptosis–inducing activity toward acute myeloid leukemia IPC-81 cells, but not toward normal fibroblasts.

1. Introduction

The Proviral integration site for Moloney murine leukemia virus (Pim) kinase family is composed of three highly homolog isoforms (Pim-1, Pim-2, Pim-3). Pim kinases are particularly involved in cell cycle regulation and antiapoptotic activity. Moreover they are upregulated in numerous human cancers such as acute myeloid leukemia (AML) [1]. For these reasons Pim kinases are considered as important targets for the development of new anticancer drugs [2,3]. Recently, we identified pyrrolo[2,3-*a*]carbazole-3-carbaldehyde derivatives as a new series of Pim inhibitors [4–7]. The lead compound **A** (Fig. 1A) was found to be a potent selective Pim inhibitor when tested toward a panel of 66 protein kinases [4]. A structure–activity relationship (SAR) study was performed on about forty derivatives diversely substituted at the C-3, C-6, C-7, C-8, C-9 and N-10 positions (Fig. 1A). In particular, the results showed that a formyl group at the 3-position is essential for Pim kinase inhibition. Actually, the study of the interactions involved between **A** and Pim-1 ATP binding site identified only one hydrogen bond established between the formyl group and the conserved active site residue Lys67 [4]. The SAR studies also showed that, as expected on the basis of the binding mode of this series with Pim-1 ATP site, N-1 and N-10 positions can be substituted without loss of Pim kinase inhibition, because they are oriented toward the outside of the ATP binding pocket and not involved in direct interaction within the protein (Fig. 1B) [6,7]. In particular, compound **B** bearing a butyl chain bridging the two nitrogens atoms (Fig. 1A), was found to be a highly potent inhibitor of Pim-1/Pim-3 with IC_{50} values of 8 nM and 13 nM, respectively [6]. Moreover, when tested toward a larger panel of kinases and for its DNA binding ability and topoisomerase inhibitory potency, compound **B** demonstrated an acceptable selectivity profile. Lastly, compound **B** was able to synergize with the antitumor drug doxorubicin, at non-toxic concentrations, in killing human colon carcinoma cells [8].

Due to the great interest of compound **B**, we undertook the synthesis of new analogues containing various alkyl chains linking N-1 and N-10 positions (Fig. 1A). The ability of new synthesized compounds to inhibit Pim kinases was evaluated as well as their ability to induce cell death and inhibit cell proliferation in leukemia cells and in normal fibroblasts.

2. Results and discussion

First of all, we easily prepared compound **3**, an analogue of **B** containing a three-carbon bridge, according to the synthetic method previously used for the synthesis of **B** (Scheme 1) [6]. For this, compound **1** [4] was treated in the presence of 1,

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3-dibromopropane and ^{t}BuONa to give the bridged derivative **2** that was further formylated using $POCl_3$ in *N*,*N*-dimethylformamide.

When the same method was applied to 1,5-dibromopentane and 1,6-dibromohexane, only alkylation at the N-10 position took place and no concomitant alkylation/deprotection/cyclization sequence was observed. Therefore, we considered a sequential approach starting with the synthesis of the N-10-monoalkylated derivative prior to benzenesulfonyl cleavage and cyclization. Thus, compound **1** was treated in the presence of 1,5-dibromopentane or 1,6-dibromohexane and NaH to give compounds **4** and **5** in 87% and 52% yields, respectively (Scheme 2). When we attempted the deprotection of **4** in the presence of TBAF [10], a mixture of fluorinated analogue **6** and alkenyl derivative **7** was obtained with no trace of the target brominated product. As fluorinated compounds are known to present good pharmacokinetic properties, compound **6** was formylated to evaluate the biological activities of its derivative **8**.

The removal of benzenesulfonyl group from **4** and **5** was finally carried out using magnesium in methanol [11] to provide deprotected analogues **9**/**10** and dehalogenated compounds **11**/**12** whose formation was anticipated according to the reaction conditions used (Scheme 3). Compounds **11** and **12** were also formylated to evaluate the Pim kinase inhibitory potencies of analogues **13** and **14**. The cyclization reaction from compound **9** was achieved in the presence of aqueous sodium hydroxide and tetrabutylammonium bromide [12] as phase-transfer agent to afford compound **15** in 22% yield. Unfortunately, despite numerous trials from **10** in various basic conditions (NaOH/TBAB, NaH...), the cyclized analogue bearing a C6 bridge was never obtained and ethylenic compound **16** was the only product observed. Finally, to evaluate the kinase inhibitory potencies of their carbaldehyde analogues, compounds **15** and **16** were formylated leading to **17** and **18** in 96% and 77% yields, respectively.

The kinase inhibitory potencies of compounds **3**, **8**, **13**, **14**, **17** and **18** were evaluated at 10 μM and 1 μM concentrations in duplicate assays against all three Pim family kinases at the International Centre for Kinase Profiling (Dundee, UK) [13]. IC_{50} values were determined for compounds that when tested at 1 μM inhibited the kinase >90%. As shown in Table 1, Pim-1 and Pim-3 were the most inhibited isoforms. Even if all of them demonstrated a substantial Pim kinase inhibitory potency, none of the tested compounds bearing only one substituent at the N-10 position were found to be more active than the bridged derivative **B**. Actually, the best inhibitory potencies toward Pim-1 in this series were observed for bridged compounds **3** and **17** with IC_{50} values of 42 nM and 9 nM, respectively. Compounds **3** and **17** were also the best Pim-3 inhibitors with IC_{50} values of 45 nM and 26 nM, respectively. In comparison to **B**, best active compound **17** exhibited similar potencies toward Pim-1 and Pim-3 whereas compound **3** was less active especially toward Pim-1 with a 5-fold lower potency.

To better understand the lower activity of compound **3** toward Pim-1 and Pim-3 compared to those of **17** and **B**, molecular modeling studies were performed. For compounds **3** and **17**, docking experiments in Pim-1 and Pim-3 were achieved taking into account the different conformations of the flexible bridge. Similar results were found for the two enzymes. In Fig. 2 are presented the two best docking models obtained for both compounds within Pim-1 ATP-binding pocket. As previously shown for compounds **A** [4] and **B** [8], the pyrrolocarbazole scaffold of **3** and **17** is inserted into the cleft with stabilizing hydrophobic interactions involving the pyrrolocarbazole moiety and a hydrogen bond between the ormyl group and Lys67 residue. Interestingly, the bridge of

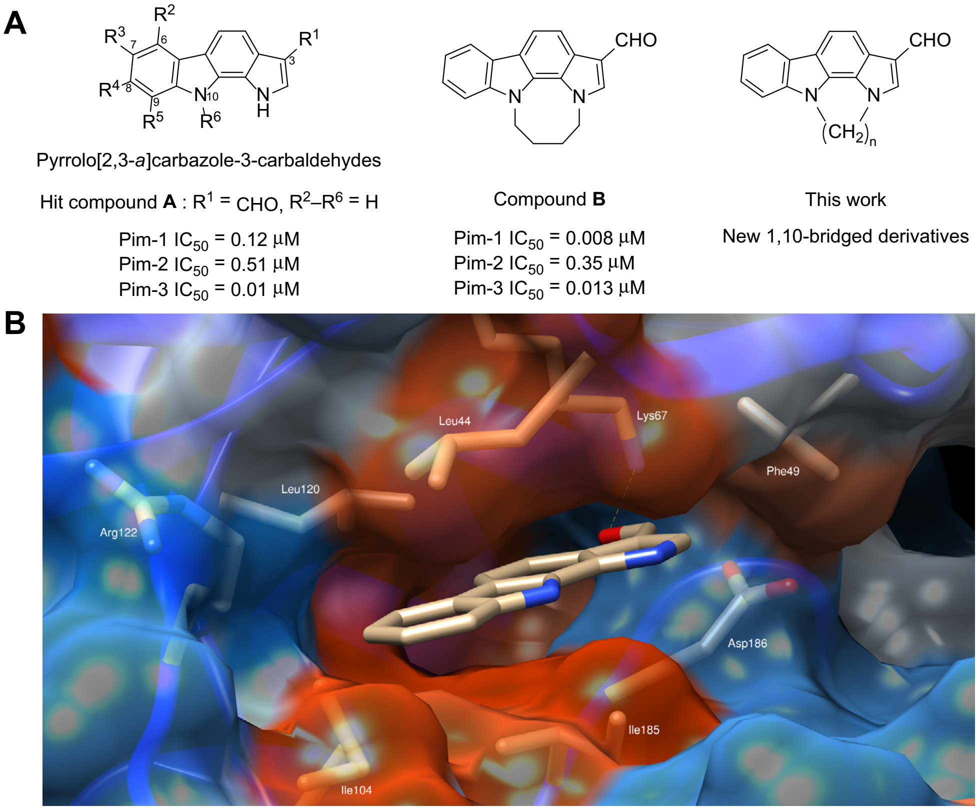
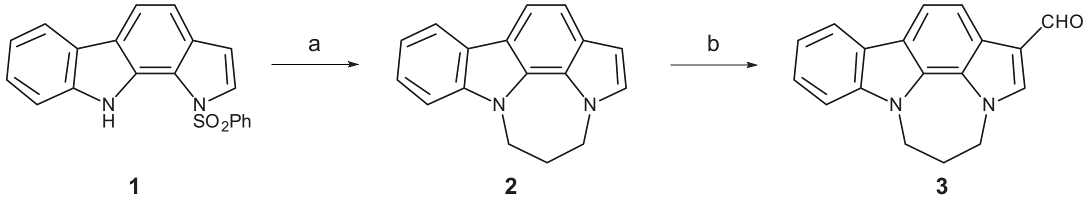
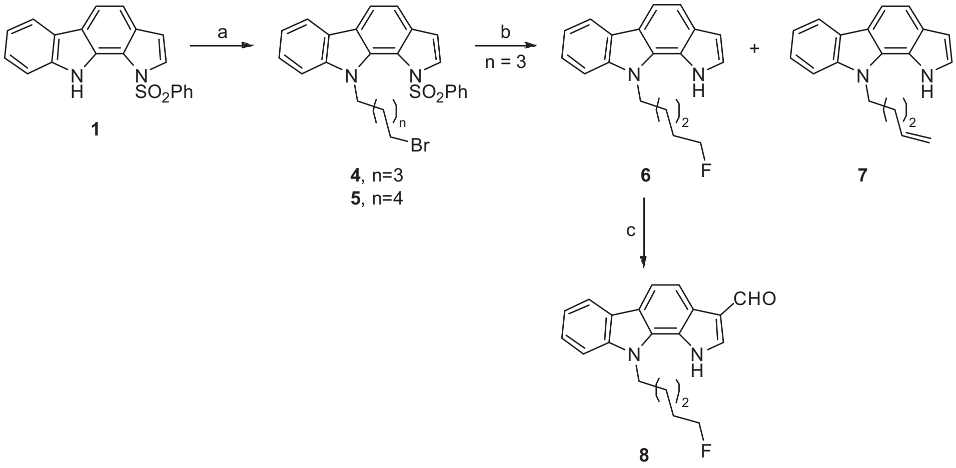


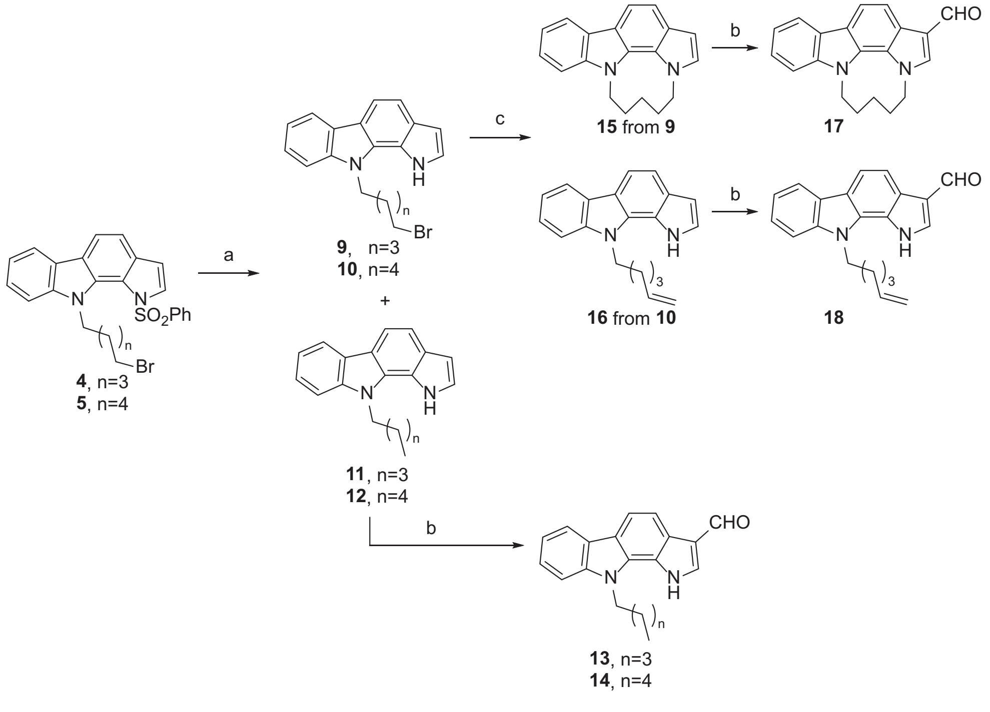
Fig. 1. (A) General structures of pyrrolo[2,3-*a*]carbazole-3-carbaldehyde series and 1,10-bridged derivative **B**. (B) Hydrophobicity surface representation of Pim-1 ATP binding site in complex with compound **A** (PDB code 3JPV [4]). Surfaces are colored in orange (hydrophobic) and in blue (hydrophilic). Hydrogen bond is indicated in yellow dashed line. Image was produced using UCSF Chimera [9].



Scheme 1. Synthesis of bridged compounds **2** and **3**. Reagents and conditions: (a) 1,3-dibromopropane, ^{t}BuONa, toluene, 35%; (b) (i) $POCl_3$, DMF, (ii) NaOH, 51%.



Scheme 2. Preparation of compounds **4–8**. Reagents and conditions: (a) NaH, 1,5-dibromopentane or 1,6-dibromohexane, **4** = 87%, **5** = 52%; (b) TBAF, THF, **6** = 65%, **7** = 21%; (c) (i) $POCl_3$, DMF, (ii) NaOH, 46%.



Scheme 3. Preparation of compounds **9–18**. Reagents and conditions: (a) Mg, MeOH, **9** = 30%, **11** = 14%, **10** = 60%, **12** = 27%; (b) (i) $POCl_3$, DMF, (ii) NaOH, **13** = 59%, **14** = 62%, **17** = 96%, **18** = 77%; (c) 50% aq. NaOH, TBAB, toluene, **15** = 22%, **16** = 15%.

compound **17** is folded toward Ile185 side chain, making additional hydrophobic interactions which were not found in the case of compound **3**. Therefore, we could hypothesize that these supplementary interactions could play a role in the best inhibitory potency of compound **17** compared to the one of **3**.

To characterize the intact cell action for the two most potent derivatives (**3** and **17**), they were tested for ability to induce death of acute myeloid leukemia IPC-81 cells. We found that compounds **3** and **17** both could induce cell death with typical apoptotic features, like nuclear condensation and fragmentation. When

Table 1
% ±SD of residual kinase activity at 10 μM and 1 μM of tested compounds (IC_{50} ± SD (μM) in brackets when determined). clogP were calculated using ACD/ChemSketch freeware version [14].

Cpds	clogP	Kinase inhibitory potencies					
		Pim-1		Pim-2		Pim-3	
		10 μM	1 μM	10 μM	1 μM	10 μM	1 μM
3	3.85	1.5 ± 0.2	5.5 ± 0.7	25 ± 6	69 ± 6	2.8 ± 0.3	9 ± 1
		(0.042 ± 0.005)				(0.05 ± 0.01)	
8	4.80	6.0 ± 0.6	33 ± 3	54.4 ± 0.0	96 ± 1	3.3 ± 0.9	11.8 ± 0.1
13	5.58	8 ± 2	44 ± 3	63 ± 3	105 ± 14	4.2 ± 0.2	15.6 ± 0.1
14	6.11	22 ± 2	74 ± 6	80 ± 5	102 ± 1	7.1 ± 0.3	29 ± 1
17	4.74	1.3 ± 0.1	2.6 ± 0.5	21.3 ± 0.2	56 ± 7	2.6 ± 0.1	2.9 ± 0.4
		(0.009 ± 0.002)				(0.026 ± 0.001)	
18	5.67	9 ± 1	46 ± 4	58.1 ± 0.5	92 ± 17	4.5 ± 0.4	16 ± 1

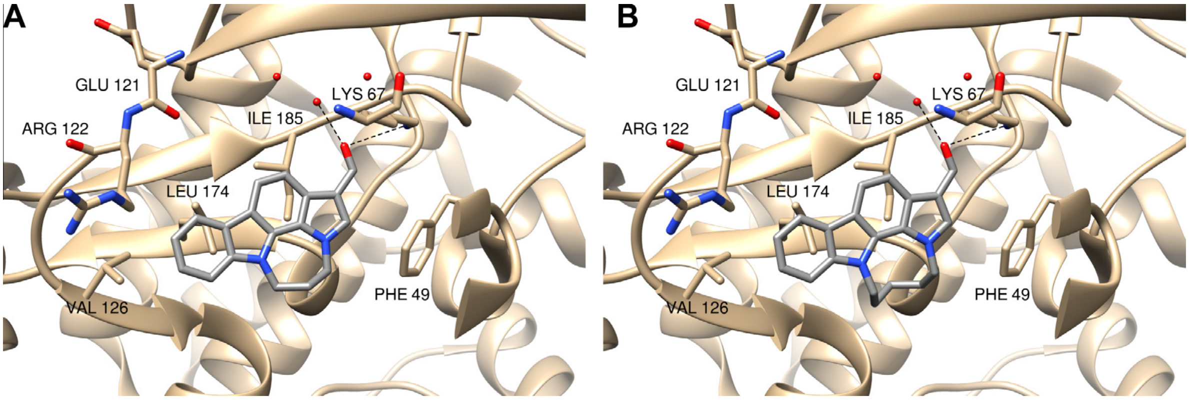


Fig. 2. Docking models of compounds **3** (A) and **17** (B) within the ATP-binding pocket of Pim-1.

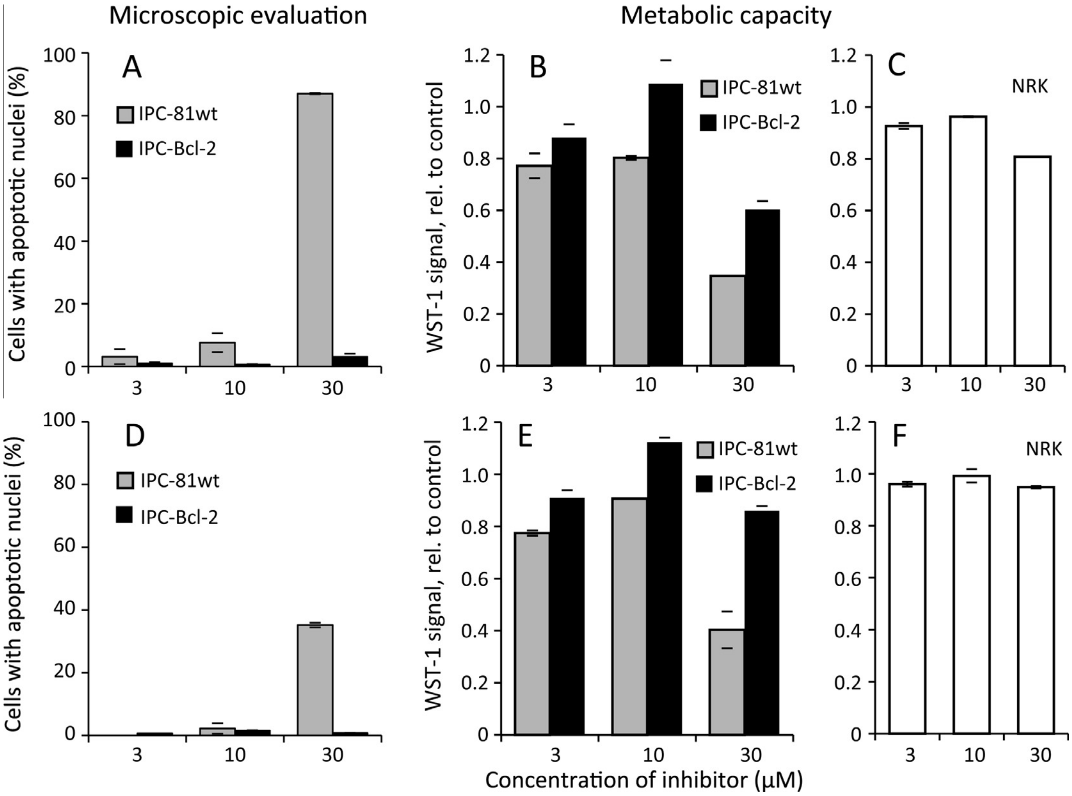


Fig. 3. Bioactivity of compounds **17** (A–C) and **3** (D–F) against acute myeloid leukemia (IPC-81wt and IPC-Bcl-2) and normal rat kidney (NRK) cells. The cells were incubated with the given concentrations of Pim kinase inhibitors and evaluated for percent apoptotic nuclei (A and D) or metabolic capacity by the WST-1 assay (B, C, E and F). The data are average of two separated experiments and the upper and lower values are indicated.

tested at a medium concentration of 30 μM compound **17** gave higher apoptosis than compound **3** (Fig. 3A and D).

The apoptosis was completely inhibited in cells expressing the anti-apoptotic survival protein Bcl-2 (Fig. 3A and D), confirming that the death induced was of the apoptotic type. The metabolic capacity of the cells was determined by the WST-1 assay, which is based on the mass of housekeeping mitochondrial enzymes. We found that the IPC wild-type cells were affected by a 30 μM solution of compounds **3** or **17** also using this assay (Fig. 3B and E). Intriguingly, the IPC-cells overexpressing Bcl-2 also showed a moderate loss of metabolic capacity (Fig. 3B and E). This may reflect reduced cell number due to decreased proliferation since Bcl-2 is not expected to protect cells against anti-mitotic effects. Neither of the compounds induced apoptosis in normal rat kidney (NRK) fibroblasts assessed by microscopy (data not shown), or decreased the metabolic capacity (Fig. 3C and F) of these cells.

Finally, clogP values were calculated for all formylated pyrrolocarbazole derivatives. As indicated in Table 1, all compounds showed clogP comprised between 3.85 and 6.11. More particularly, the logP calculated for the most interesting compounds of this series (**3** and **17**) were in accordance with the Lipinski's rule of five [15].

3. Conclusion

In conclusion, new pyrrolocarbazole-3-carbaldehydes substituted at N-1 and/or N-10 positions by various alkyl chains were synthesized. The evaluation of the Pim kinase inhibitory potency of the target pyrrolo[2,3-*a*]carbazole-3-carbaldehydes, as well as the one of the side products obtained, demonstrated that all the compounds tested were reasonable Pim-1 and Pim-3 kinase inhibitors. Nevertheless, the best Pim-1 and Pim-3 activities were observed for bridged derivatives **3** and **17** with nanomolar inhibitory potencies. These derivatives also showed death-inducing activity against the IPC-81 AML cell line, which is a good predictor of leukemia therapy *in vivo* [16]. Since there was no observable effect on normal rat kidney cells, we believe that compounds **3** and **17** should be further explored as anti-AML drug candidates or lead compounds.

The results obtained in this study confirmed that the introduction of an aliphatic bridge between N-1 and N-10 positions of the pyrrolo[2,3-*a*]carbazole scaffold could lead to interesting Pim kinase inhibitory potencies. The preparation of new analogues to enlarge the SAR performed in this series is currently under investigation.

4. Experimental part

4.1. Chemistry

Starting materials were obtained from commercial suppliers and used without further purification. IR spectra were recorded on Shimadzu FTIR-8400S or Perkin–Elmer Spectrum 65 FT-IR spectrometers ($\bar{\nu}$ in cm^{-1}). NMR spectra, performed on a Bruker AVANCE 400 spectrometer (^1H: 400 MHz, ^{13}C: 100 MHz), or a Bruker AVANCE 500 spectrometer (^1H: 500 MHz, ^{13}C: 126 MHz), are reported in ppm using the solvent residual peak as an internal standard; the following abbreviations are used: singlet (s), doublet (d), triplet (t), doublet of doublet (dd), doublet of doublet of doublet (ddd), doublet of triplet (dt), multiplet (m), broad signal (br s). High resolution mass spectra were determined on a high-resolution Micro Q-Tof apparatus (UBP-START, Université Blaise Pascal, Clermont-Ferrand, France). Chromatographic purifications were performed by column chromatography using 40–63 μm silica gel. Reactions were monitored by TLC using fluorescent silica gel plates (60 F254 from Merck). Melting points were measured on a Stuart SMP3 apparatus and are uncorrected.

4.2. 5,6-Dihydro-4H-indolo[1,2,3-*ef*]pyrrolo[3,2,1-*jk*][1,5]benzodiazepine (**2**)

To a solution of **1** (200 mg, 0.578 mmol) and 1,3-dibromopropane (234 μL, 2.31 mmol) in toluene (20 mL) was added ^{t}BuONa (277 mg, 2.89 mmol). The reaction mixture was stirred at 100 °C for 4 h. After cooling, water was added (50 mL) and the product was extracted with EtOAc (120 mL). The combined organic fractions were washed with a brine solution, dried over $MgSO_4$ and evaporated. Column chromatography (Pentane/EtOAc, 8:1) provided **2** (50.1 mg, 0.20 mmol, 35%) as a beige solid. Mp = 151–152 °C; IR (ATR): 1646, 1490, 1449, 1339, 1187 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 2.54–2.58 (2H, m), 4.52–4.58 (4H, m), 6.58 (1H, d, *J* = 3.2 Hz), 7.18 (1H, ddd, J_1 = 8 Hz, J_2 = 7 Hz, J_3 = 1 Hz), 7.32–7.38 (3H, m), 7.61 (1H, d, *J* = 8.0 Hz), 7.74 (1H, d, *J* = 8.0 Hz), 8.08 (1H, d, *J* = 7.7 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 28.7, 46.3, 50.1 (CH_2), 102.6, 109.2, 112.0, 112.1, 118.8, 119.2, 123.4, 127.1 (CH_{arom}), 115.3, 122.5, 123.9, 126.6, 127.5, 138.6 (C_{arom}); HRMS (ESI+) calcd for $C_{17}H_{15}N_2$ $(M + H)^+$ 247.1235, found 247.1232.

4.3. 5,6-Dihydro-4H-indolo[1,2,3-*ef*]pyrrolo[3,2,1-*jk*][1,5]benzodiazepine-1-carbaldehyde (**3**)

To a round-bottom flask containing 1.5 mL of anhydrous DMF at 0 °C under argon was slowly added phosphorus oxychloride (44.6 μL, 0.477 mmol). The solution was stirred for 1 h at 0 °C before adding of solution of **2** (39.1 mg, 0.159 mmol) in anhydrous DMF (1 mL). The reaction mixture was stirred at room temperature for 1 h before addition of a saturated aqueous $NaHCO_3$ solution (30 mL). After stirring for 1 h 30, the mixture was filtrated under vacuum and the yellow solid obtained was added to a 5 M aqueous NaOH solution (30 mL). The mixture was stirred at 50 °C during 16 h. After cooling, the mixture was extracted with EtOAc (90 mL). The combined organic fractions were washed with water, dried over $MgSO_4$ and evaporated. Column chromatography (CH_2Cl_2/MeOH, 98:2) provided **3** (22.1 mg, 0.08 mmol, 51%) as beige solid. Mp = 201–202 °C; IR (ATR): 1653, 1532, 1449, 1395, 1329 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 2.59–2.64 (2H, m), 4.61–4.63 (2H, m), 4.67–4.70 (2H, m), 7.24 (1H, ddd, J_1 = 8 Hz, J_2 = 7 Hz, J_3 = 1 Hz), 7.44 (1H, ddd, J_1 = 8.5 Hz, J_2 = 7 Hz, J_3 = 1 Hz), 7.66 (1H, d, *J* = 8.5 Hz), 7.93 (1H, d, *J* = 8.5 Hz), 8.01 (1H, d, *J* = 8.5 Hz), 8.16 (1H, d, *J* = 8 Hz), 8.31 (1H, s), 9.99 (1H, s, CHO); ^{13}C NMR (100 MHz, DMSO-d_6): 27.8, 46.3, 50.9 (CH_2), 109.4, 111.9, 115.2, 119.2, 119.6, 124.3, 138.6 (CH_{arom}), 117.5, 118.8, 123.1, 123.3, 123.6, 127.1, 138.9 (C_{arom}), 184.7 (CHO); HRMS (ESI+) calcd for $C_{18}H_{15}N_2O$ $(M + H)^+$ 275.1184 found 275.1190.

4.4. 1-(Benzenesulfonyl)-10-(5-bromopentyl)-1,10-dihydropyrrolo[2,3-*a*]carbazole (**4**)

To a solution of **1** (200 mg, 0.58 mmol) in 1,5-dibromopentane (1.25 mL, 9.24 mmol) was added NaH 60% dispersion in mineral oil (92.4 mg, 2.31 mmol). The reaction mixture was stirred at 100 °C for 4 h before cooling and addition of a 1 M aqueous HCl solution (20 mL). The aqueous layer was extracted with EtOAc (120 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated. Column chromatography (Cyclohexane/EtOAc, 100:0 to 97.5:2.5) provided **4** (248.1 mg, 0.50 mmol, 87%) as a light brown oil. IR (ATR): 2930, 1448, 1364, 1325, 1185, 1170 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 0.87–0.94 (2H, m), 1.29–1.36 (2H, m), 1.53–1.60 (2H, m), 3.22 (2H, t, *J* = 6.4 Hz), 4.92 (2H, t, *J* = 7.2 Hz), 6.94 (1H, d, *J* = 3.6 Hz), 7.16 (2H, dd, J_1 = 8.0 Hz, J_2 = 0.8 Hz), 7.23–7.31 (4H, m), 7.44–7.54 (3H, m), 7.78 (1H, d, *J* = 8.0 Hz), 8.11 (1H, d, $J = 8.0$ Hz), 8.21 (1H, d, $J = 7.6$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 24.5, 26.7, 31.4, 34.6, 45.7 (CH_2), 111.7, 113.2, 118.4, 119.1, 120.0, 120.2, 125.6, 126.9 (2C), 128.4 (2C), 131.3, 134.2 (CH_{arom}), 123.0, 123.4, 124.1, 132.9, 133.4, 133.5, 141.7 (C_{arom}); HRMS (ESI+) calcd for $C_{25}H_{24}{}^{79}BrN_2O_2S$ $(M + H)^+$ 495.0742 found 495.0768.

*4.5. 1-(Benzenesulfonyl)-10-(6-bromohexyl)-1,10-dihydropyrrolo[2,3-a]carbazole (**5**)*

Following the same procedure as described for **4**, compound **5** (768 mg, 1.51 mmol, 52%) was obtained as a orange yellow oil after column chromatography (CH_2Cl_2/MeOH, 98:2) from **1** (1 g, 2.89 mmol) and 1,6-dibromohexane (7.0 mL, 46.24 mmol). IR (ATR): 2935, 1747, 1367, 1228, 1196 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 0.71–0.79 (2H, m), 1.08–1.15 (2H, m), 1.27–1.36 (2H, m), 1.39–1.47 (2H, m), 3.26 (2H, t, $J = 6.8$ Hz), 4.90 (2H, t, $J = 6.8$ Hz), 6.93 (1H, d, $J = 3.6$ Hz), 7.16 (2H, dd, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz), 7.22–7.30 (4H, m), 7.48–7.53 (3H, m), 7.76 (1H, d, $J = 8.0$ Hz), 8.11 (1H, d, $J = 8.0$ Hz), 8.20 (1H, d, $J = 8.0$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 24.7, 26.5, 27.1, 31.8, 34.5, 45.6 (CH_2), 111.6, 113.0, 118.3, 119.0, 119.8, 120.1, 125.5, 126.8 (2C), 128.4 (2C), 131.2, 134.1 (CH_{arom}), 122.9, 123.3, 124.0, 132.9, 133.36, 133.39, 141.6 (C_{arom}); HRMS (ESI+) calcd for $C_{26}H_{26}{}^{79}BrN_2O_2S$ $(M + H)^+$ 509.0898, found 509.0882.

*4.6. 10-(5-Fluoropentyl)-1,10-dihydropyrrolo[2,3-a]carbazole (**6**) and 10-(Pent-4-en-1-yl)-1,10-dihydropyrrolo[2,3-a]carbazole (**7**)*

To a solution of **4** (137.6 mg, 0.28 mmol) in THF (20 mL) was added a 1 M solution of TBAF in THF (0.278 μL, 0.28 mmol). The reaction mixture was refluxed during 20 h before adding three more equiv. of TBAF (0.834 μL, 0.834 mmol). The mixture was then refluxed for 42 h. After cooling, water (30 mL) was added before extraction with EtOAc (120 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated. Column chromatography (Cyclohexane/EtOAc, 100:0 to 88:12) provided **6** (53.5 mg, 0.18 mmol, 65%) and **7** (16 mg, 0.06 mmol, 21%) as brown oils.

Compound 6: IR (ATR): 3518–3360, 2925, 2852, 1630, 1469, 1388, 1335 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.35–1.43 (2H, m), 1.53–1.67 (2H, m), 1.76–1.85 (2H, m), 4.33 (2H, dt, $J_{H-F} = 47.5$ Hz, $J_{H-H} = 6.0$ Hz), 4.73 (2H, t, $J = 6.8$ Hz), 6.64 (1H, dd, $J_1 = 2.8$ Hz, $J_2 = 1.6$ Hz), 7.17 (1H, t, $J = 7.2$ Hz), 7.33–7.42 (3H, m), 7.62 (1H, d, $J = 8.0$ Hz), 7.76 (1H, d, $J = 8.4$ Hz), 8.07 (1H, d, $J = 7.6$ Hz), 11.35 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 22.1 (d, $J_{CF} = 4.9$ Hz), 29.7 (d, $J_{CF} = 19.0$ Hz), 30.1, 43.7, 83.7 (d, $J_{CF} = 160.8$ Hz) (CH_2), 102.9, 109.3, 112.2, 112.4, 118.7, 119.1, 123.5, 124.2 (CH_{arom}), 116.3, 120.9, 123.7, 127.2, 127.6, 138.8 (C_{arom}); HRMS (ESI+) calcd for $C_{19}H_{20}FN_2$ $(M + H)^+$ 295.1611 found 295.1605.

Compound 7: IR (ATR): 3510–3372, 2930, 1635, 1468, 1389, 1338 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.82–1.90 (2H, m), 1.99–2.10 (2H, m), 4.73 (2H, t, $J = 6.8$ Hz), 4.90–4.97 (2H, m), 5.74–5.85 (1H, m), 6.64 (1H, dd, $J_1 = 2.8$ Hz, $J_2 = 1.6$ Hz), 7.17 (1H, t, $J = 7.6$ Hz), 7.33–7.44 (3H, m), 7.60 (1H, d, $J = 8.4$ Hz), 7.77 (1H, d, $J = 6.8$ Hz), 8.08 (1H, d, $J = 7.6$ Hz), 11.36 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 29.4, 30.2, 43.3 (CH_2), 114.9 (=CH_2), 102.9, 109.2, 112.1, 112.4, 118.7, 119.0, 123.4, 124.2, 138.0 (CH_{arom} + CH_{alkene}), 116.3, 120.9, 123.7, 127.2, 127.6, 138.7 (C_{arom}); HRMS (ESI+) calcd for $C_{19}H_{19}N_2$ $(M + H)^+$ 275.1548 found 275.1554.

*4.7. 10-(5-Fluoropentyl)-1,10-dihydropyrrolo[2,3-a]carbazole-3-carbaldehyde (**8**)*

Using the same procedure as described for **3**, compound **8** ($m = 21.5$ mg, 0.07 mmol, 46%) was obtained as a beige solid after column chromatography (CH_2Cl_2/MeOH, 98:2) from **6** (44 mg, 0.15 mmol). Mp = 214–215 °C; IR (ATR): 3433–3117, 2925, 2858, 1630, 1426 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.33–1.42 (2H, m), 1.52–1.66 (2H, m), 1.76–1.84 (2H, m), 4.32 (2H, dt, $J_{H-F} = 47.6$ Hz, $J_{H-H} = 6.0$ Hz), 4.75 (2H, t, $J = 6.8$ Hz), 7.22 (1H, t, $J = 7.2$ Hz), 7.42 (1H, t, $J = 7.6$ Hz), 7.68 (1H, d, $J = 8.0$ Hz), 7.97–8.03 (2H, m), 8.14 (1H, d, $J = 7.6$ Hz), 8.38 (1H, d, $J = 7.2$ Hz), 10.06 (1H, s, CHO), 12.33 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 22.0 (d, $J_{CF} = 5.3$ Hz), 29.7 (d, $J_{CF} = 19.2$ Hz), 29.9, 43.7, 83.7 (d, $J_{CF} = 160.7$ Hz) (CH_2), 109.5, 112.3, 115.2, 119.1, 119.5, 124.4, 137.4 (CH_{arom}), 118.4, 119.1, 122.0, 123.1, 123.8, 126.8, 139.2 (C_{arom}), 185.2 (CHO); HRMS (ESI+) calcd for $C_{20}H_{20}FN_2O$ $(M + H)^+$ 323.1560 found 323.1544.

*4.8. 10-(5-bromopentyl)-1,10-dihydropyrrolo[2,3-a]carbazole (**9**) and 10-Pentyl-1,10-dihydropyrrolo[2,3-a]carbazole (**11**)*

To a solution of **4** (1.14 g, 2.30 mmol) in 80 mL of anhydrous MeOH at room temperature under argon were added magnesium turnings (0.33 g, 13.80 mmol). The suspension was stirred for 3 days at room temperature in the dark. A saturated solution of ammonium chloride was then slowly added and the aqueous layer was extracted with CH_2Cl_2. The combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (Cyclohexane/EtOAc, 99:1 to 95:5) to give **9** (253 mg, 0.71 mmol, 30%) as a brown yellow oil and **11** (91 mg, 0.33 mmol, 14%) as a beige powder. Starting material (524 mg) was also recovered unchanged.

Compound 9: IR (ATR): 2923, 2853, 1467, 1185 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.41–1.49 (2H, m), 1.72–1.82 (4H, m), 3.42 (2H, t, $J = 6.8$ Hz), 4.72 (2H, t, $J = 7.2$ Hz), 6.64 (1H, dd, $J_1 = 2.8$ Hz, $J_2 = 1.6$ Hz), 7.17 (1H, t, $J = 7.2$ Hz), 7.32–7.43 (3H, m), 7.60 (1H, d, $J = 8.0$ Hz), 7.76 (1H, d, $J = 8.4$ Hz), 8.06 (1H, d, $J = 7.6$ Hz), 11.35 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.7, 29.5, 32.0, 34.9, 43.5 (CH_2), 102.8, 109.2, 112.1, 112.3, 118.6, 118.9, 123.4, 124.1 (CH_{arom}), 116.2, 120.9, 123.6, 127.1, 127.6, 138.6 (C_{arom}); HRMS (ESI+) calcd for $C_{19}H_{20}{}^{79}BrN_2$ $(M + H)^+$ 355.0810, found 355.0814.

Compound 11: Mp: 99–100 °C; IR (ATR): 3505–3410, 2926, 1469, 1388, 1337 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 0.76 (3H, t, $J = 6.8$ Hz), 1.22–1.28 (4H, m), 1.73–1.79 (2H, m), 4.70 (2H, t, $J = 6.8$ Hz), 6.63 (1H, dd, $J_1 = 2.8$ Hz, $J_2 = 2.0$ Hz), 7.16 (1H, t, $J = 7.2$ Hz), 7.32–7.42 (3H, m), 7.59 (1H, d, $J = 8.0$ Hz), 7.75 (1H, d, $J = 8.4$ Hz), 8.06 (1H, d, $J = 8.0$ Hz), 11.32 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 13.9 (CH_3), 22.1, 28.3, 30.1, 43.8 (CH_2), 102.9, 109.2, 112.2, 112.4, 118.6, 119.0, 123.4, 124.2 (CH_{arom}), 116.2, 121.0, 123.7, 127.3, 127.6, 138.8 (C_{arom}); HRMS (ESI+) calcd for $C_{19}H_{21}N_2$ $(M + H)^+$ 277.1705, found 277.1711.

*4.9. 10-(6-Bromohexyl)-1,10-dihydropyrrolo[2,3-a]carbazole (**10**) and 10-Hexyl-1,10-dihydropyrrolo[2,3-a]carbazole (**12**)*

Following the same procedure as described for **9** and **11**, compounds **10** (198 mg, 0.54 mmol, 60%) and **12** (70 mg, 0.24 mmol, 27%) were obtained as green yellow oils from derivative **5** (452 mg, 0.887 mmol). Traces of starting material were also observed on TLC (not isolated).

Compound 10: IR (ATR): 3500–3289, 2967–2807, 1703, 1335 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.32–1.35 (4H, m), 1.65–1.79 (4H, m), 3.43 (2H, t, $J = 6.8$ Hz), 4.70 (2H, t, $J = 6.8$ Hz), 6.63 (1H, dd, $J_1 = 2.8$ Hz, $J_2 = 1.6$ Hz), 7.16 (1H, t, $J = 7.2$ Hz), 7.32–7.43 (3H, m), 7.60 (1H, d, $J = 8.0$ Hz), 7.75 (1H, d, $J = 8.4$ Hz), 8.06 (1H, d, $J = 7.4$ Hz), 11.32 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 25.0, 27.2, 30.0, 32.0, 34.8, 43.6 (CH_2), 102.8, 109.1, 112.0, 112.3, 118.5, 118.9, 123.3, 124.1 (CH_{arom}), 116.2, 120.8, 123.6, 127.1, 127.5, 138.6 (C_{arom}); HRMS (ESI+) calcd for $C_{20}H_{22}{}^{79}BrN_2$ $(M + H)^+$ 369.0966, found 369.0970.

Compound 12: IR (ATR): 3516–3307, 3012–2783, 1448, 1334 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 0.76 (3H, t, J = 7.8 Hz), 1.19–1.33 (6H, m), 1.72–1.79 (2H, m), 4.70 (2H, t, J = 7.2 Hz), 6.63 (1H, dd, J_1 = 2.8 Hz, J_2 = 1.6 Hz), 7.16 (1H, t, J = 7.2 Hz), 7.31–7.42 (3H, m), 7.59 (1H, d, J = 7.6 Hz), 7.75 (1H, d, J = 8.4 Hz), 8.06 (1H, d, J = 7.6 Hz), 11.31 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 13.7 (CH_3), 21.9, 25.6, 30.2, 31.0, 43.7 (CH_2), 102.7, 109.1, 112.0, 112.2, 118.5, 118.9, 123.3, 124.1 (CH_{arom}), 116.1, 120.9, 123.6, 127.2, 127.5, 138.7 (C_{arom}); HRMS (ESI+) calcd for $C_{20}H_{23}N_2$ $(M + H)^+$ 291.1861, found 291.1853.

*4.10. 10-pentyl-1,10-dihydropyrrolo[2,3-a]carbazole-3-carbaldehyde (**13**) and 10-Hexyl-1,10-dihydropyrrolo[2,3-a]carbazole-3-carbaldehyde (**14**)*

Following the same procedure as described for **3**, compounds **13** (59 mg, 0.194 mmol, 59%) and **14** (68 mg, 0.213 mmol, 62%) were obtained as gray powders from derivatives **11** (91 mg, 0.329 mmol) and **12** (68 mg, 0.327 mmol) respectively after column chromatography (Cyclohexane/EtOAc, 1:1 to 1:5).

Compound 13: Mp: 225–226 °C; IR (ATR): 1747, 1631, 1224 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 0.74 (3H, t, J = 7.2 Hz), 1.15–1.32 (4H, m), 1.72–1.79 (2H, m), 4.73 (2H, t, J = 6.8 Hz), 7.21 (1H, t, J = 7.6 Hz), 7.41 (1H, t, J = 8.0 Hz), 7.64 (1H, d, J = 8.0 Hz), 8.01 (2H, s), 8.14 (1H, d, J = 8.0 Hz), 8.37 (1H, d, J = 2.4 Hz), 10.07 (1H, s, CHO), 12.32 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 13.7 (CH_3), 21.9, 28.1, 29.8, 43.7 (CH_2), 109.4, 112.2, 115.2, 119.0, 119.5, 124.3, 137.3 (CH_{arom}), 118.3, 119.1, 121.9, 123.0, 123.7, 126.7, 139.1 (C_{arom}), 185.1 (CHO); HRMS (ESI+) calcd for $C_{20}H_{21}N_2O$ $(M + H)^+$ 305.1654, found 305.1649.

Compound 14: Mp: 192–193 °C; IR (ATR): 3368–3112, 2973–2771, 1631, 1429, 1113 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 0.72 (3H, t, J = 7.2 Hz), 1.11–1.30 (6H, m), 1.71–1.78 (2H, m), 4.72 (2H, t, J = 6.8 Hz), 7.21 (1H, t, J = 7.2 Hz), 7.41 (1H, t, J = 7.2 Hz), 7.65 (1H, d, J = 8.0 Hz), 8.01 (2H, s), 8.14 (1H, d, J = 7.6 Hz), 8.37 (1H, s), 10.07 (1H, s, CHO), 12.31 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 13.7 (CH_3), 21.9, 25.6, 30.0, 30.9, 43.7 (CH_2), 109.4, 112.2, 115.2, 119.0, 119.5, 124.3, 137.3 (CH_{arom}), 118.3, 119.1, 121.9, 123.0, 123.7, 126.7, 139.1 (C_{arom}), 185.2 (CHO); HRMS (ESI+) calcd for $C_{21}H_{23}N_2O$ $(M + H)^+$ 319.1810, found 319.1812.

*4.11. 5,6,7,8-Tetrahydro-4H-indolo[1,2,3-gh]pyrrolo[3,2,1-lm][1,7]benzodiazonine (**15**)*

To a solution of **9** (227 mg, 0.64 mmol) in 2 mL of toluene were successively added *tetra*-butylammonium bromide (82 mg, 0.256 mmol) and 50% aqueous NaOH solution (844 μL). The reaction mixture was vigorously stirred at room temperature for 15 h before addition of water and EtOAc. The product was extracted with EtOAc and the combined organic fractions were washed with saturated NaCl solution, dried over $MgSO_4$ and evaporated under reduced pressure. Column chromatography (Cyclohexane/EtOAc, 100:0 to 92:8) provided **15** (38 mg, 0.14 mmol, 22%) as a beige yellow powder. Mp: 112–114 °C; IR (ATR): 2957–2824, 1738, 1365 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.35–1.41 (2H, m), 1.95–2.08 (4H, m), 4.52–5.18 (4H, m), 6.61 (1H, d, J = 2.8 Hz), 7.18 (1H, t, J = 7.6 Hz), 7.33–7.41 (3H, m), 7.61 (1H, d, J = 8.4 Hz), 7.83 (1H, d, J = 8.4 Hz), 8.10 (1H, d, J = 7.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 19.4, 30.5, 32.5, 45.2, 50.6 (CH_2), 102.5, 108.9, 112.1, 113.1, 118.76, 118.78, 123.8, 130.4 (CH_{arom}), 117.5, 123.2, 123.4, 129.2, 129.9, 138.5 (C_{arom}); HRMS (ESI+) calcd for $C_{19}H_{19}N_2$ $(M + H)^+$ 275.1548, found 275.1556.

*4.12. 10-(Hex-5-en-1-yl)-1,10-dihydropyrrolo[2,3-a]carbazole (**16**)*

Following the same procedure as described for **15**, compound **16** (27 mg, 0.094 mmol, 15%) was obtained as a pale yellow powder from derivative **10** (230 mg, 0.623 mmol) after column chromatography (Cyclohexane/EtOAc, 98:2 to 85:15). Mp: 86–87 °C; IR (ATR): 3484–3371, 2962–2775, 1467, 1386 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.35–1.43 (2H, m), 1.72–1.80 (2H, m), 1.94–2.00 (2H, m), 4.72 (2H, t, J = 6.8 Hz), 4.84–4.92 (2H, m), 5.62–5.73 (1H, m), 6.62 (1H, dd, J_1 = 2.4 Hz, J_2 = 1.6 Hz), 7.16 (1H, t, J = 7.2 Hz), 7.33–7.42 (3H, m), 7.60 (1H, d, J = 8.0 Hz), 7.75 (1H, d, J = 7.6 Hz), 8.06 (1H, d, J = 8.0 Hz), 11.32 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 25.2, 29.8, 32.9, 43.5 (CH_2), 114.8 ($=CH_2$), 102.8, 109.2, 112.1, 112.3, 118.5, 118.9, 123.3, 124.1, 138.4 ($CH_{arom} + CH_{alkene}$), 116.2, 120.8, 123.6, 127.2, 127.5, 138.7 (C_{arom}); HRMS (ESI+) calcd for $C_{20}H_{21}N_2$ $(M + H)^+$ 289.1705, found 289.1714.

*4.13. 5,6,7,8-Tetrahydro-4H-indolo[1,2,3-gh]pyrrolo[3,2,1-lm][1,7]benzodiazonine-1-carbaldehyde (**17**)*

Following the same procedure as described for **3**, compound **17** (37 mg, 0.122 mmol, 96%) was obtained as a white powder from derivative **15** (35 mg, 0.127 mmol) after column chromatography (Cyclohexane/EtOAc, 1:1 to 1:2). Mp: 246–247 °C; IR (ATR): 1649, 1384, 1162 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.25–1.42 (2H, m), 1.99–2.09 (4H, m), 4.65–5.18 (4H, m), 7.24 (1H, t, J = 7.2 Hz), 7.43 (1H, t, J = 7.6 Hz), 7.67 (1H, d, J = 8.0 Hz), 8.08–8.20 (3H, m), 8.33 (1H, s), 9.99 (1H, s, CHO); ^{13}C NMR (100 MHz, DMSO-d_6): 19.2, 30.4, 32.0, 45.1, 51.8 (CH_2), 109.1, 112.5, 115.3, 119.17, 119.19, 124.75, 142.4 (CH_{arom}), 117.5, 119.6, 122.7, 124.70, 125.9, 128.8, 138.8 (C_{arom}), 184.5 (CHO); HRMS (ESI+) calcd for $C_{20}H_{19}N_2O$ $(M + H)^+$ 303.1497, found 303.1503.

*4.14. 10-(Hex-5-en-1-yl)-1,10-dihydropyrrolo[2,3-a]carbazole-3-carbaldehyde (**18**)*

Following the same procedure as described for **3**, compound **18** (17 mg, 0.054 mmol, 77%) was obtained as a gray powder from derivative **16** (20 mg, 0.069 mmol) after column chromatography (Cyclohexane/EtOAc, 1:1). Mp: 188–189 °C; IR (ATR): 3375–3160, 1630, 1431, 1114 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.32–1.39 (2H, m), 1.74–1.80 (2H, m), 1.94–2.00 (2H, m), 4.75 (2H, t, J = 6.8 Hz), 4.83–4.91 (2H, m), 5.63–5.70 (1H, m), 7.22 (1H, t, J = 7.6 Hz), 7.42 (1H, t, J = 7.2 Hz), 7.66 (1H, d, J = 8.0 Hz), 8.01 (2H, s), 8.14 (1H, d, J = 7.6 Hz), 8.37 (1H, s), 10.07 (1H, s, CHO), 12.31 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 25.2, 29.6, 32.9, 43.6 (CH_2), 114.8 ($=CH_2$), 109.5, 112.2, 115.2, 119.02, 119.5, 124.3, 137.3, 138.3 ($CH_{arom} + CH_{alkene}$), 118.3, 119.06, 121.9, 123.0, 123.7, 126.7, 139.1 (C_{arom}), 185.2 (CHO); HRMS (ESI+) calcd for $C_{21}H_{21}N_2O$ $(M + H)^+$ 317.1654, found 317.1639.

4.15. In vitro kinase inhibition assays

Kinase assays were performed by the International Centre for Kinase Profiling (Dundee, UK). The procedures for the *in vitro* protein kinase assays and for the expression and activation of the protein kinases have been described previously [13].

Source and purification of kinases: All protein kinases were of human origin and encoded full-length proteins. All proteins were either expressed as GST (glutathione transferase) fusion proteins in *Escherichia coli* or as hexahistidine (His6)-tagged proteins in Sf21 (*Spodoptera frugiperda* 21) insect cells. GST fusion proteins were purified by affinity chromatography on glutathione–Sepharose, and His6-tagged proteins on nickel/nitrilotriacetate–agarose.

Protein kinase assays: All assays (25.5 μL volume) were carried out robotically at room temperature (21 °C) and were linear with respect to time and enzyme concentration under the conditions used. Assays were performed for 30 min. using Multidrop Micro reagent dispensers (Thermo Electron Corporation, Waltham, MA, U.S.A.) in a 96-well format. The concentration of magnesium acetate in the assays was 10 mM and [γ-^{33}P]ATP (800 c.p.m./pmol) was used at 5 μM for Pim-2 and 20 μM for Pim-1 and Pim-3, in order to be at or below the K_m for ATP for each enzyme.

The assays were initiated with MgATP, stopped by the addition of 5 μL of 0.5 M orthophosphoric acid and spotted on to P81 filter plates using a unifilter harvester (PerkinElmer, Boston, MA, U.S.A.). Kinase substrate was RSRHSSYPAGT (300 μM) for Pim-1, Pim-2 and Pim-3. Inhibitors were dissolved in DMSO and compounds were incubated for 5 min with enzyme before addition of ATP. The enzymes were diluted in a buffer consisting of 50 mM Tris/HCl, pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA and 0.1% 2-mercaptoethanol and assayed in a buffer comprising 50 mM Tris/HCl, pH 7.5, 0.1 mM EGTA and 0.1% 2-mercaptoethanol.

The IC_{50} values of inhibitors were determined after carrying out assays at 10 different concentrations of each compound.

4.16. Cell maintenance and experimental conditions

The leukemia cell lines IPC-81 [17] and its Bcl-2 overexpressing variant IPC-Bcl2 [18] were cultured in DMEM medium (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% horse serum (Invitrogen, Carlsbad, CA, USA), and antibiotics (50 U/mL of penicillin and 0.05 mg/mL streptomycin). NRK cells (ATCC no: CRL-6509) were cultured in DMEM supplemented with fetal bovine serum (Invitrogen, Carlsbad, CA, USA) and antibiotics. Both cell lines were kept in a humidified atmosphere at 37 °C during culturing and experiments. For experiments the IPC-cells were seeded in 0.1 ml fresh medium in 96-well tissue culture plates at 15,000 cells/well the day of the experiments, while NRK cells were seeded at 7000 cells/well and left overnight to attach before experiments were conducted. Compounds were dissolved in DMSO at 10 mM and diluted in medium before added to the cells. After 24 h of incubation, the metabolic activity was recorded by the WST-1 assay (Roche Applied Sciences, Mannheim, Germany) according to the manufacturer instructions. The cells were next fixed in 2% buffered formaldehyde (pH 7.4) and the nuclei stained with the DNA dye Hoechst 33,342 (0.01 mg/mL, Sigma–Aldrich). Cell death was assessed by UV-microscopic evaluation of nuclear morphology as described previously [19].

4.17. Molecular modeling

Conformational analysis of compounds **3** and **17** were conducted with the Random Search module of Sybylx2.1 [20] using the conjugated gradient method in Tripos force field with Gasteiger–Hückel charges (Dielectric Constant: 78, Energy Cutoff: 5.0 kcal mol^{-1}, maximum cycles: 5000; RMS threshold: 0.1 Å, convergence threshold: 0.05). The geometrical optimization of the preferential conformations was performed using Gaussian 09 (Revision D) [21] at the density functional theory level (DFT) [22,23] with B3LYP [24,25] Hybrid Functional and 6-31G (d,p) basis set, leading to two preferential conformations for compound **3** and seven for compound **17**.

The docking studies were performed for each conformer with AutoDock Vina [26]. Preparation of Pim-1 and Pim-3 models has already been previously detailed [27] and water molecules were removed except three back ATP-binding pocket water molecules. Pim-1/Pim-3 and inhibitor (**3**, **17**) pdbqt files were prepared with AutoDockTools (ADT) [28] using Gasteiger–Hückel charges. Docking experiments were performed using the following AutoDock Vina parameters: number of binding modes: 9, maximum energy difference: 3 kcal mol^{-1}, exhaustiveness of search: 8.

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