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Original article

Synthesis and biological activities of 4-substituted pyrrolo[2,3-*a*]carbazole Pim kinase inhibitors

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ABSTRACT

Pyrrolo[2,3-*a*]carbazole-3-carbaldehydes are potent Pim kinase inhibitors with in vitro antiproliferative activities. In the present study, we report the synthesis and biological activities (Pim kinase inhibition and in vitro antiproliferative potency) of new 4-substituted pyrrolo[2,3-*a*]carbazoles. The results demonstrated that the Pim kinase inhibitory potency (especially Pim-3) can be conserved for pyrrolo [2,3-*a*]carbazoles bearing a methoxycarbonyl group at the 4-position without a formyl at the 3-position. Moreover, compound **27** that was found to be active against Pim-1 and Pim-3 kinases showed antiproliferative activities in the micromolar range.

1. Introduction

As part of our studies directed toward the synthesis of biologically active heteroaromatic compounds, we were interested in the preparation of 4-substituted pyrrolo[2,3-*a*]carbazoles as Pim kinase inhibitors. Recently, we reported the synthesis and biological activities of pyrrolo[2,3-*a*]carbazole-3-carbaldehyde **A** that is a potent and selective inhibitor of the Pim kinase family members (Fig. 1) [1]. The structure–activity relationship study achieved around this scaffold showed that this series of compounds constitute potent inhibitors of Pim kinases and attractive molecules for drug development, for instance to inhibit the invasiveness of Pim-overexpressing cancer cells [1–4]. To enlarge the structural diversity introduced on this scaffold, we undertook the preparation of pyrrolo[2,3-*a*]carbazoles bearing carboxy or methoxycarbonyl groups at the 4-position, that could replace the formyl group at the 3-position of the parent series and possibly reinforce the interaction between these compounds and Pim kinase ATP-binding site. Then, the potencies of these new compounds toward the three Pim isoforms were evaluated. In addition, as over-expression of Pim kinases has been observed in various solid tumors such as prostate, the in vitro antiproliferative activities of these compounds were studied toward a human fibroblast primary culture and three human solid cancer cell lines: PC3 and DU145 (prostatic carcinoma) as well as PA1 (ovarian carcinoma).

2. Chemistry

To prepare the 6-amino derivatives, we first envisaged a synthetic pathway starting from commercially available 4-nitroindole-3-carbaldehyde (Scheme 1). Firstly, the indole nitrogen atom was protected using benzenesulfonyl chloride and cesium carbonate in refluxing acetonitrile leading to compound **1** in 94% yield. Methyl 2-bromoacrylate **2** was subsequently obtained in 95% yield from a Wittig type reaction as reported by Bourderioux et al. [5]. All our assays to achieve Suzuki coupling between **2** and pinacol boronate ester **5** failed. Thus, the nitro group of compound **2** was reduced in the presence of iron powder and ammonium

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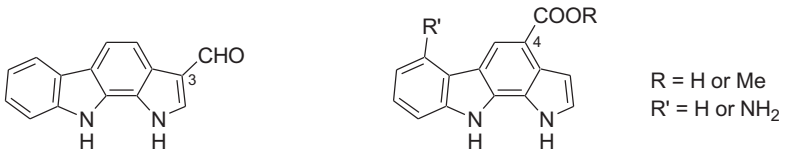


A, pyrrolo[2,3-*a*]carbazole-3-carbaldehyde 4-substituted pyrrolo[2,3-*a*]carbazoles

Fig. 1. Structure of compound **A** and 4-substituted pyrrolo[2,3-*a*]carbazoles.

chloride to give compound **3** in 90% yield [6], and the amino group was protected in the presence of Boc_2O and DMAP leading to compound **4** in 70% yield. Subsequent Suzuki cross-coupling between bromo derivative **4** and compound **5** [7–12], using potassium carbonate and tetrakis(triphenylphosphine)palladium(0) [5] allowed the preparation of compound **6** in 76% yield. Treatment of compound **6** with TBAF [13] led to compound **8** in 56% yield. Unexpectedly, pyrrolocarbazole derivative **7** was also isolated in 5% yield and led to the ring-opening product **9** in 73% yield when the deprotection of the amino group was conducted in the presence of TFA. When we carried on the synthesis from compound **8** in order to cleave the benzenesulfonyl group using magnesium turnings in methanol [14], the reaction yielded reduced compound **10** in 47% yield [15,16].

Due to the difficulties encountered for the benzenesulfonyl group cleavage of compound **8** and the amino deprotection of compound **7**, we decided to perform the synthesis using a *tert*-butoxycarbonyl as indole nitrogen protecting group and an azido group as precursor of the final amino function (Scheme 2). Protected derivative **11** was easily prepared according to previously described procedures [17,18] and its methyl bromoacrylate derivative **12** was prepared as described above. Nitro group of compound **12** was then reduced to give compound **13** in 69% yield [6], and the azido group was introduced by adding sodium azide to the diazonium salt prepared from compound **13** in the presence of sodium nitrite in acetic acid [19]. Suzuki cross-coupling between compound **14** and boronate **5** led to product **15** in 60% yield using the same procedure as the one described above. After several trials, we noticed that the deprotection steps should be completed after the reduction of the azido group to proceed in acceptable yields. Therefore, **16** was prepared in 61% yield by reduction of **15** using stannous chloride [20–22]. Subsequent deprotection of TIPS group in the presence of TBAF afforded **17** in 89% yield. After *t*Boc cleavage using K_2CO_3 in MeOH/H_2O [23], photo-induced cyclization of compound **18** was performed under irradiation using a 400 W medium-pressure Hg lamp fitted with a Pyrex filter, in the presence of a catalytic amount of I_2 [24]. The expected pyrrolo[2,3-*a*]carbazole **19** was obtained, as confirmed by a $^1H-^1H$ NOESY NMR experiment showing NOE correlations between all neighboring protons of the scaffold (Fig. 2). Finally, carboxylic acid **20** was prepared in 60% yield after saponification of ester **19** using sodium hydroxide.

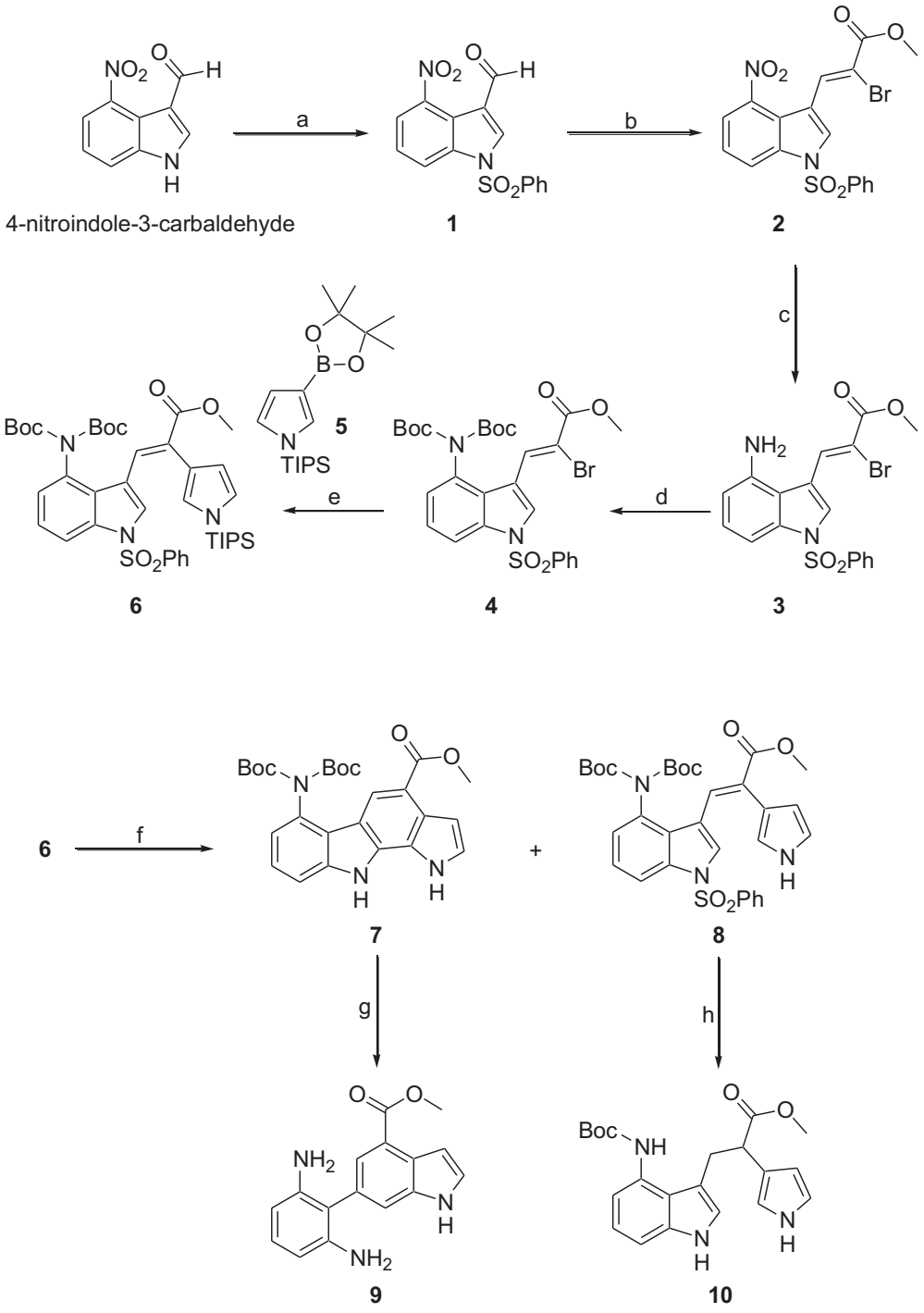
Finally, the preparation of 1,10-dihydropyrrolo[2,3-*a*]carbazole-4-carboxylic acid **29** was achieved in seven steps from commercially available indole-3-carbaldehyde using a similar synthetic pathway (Scheme 3). Firstly, compound **22** was prepared as described above after *t*Boc protection of the indole nitrogen atom and introduction of the methyl bromoacrylate side chain [5]. Subsequent Suzuki cross-coupling with pinacol boronate ester **5** using potassium carbonate and tetrakis(triphenylphosphine) palladium(0) led to compound **23** in 77% yield. Cleavage of the triisopropylsilyl and *tert*-butoxycarbonyl groups in the presence of TBAF and K_2CO_3/MeOH/H_2O, respectively, led to compound **25** in 70% yield from compound **23**. When the *tert*-butoxycarbonyl deprotection step was carried out for an extended time, a partial hydrolysis of the methoxycarbonyl group was also observed, leading to the corresponding carboxylic acid **26**. Finally, cyclization of compound **25** using a 400 W medium-pressure Hg lamp (pyrex filter) in the presence of a catalytic amount of I_2 led to compound **27** and side product **28** in 59% and 29% yields, respectively. As for compound **19**, the pyrrolo[2,3-*a*]carbazole structure of **27** was confirmed by a $^1H-^1H$ NOESY experiment (Fig. 2). Carboxylic acid **29** was prepared after saponification of the corresponding methyl ester using sodium hydroxide.

3. Results and discussion

The kinase inhibitory potencies of compounds **19**, **20**, **27**, and **29** were evaluated at 10 and 1 μM concentrations in duplicate assays against all three Pim family kinases. Kinase assays were performed by the International Centre for Kinase Profiling (Dundee, UK) as previously described [25]. The percentages of residual kinase activities are reported in Table 1. IC_{50} values were determined when the remaining kinase activity was less than 60% when the compounds were tested at 1 μM.

As evident from Table 1, the results obtained indicate that, compared to parent compound **A**, these new derivatives were less active toward Pim kinases. Nevertheless, it is noteworthy that compound **27**, bearing a methoxycarbonyl group at the 4-position demonstrated inhibitory potencies toward Pim-1 and Pim-3 with IC_{50} values of 3 μM and 0.5 μM, respectively. Moreover, the corresponding 6-amino analog **19** was also found to be active toward Pim-3 with an IC_{50} value of 0.41 μM. In both cases, the methyl ester derivatives were more efficient toward Pim kinases than the carboxylic acid analogs, the 6-position being substituted or not by an amino group. The putative binding mode between the ATP-binding site of Pim-3 and compound **27** was examined by molecular modeling experiments.

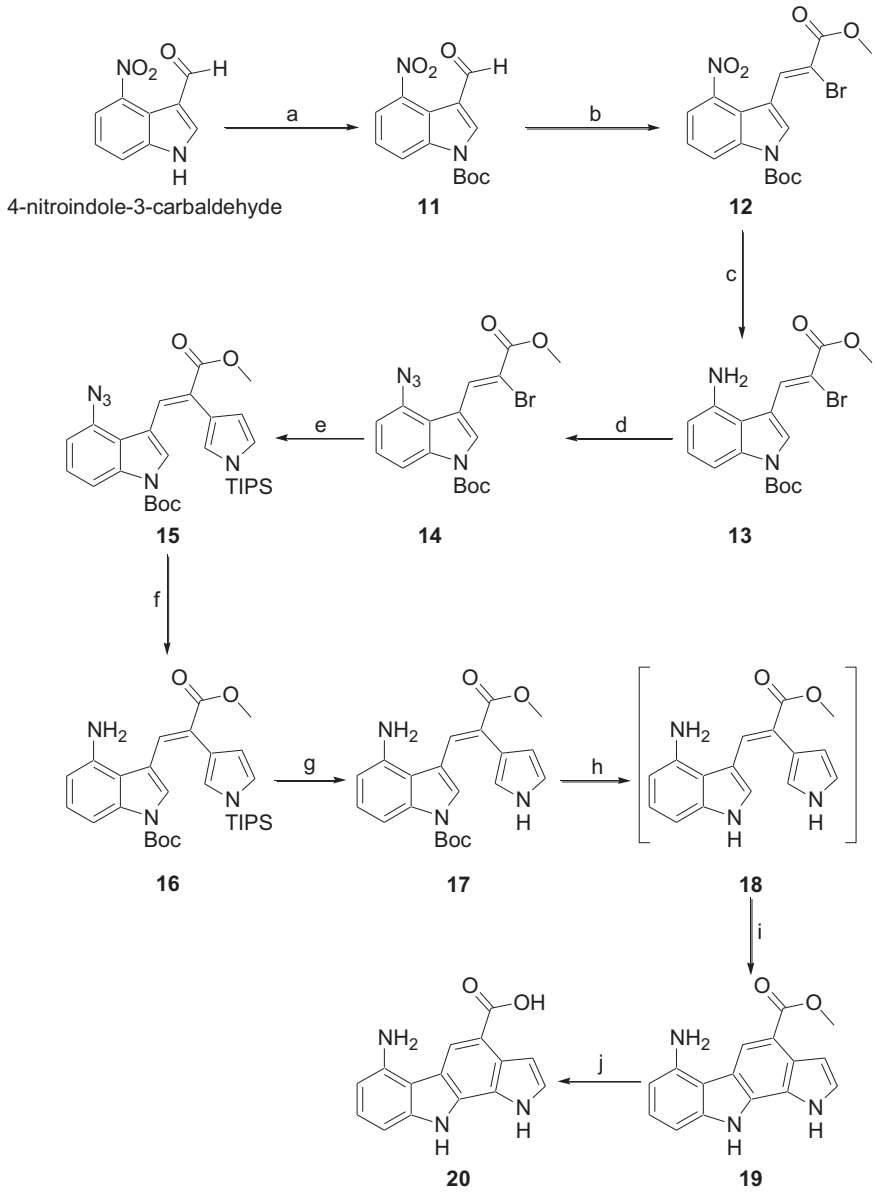
As shown in Fig. 3A, the sequence analysis of the three Pim kinase isoforms Pim-1, Pim-2 and Pim-3 revealed a high degree of homology. As no three dimensional structure of Pim-3 has been solved so far, we decided to generate a model of Pim-3 active site by sequence homology modeling from a known Pim-1 X-ray crystal structure. Most of the Pim-1 structures available in the Protein Data Bank (PDB) present a good resolution and can be used. We started from the 2C3I model [27] that showed a 1.9 Å resolution and a well defined gatekeeper loop containing the Phe49. The Pim-3 Q86V86 sequence available in UniProtKB database [28], presenting 73.6% of sequence identity with Pim-1, was used for the construction of the Pim-3 model. In order to validate our method, we also generated a Pim-2 homology model from the Q9P1W9 sequence available in UniProtKB (59.9% of sequence identity with Pim-1) [29] and the 2C3I crystal structure. The Pim-2 homology model was then compared to the 2IWI Pim-2 crystal structure available in the PDB [30]. As shown Fig. 3B, an RMSD of 1.917 Å was found for all atoms in the superimposition of the generated Pim-2 model and 2IWI structure, thus validating our method. Docking experiments were then performed for compound **27** using our Pim-3 model. The



(a) $PhSO_2Cl$, Cs_2CO_3, CH_3CN, reflux, 94% (b) $Ph_3P{=}CHCO_2Me$, NBS, K_2CO_3, CH_2Cl_2, –20 °C to rt, 95% (c) Fe, NH_4Cl, isopropanol/water, 60 °C, 90% (d) DMAP, Boc_2O, CH_2Cl_2, rt, 70% (e) K_2CO_3, $Pd(PPh_3)_4$, H_2O/dioxane, 70°C, 76% (f) TBAF, THF, 0°C, 5% (**7**) and 56% (**8**) (g) TFA, rt, 73% (h) Mg, I_2, MeOH, reflux, 47%.

Scheme 1. Preparation of compounds **1**–**4** and **6**–**10**.

structure corresponding to the best docking score (6.32) was not retained in regards to the conformation of **27** and its location outside of the hydrophobic ATP adenine binding cleft. Thus, another complex (score of 5.27) showing **27** in a preferred conformation and bound to the hydrophobic pocket was selected and minimized. As shown in Fig. 3C, compound **27** interacts within Pim-3 ATP-binding site *via* hydrophobic interactions between the ester methyl group and Phe51 and two hydrogen bonds. The ester carbonyl is H-bonded with Lys69 side chain, and the hydrogen at the N1 position with Glu124 backbone carbonyl. Compared to



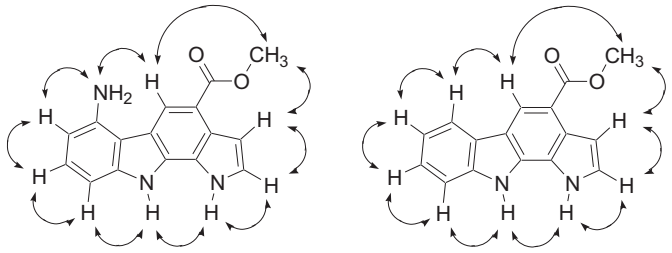
(a) DMAP, Boc_2O, CH_3CN, rt, 99% (b) $Ph_3P{=}CHCO_2Me$, NBS, K_2CO_3, CH_2Cl_2, –20 °C to rt, 98% (c) Fe, NH_4Cl, isopropanol/water, 60 °C, 69% (d) $NaNO_2$, NaN_3, H_2O, AcOH, 0°C, 97% (e) K_2CO_3, $Pd(PPh_3)_4$, H_2O/dioxane, **5**, 70°C, 60% (f) $SnCl_2$, MeOH/THF, 0°C to reflux, 61% (g) TBAF, THF, 0°C, 89% (h) K_2CO_3, MeOH/water, 70°C (i) I_2 (cat), CH_3CN, rt, 41% from **17** (j) NaOH 1N, MeOH/THF, reflux, 60%.

Scheme 2. Preparation of compounds **11**–**20**.

compound **A** bound to Pim-1 (3JPV), due to the presence of the methyl ester group at the 4-position, the pyrrolocarbazole scaffold of **27** was flipped over, orienting the NH groups toward the bottom of the pocket.

Finally, in vitro antiproliferative activities of compounds **19**, **20**, **27**, and **29** were evaluated using the resazurin reduction test [31]. All the compounds were firstly tested toward PA1 (ovarian carcinoma) and PC3 (prostate carcinoma) cancer cells at 1 μM and 10 μM concentrations. When the growth inhibition was found to be more than 50% with compounds tested at 10 μM, IC_{50} values were determined toward PA1, PC3, DU145, another prostatic carcinoma, and a human fibroblast primary culture (Table 1). Carboxylic acids **20** and **29**, that were found to be inactive against Pim kinases, did not show any antiproliferative activity when they were tested at 1 μM and 10 μM toward PA1 and PC3 cells. Compound **19** that was found to inhibit Pim-3 was also inactive toward the cell lines tested. Contrarily, methyl ester derivative **27**, that was found to be active against Pim-1 and Pim-3, showed interesting antiproliferative activities in the micromolar range toward PA1 and PC3 cells as well as against the human fibroblast primary culture tested.

In conclusion, new 4-substituted pyrrolo[2,3-*a*]carbazoles, substituted or not at the 6-position by an amino group, were synthesized and evaluated for their biological activities. The results obtained in this preliminary relationship study demonstrated that



19 **27**

Fig. 2. $^{1}H-^{1}H$ NOE correlations (indicated by arrows) determined for compounds **19** and **27**. For details, see Supporting information.

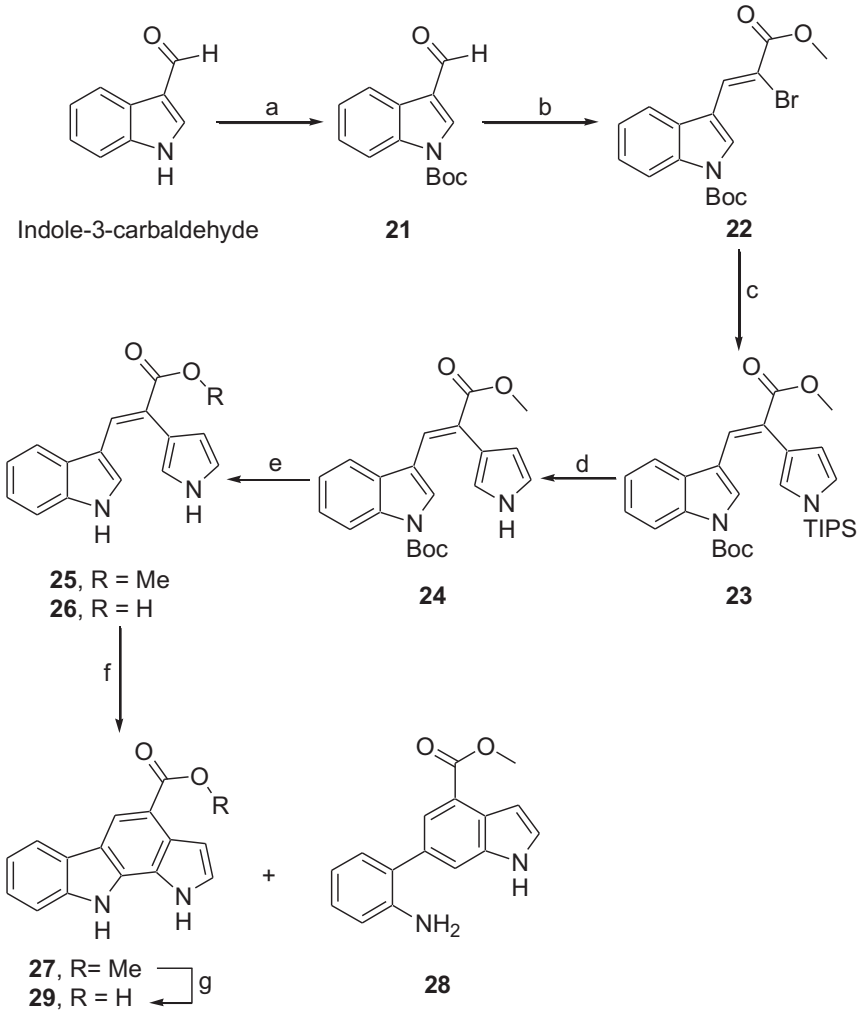
the Pim kinase inhibitory potency (especially Pim-3) can be conserved for a pyrrolo[2,3-*a*]carbazole bearing a methoxycarbonyl group at the 4-position without a formyl at the 3-position. Moreover, compounds **27** that was found to be active against Pim-1 and Pim-3 kinases showed antiproliferative activities in the micromolar range. Finally, complementary molecular modeling studies are currently undertaken in our group to understand why methyl esters **19** and **27** are more active toward Pim kinases compared to their carboxylic acid analogs **20** and **29**.

4. Experimental

4.1. Chemistry

4.1.1. General

Starting materials were obtained from commercial suppliers and used without further purification. IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer ($\bar{\nu}$ in cm^{-1}). NMR spectra, performed on a Bruker AVANCE 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), or a Bruker AVANCE 500 (^{1}H: 500 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), septet (sept), doublet of doublet (dd), doublet of doublet of doublet (ddd), multiplet (m), broad signal (br s). High-resolution mass spectra



(a) DMAP, Boc_2O, CH_3CN, rt, 99% (b) $Ph_3P{=}CHCO_2Me$, NBS, K_2CO_3, CH_2Cl_2, –20 °C to rt, 85% (c) **5**, K_2CO_3, $Pd(PPh_3)_4$, H_2O/dioxane, 70 °C, 77% (d) TBAF, THF, 0 °C, 92% (e) K_2CO_3, MeOH/H_2O, 70 °C, 76% of **25** (f) I_2, CH_3CN, rt, 59% of **27**, 29% of **28** (g) NaOH 1 N, MeOH, THF, Reflux, 76%.

Scheme 3. Preparation of compounds **21**–**29**.

Table 1
Kinase inhibitory potencies: % of residual kinase activity at 10 μM and 1 μM (IC_{50} (μM) in brackets when determined) and antiproliferative activities of compounds **A**, **19**, **20**, **27** and **29** (IC_{50} in μM). nd: Not determined.

Cpds	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
19	39 ± 1 (nd)	90 ± 7	77 ± 4 (nd)	93 ± 8	16 ± 2 (0.41 ± 0.06)	40 ± 2	nd	nd	nd	nd
20	87 ± 1 (nd)	103 ± 1	89 ± 11 (nd)	101 ± 1	55 ± 1 (nd)	103 ± 3	nd	nd	nd	nd
27	15 ± 1 (3 ± 1)	57 ± 5	75 ± 1 (nd)	95 ± 6	24 ± 2 (0.5 ± 0.1)	47 ± 14	8 ± 4	27 ± 5	6 ± 4	> 50
29	70 ± 18 (nd)	90 ± 3	93 ± 8 (nd)	100 ± 0	65 ± 13 (nd)	101 ± 20	nd	nd	nd	nd

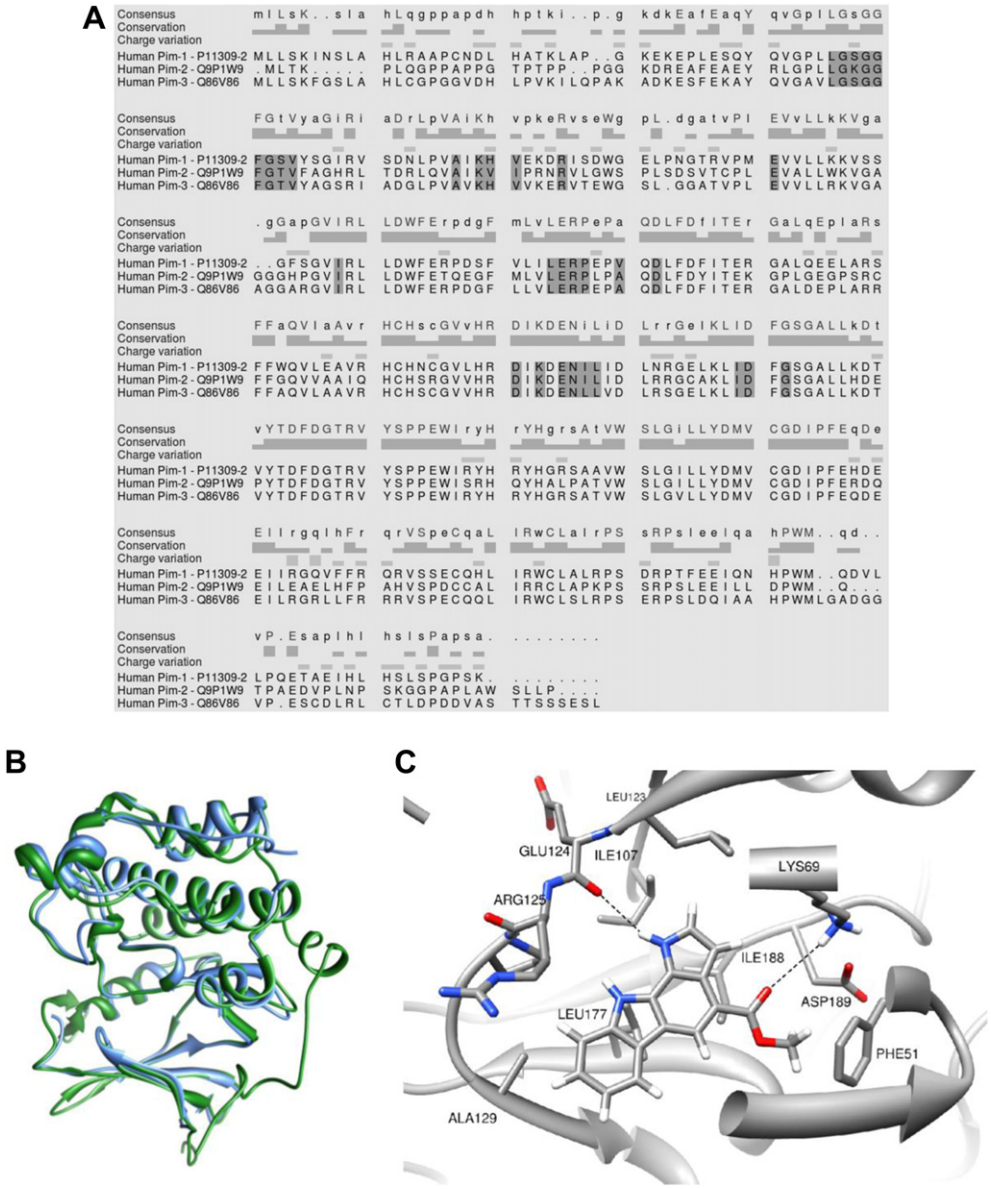


Fig. 3. A) Human Pim kinases: alignment of amino acid sequences (from Swiss-Prot, accession numbers: Pim-1 (P11309-2), Pim-2 (Q9P1W9), Pim-3 (Q86V86)). The ATP-binding domain, defined as amino acid residues located within a distance < 5 A from AMP-PNP bounded to Pim-1 (PDB code: 1YXT, 1XR1, 2BZK) is highlighted in gray. B) Superimposition of Pim-2 model (blue) and 2IWI Pim-2 structure (green). C) Docking model of compound **27** bound to the Pim-3 ATP-binding site. Molecular graphics images were produced using UCSF Chimera [26]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(ESI+) were determined on a high-resolution Micro Q-Tof apparatus (CRMP, 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 Reichert microscope and are uncorrected. Photochemical reactions were carried out at room temperature in an annular reactor equipped with a water-cooling circuit using a 400 W medium-pressure Hg lamp fitted with a Pyrex filter. A solution of the reactant was deoxygenated for 45 min with an argon stream then irradiated for the appropriate time indicated below.

4.1.2. 4-Nitro-1-(phenylsulfonyl)-1H-indole-3-carbaldehyde **1**

To a solution of 4-nitro-1*H*-indole-3-carbaldehyde (150 mg, 0.79 mmol) in CH_3CN (4 mL) was added Cs_2CO_3 (513 mg, 1.58 mmol). The suspension was refluxed under argon for 45 min. Oil bath was removed before addition of benzenesulfonyl chloride (0.11 mL, 0.87 mmol) and then reflux was continued for 1 h. The reaction mixture was allowed to reach room temperature, filtered and washed with CH_2Cl_2. Water was added and the product was extracted with CH_2Cl_2. Combined organic fractions were dried over $MgSO_4$ and evaporated. The residue was triturated in small volumes of cyclohexane and filtered off to give **1** (245 mg, 0.74 mmol, 94%) as a beige powder. Mp = 154–156 °C; IR (ATR): 1680, 1519, 1451, 1381, 1177, 1139 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 7.65–7.70 (m, 3H), 7.81 (t, 1H, J = 7.6 Hz), 8.00 (d, 1H, J = 8.0 Hz), 8.22 (d, 2H, J = 8.0 Hz), 8.39 (d, 1H, J = 8.0 Hz), 9.01 (s, 1H), 10.05 (s, 1H, CHO); ^{13}C NMR (100 MHz, DMSO-d_6): 118.3 (CH), 120.1 (C), 120.3 (CH), 123.0 (C), 126.3 (CH), 127.5 (2CH), 129.9 (C), 130.3 (2CH), 135.6 (C), 135.8 (CH), 138.51 (CH), 138.53 (C), 185.5 (C=O); HRMS (ESI+) calcd for $C_{15}H_{10}N_2NaO_5S$ $(M + Na)^+$ 353.0208, found 353.0225.

4.1.3. Methyl 2-bromo-3-[4-nitro-1-(phenylsulfonyl)-1H-indol-3-yl] prop-2-enoate **2**

To a solution of methyl(phosphoranylidene)acetate (377 mg, 1.13 mmol) in anhydrous CH_2Cl_2 (8 mL) at −20 °C under argon was added *N*-bromosuccinimide (221 mg, 1.24 mmol). The solution was stirred for 45 min before addition of compound **1** (186 mg, 0.56 mmol) and K_2CO_3 (257 mg, 1.86 mmol). The reaction mixture was allowed to reach room temperature and stirring was continued for 48 h. It was filtered through a short pad of Celite and washed with CH_2Cl_2. Filtrate was concentrated under reduced pressure. Residue was purified by column chromatography (EtOAc/cyclohexane, from 1:3 to 1:1) to give **2** (250 mg, 0.54 mmol, 95%) as a yellow powder. Mp = 162–163 °C; IR (ATR): 1717, 1526, 1448, 1380, 1354, 1245, 1154 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 3.85 (s, 3H, CH_3), 7.61–7.67 (m, 3H), 7.77 (t, 1H, J = 7.2 Hz), 8.09 (d, 1H, J = 8.0 Hz), 8.15 (d, 2H, J = 8.0 Hz), 8.29 (s, 1H), 8.44 (d, 1H, J = 8.0 Hz), 8.64 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 53.6 (CH_3), 113.6 (C), 114.4 (C), 119.1 (CH), 121.0 (C), 121.1 (CH), 125.4 (CH), 127.2 (2CH), 130.1 (2CH), 131.2 (CH), 135.0 (CH), 135.2 (C), 135.5 (CH), 135.8 (C), 142.4 (C), 162.6 (C=O); HRMS (ESI+) calcd for $C_{18}H_{13}{}^{79}BrN_2NaO_6S$ $(M + Na)^+$ 486.9575, found 486.9588.

4.1.4. Methyl 3-[4-amino-1-(phenylsulfonyl)-1H-indol-3-yl]-2-bromoprop-2-enoate **3**

To a solution of compound **2** (330 mg, 0.71 mmol) in isopropanol (15 mL) were successively added iron powder (238 mg, 4.25 mmol), ammonium chloride (15 mg, 0.28 mmol) and water (1.5 mL). The mixture was vigorously stirred at 60 °C for 2 h. The reaction mixture was filtered through a short pad of Celite and was washed with water and EtOAc. The product was extracted with EtOAc and the combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, from 1:4 to 1:3) to give **3** (280 mg, 0.64 mmol, 90%) as a yellow powder. Mp = 131–133 °C; IR (ATR): 3352, 1718, 1609, 1431, 1370, 1287, 1246, 1167 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 3.83 (s, 3H, CH_3), 5.24 (br s, 2H, NH_2), 6.61 (d, 1H, J = 8.0 Hz), 7.09 (t, 1H, J = 8.0 Hz), 7.23 (d, 1H, J = 8.0 Hz), 7.62 (t, 2H, J = 8.0 Hz), 7.72 (t, 1H, J = 8.0 Hz), 8.01 (d, 2H, J = 8.0 Hz), 8.40 (s, 1H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 53.4 (CH_3), 102.5 (CH), 111.2 (CH), 112.4 (C), 116.5 (C), 117.0 (C), 125.3 (CH), 126.4 (CH), 126.8 (2CH), 129.9 (2CH), 133.7 (CH), 134.88 (CH), 134.94 (C), 136.2 (2C), 162.9 (C=O); HRMS (ESI+) calcd for $C_{18}H_{16}{}^{79}BrN_2O_4S$ $(M + H)^+$ 435.0014, found 435.0022.

4.1.5. Methyl 3-{4-[bis(tert-butoxycarbonyl)amino]-1-(phenylsulfonyl)-1H-indol-3-yl}-2-bromoprop-2-enoate **4**

To a solution of compound **3** (1.22 g, 2.80 mmol) in anhydrous CH_2Cl_2 (30 mL) at room temperature under argon were added Boc_2O (1.53 g, 7.00 mmol) and then DMAP (34 mg, 0.28 mmol). This solution was stirred at room temperature for 48 h. Water was added to the reaction medium and the product was extracted with CH_2Cl_2. Combined organic fractions were dried over $MgSO_4$ and concentrated. Residue was purified by column chromatography (EtOAc/cyclohexane, 1:3) to give **4** (1.25 g, 1.96 mmol, 70%) as a yellow oil. IR (ATR): 1726, 1448, 1368, 1260, 1238, 1153, 984 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 1.27 (s, 18H, 6CH_3 Boc), 3.82 (s, 3H, CH_3), 7.21 (d, 1H, J = 8.0 Hz), 7.45 (t, 1H, J = 8.0 Hz), 7.63 (t, 2H, J = 8.4 Hz), 7.74 (t, 1H, J = 7.6 Hz), 8.01 (d, 1H, J = 8.0 Hz), 8.10 (d, 2H, J = 8.0 Hz), 8.22 (s, 1H), 8.59 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 27.2 (6CH_3 Boc), 53.5 (CH_3 ester), 82.7 (2C Boc), 113.3 (CH), 114.1 (C), 114.8 (C), 125.2 (CH), 126.0 (CH), 126.3 (C), 127.0 (2CH), 128.2 (CH), 130.0 (2CH), 131.4 (CH), 131.8 (C), 134.3 (C), 135.3 (CH), 135.9 (C), 150.7 (2C=O Boc), 162.5 (C=O ester); HRMS (ESI+) calcd for $C_{28}H_{31}{}^{79}BrN_2NaO_8S$ $(M + Na)^+$ 657.0882, found 657.0896.

4.1.6. Methyl 3-{4-[bis(tert-butoxycarbonyl)amino]-1-(phenylsulfonyl)-1H-indol-3-yl}-2-[1-(triisopropylsilyl)-1H-pyrrol-3-yl] prop-2-enoate **6**

To a solution of compounds **4** (215 mg, 0.34 mmol) and **5** (165 mg, 0.47 mmol) in 1,4-dioxane (7 mL) at room temperature under argon were added water (2.5 mL) and K_2CO_3 (187 mg, 1.35 mmol). The solution was degassed with argon for 20 min, tetrakis(triphenylphosphine)palladium(0) (39 mg, 0.03 mmol) was added. Reaction mixture was heated overnight at 70 °C. Water and EtOAc were added and the mixture was filtered through a short pad of Celite which was next washed several times with EtOAc. The filtrate was washed with a saturated aqueous NaCl solution and the organic fraction was dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, 1:3 then 1:2) to give **6** (201 mg, 0.26 mmol, 76%) as a pale yellow foam. Mp = 69–70 °C; IR (ATR): 2963, 2869, 1753, 1712, 1448, 1367, 1243, 1151 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 1.04 (d, 18H, J = 7.2 Hz, 6CH_3 iPr), 1.30 (s, 18H, 6CH_3 Boc), 1.45 (sept, 3H, J = 7.2 Hz, 3CH iPr), 3.70 (s, 3H, CH_3), 5.96–5.97 (m, 1H), 6.76 (s, 1H), 6.88 (t, 1H, J = 2.4 Hz), 7.14 (d, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 1.0 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.48 (d, 1H, J = 1.0 Hz), 7.60 (t, 2H, J = 8.0 Hz), 7.70 (t, 1H, J = 7.6 Hz), 7.86 (d, 2H, J = 7.6 Hz), 7.90 (d, 1H, J = 8.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 10.7 (3CH iPr), 17.4 (6CH_3 iPr), 27.2 (6CH_3 Boc), 51.8 (CH_3 ester), 82.4 (2C Boc), 110.9 (CH), 113.0 (CH), 116.9 (C), 118.4 (C), 124.1 (CH), 124.5 (CH), 124.6 (CH), 125.3 (CH), 126.6 (2CH), 127.2 (CH), 127.4 (C), 129.0 (C), 129.8 (3CH), 131.9 (C), 134.5 (C), 134.9 (CH), 136.2 (C), 150.9 (2C=O Boc), 167.1 (C=O ester); HRMS (ESI+) calcd for $C_{41}H_{55}N_3NaO_8SSi$ $(M + Na)^+$ 800.3377, found 800.3369.

*4.1.7. Methyl 6-[bis(tert-butoxycarbonyl)amino]-1,10-dihydropyrrolo [2,3-a]carbazole-4-carboxylate **7** and methyl 3-{4-[bis(tert-butoxycarbonyl)amino]-1-(phenylsulfonyl)-1H-indol-3-yl}-2-(1H-pyrrol-3-yl)prop-2-enoate **8***

To a solution of compound **6** (180 mg, 0.23 mmol) in THF (4 mL) at 0 °C under argon was slowly added a 1 M solution of TBAF in THF (2.3 mL, 2.31 mmol). The solution was stirred at 0 °C for 10 min and then refluxed for 2.5 h. A saturated aqueous NaCl solution was added and the product was extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (CH_2Cl_2/EtOAc, from 98:2 to 90:10) to give **7** (5 mg, 0.01 mmol, 5%) as a pale yellow solid and **8** (80 mg, 0.13 mmol, 56%) as a yellow foam.

7: Yellow solid. Mp = 168–170 °C; IR (ATR): 3385, 1762, 1699, 1559, 1431, 1384, 1109 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 1.40 (s, 18H, 6CH_3 Boc), 3.90 (s, 3H, CH_3), 7.03 (d, 1H, J = 7.6 Hz), 7.14 (d, 1H, J = 1.2 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.56 (d, 1H, J = 1.2 Hz), 7.65 (d, 1H, J = 7.6 Hz), 8.44 (s, 1H), 11.18 (br s, 1H, NH), 11.49 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 27.4 (6CH_3 Boc), 51.2 (CH_3 ester), 82.3 (2C Boc), 104.0 (CH), 111.3 (CH), 112.9 (C), 114.1 (C), 118.2 (CH), 120.0 (CH), 121.3 (C), 121.4 (C), 124.3 (CH), 125.4 (C), 125.7 (CH), 129.8 (C), 132.2 (C), 139.8 (C), 151.7 (2C=O Boc), 167.3 (C=O ester); HRMS (ESI+) calcd for $C_{26}H_{29}N_3NaO_6$ $(M + Na)^+$ 502.1954, found 502.1942.

8: Yellow foam. Mp = 77–78 °C; IR (ATR): 1705, 1424, 1369, 1277, 1245, 1151 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 1.30 (s, 18H, 6CH_3 Boc), 3.70 (s, 3H, CH_3), 5.79 (br s, 1H), 6.71 (br s, 1H), 6.87 (dd, 1H, J = 7.6 Hz, J = 0.8 Hz), 7.14 (d, 1H, J = 7.8 Hz), 7.25 (s, 1H), 7.37 (t, 1H, J = 8.0 Hz), 7.49 (s, 1H), 7.63 (t, 2H, J = 7.8 Hz), 7.71 (t, 1H, J = 7.8 Hz), 7.82 (d, 2H, J = 7.8 Hz), 7.92 (d, 1H, J = 7.6 Hz), 11.02 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 27.4 (6CH_3 Boc), 52.0 (CH_3 ester), 82.0 (2C Boc), 109.5 (C), 112.1 (CH), 115.4 (CH), 118.9 (C), 120.2 (CH), 121.3 (CH), 122.0 (CH), 123.7 (C), 124.0 (C), 126.5 (2CH), 126.7 (2CH), 129.9 (2CH), 131.1 (C), 134.1 (CH), 134.6 (CH), 136.9 (C), 138.0 (C), 151.5 (2C=O Boc), 166.9 (C=O ester); HRMS (ESI+) calcd for $C_{32}H_{35}N_3NaO_8S$ $(M + Na)^+$ 644.2043, found 644.2047.

*4.1.8. Methyl 6-(2,6-diaminophenyl)-1H-indole-4-carboxylate **9***

Compound **7** (21 mg, 0.04 mmol) was placed at room temperature under argon and TFA (1 mL) was added. The solution became rapidly red and orange and was stirred at room temperature for 2.5 h before addition of a 5% aqueous $NaHCO_3$ solution to adjust the pH to 7. After extraction with EtOAc, the combined organic fractions were dried over $MgSO_4$ and evaporated. The crude residue was purified by column chromatography (EtOAc/cyclohexane, 1:1 and 2:1) to give **9** (9 mg, 0.03 mmol, 73%) as a yellow oil. IR (ATR): 3352, 1698, 1461, 1431, 1356, 1169 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 3.88 (s, 3H, CH_3), 4.14 (br s, 4H, 2NH_2), 6.04 (d, 2H, J = 8.0 Hz), 6.75 (t, 1H, J = 8.0 Hz), 6.96–6.98 (m, 1H), 7.49 (t, 1H, J = 1.6 Hz), 7.55–7.57 (m, 2H), 11.49 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 51.5 (CH_3), 102.0 (CH), 103.7 (2CH), 111.7 (C), 118.6 (CH), 121.1 (C), 124.8 (CH), 126.1 (C), 127.7 (2C), 127.8 (CH), 128.0 (CH), 137.7 (C), 145.9 (C), 167.1 (C=O); HRMS (ESI+) calcd for $C_{16}H_{16}N_3O_2$ $(M + H)^+$ 282.1243, found 282.1242.

*4.1.9. Methyl 2-(1H-pyrrol-3-yl)-3-{4-(tert-butoxycarbonyl)amino]-1H-indol-3-yl}propanoate **10***

To a solution of **8** (69 mg, 0.11 mmol) in anhydrous MeOH (4.5 mL) at 0 °C under argon were added magnesium turnings (13 mg, 0.55 mmol). The reaction mixture was stirred at room temperature for 2.5 h. A saturated aqueous NH_4Cl solution was added and the product was extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and evaporated. Crude residue was purified by column chromatography (CH_2Cl_2 then CH_2Cl_2/EtOAc, 95:5 and 90:10) to give **10** (20 mg, 0.05 mmol, 47%) as a yellow solid. Mp = 137–138 °C; IR (ATR): 3351, 1712, 1680, 1441, 1367, 1163 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 1.39 (s, 9H, 3CH_3 Boc), 3.16 (dd, 1H, J = 5.2 Hz, J = 14.8 Hz), 3.28 (d, 1H, J = 14.8 Hz), 3.47 (s, 3H, CH_3), 3.89 (dd, 1H, J = 5.2 Hz, J = 14.8 Hz), 6.04 (d, 1H, J = 2.0 Hz), 6.64–6.65 (m, 2H), 6.78 (d, 1H, J = 7.6 Hz), 6.92 (d, 1H, J = 2.0 Hz), 6.97 (t, 1H, J = 7.6 Hz), 7.16 (d, 1H, J = 7.6 Hz), 8.68 (br s, 1H, NHBoc), 10.61 (br s, 1H, NH), 10.78 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 28.0 (3CH_3 Boc), 39.4 (CH_2), 45.6 (CH), 51.0 (CH_3 ester), 78.0 (C Boc), 106.6 (CH), 109.2 (CH), 112.1 (C), 115.1 (CH), 116.9 (CH), 117.3 (CH), 120.5 (CH), 123.1 (C), 123.3 (CH), 130.1 (C), 137.6 (2C), 154.4 (C=O Boc), 174.7 (C=O ester); HRMS (ESI+) calcd for $C_{21}H_{25}N_3NaO_4$ $(M + Na)^+$ 406.1743, found 406.1749.

*4.1.10. Methyl 2-bromo-3-[4-nitro-1-(tert-butoxycarbonyl)-1H-indol-3-yl]prop-2-enoate **12***

To a solution of methyl(phosphoranylidene)acetate (345 mg, 1.03 mmol) in anhydrous CH_2Cl_2 (7 mL) at −20 °C under argon was added *N*-bromosuccinimide (202 mg, 1.14 mmol). The solution was stirred for 45 min before addition of compound **11** (150 mg, 0.52 mmol) and K_2CO_3 (238 mg, 1.72 mmol). The reaction mixture was allowed to reach room temperature and stirring was continued for 48 h. It was filtered through a short pad of Celite and washed with CH_2Cl_2. Filtrate was concentrated under reduced pressure. Residue was purified by column chromatography (EtOAc/cyclohexane, from 1:4) to give **12** (217 mg, 0.51 mmol, 98%) as a yellow powder. Mp = 128–129 °C; IR (ATR): 1746, 1723, 1522, 1436, 1372, 1354, 1150 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 1.66 (s, 9H, 3CH_3 Boc), 3.85 (s, 3H, CH_3), 7.61 (t, 1H, J = 8.0 Hz), 8.05 (d, 1H, J = 8.0 Hz), 8.32 (s, 1H), 8.53 (d, 1H, J = 8.0 Hz), 8.57 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 27.4 (3CH_3 Boc), 53.5 (CH_3 ester), 86.1 (C Boc), 112.3 (C), 112.6 (C), 120.3 (CH), 120.4 (C), 120.8 (CH), 124.9 (CH), 131.2 (CH), 134.8 (CH), 136.1 (C), 142.3 (C), 147.7 (C=O Boc), 162.7 (C=O ester); HRMS (ESI+) calcd for $C_{17}H_{17}{}^{79}BrN_2NaO_6$ $(M + Na)^+$ 447.0168, found 447.0182.

*4.1.11. Methyl 2-bromo-3-[4-amino-1-(tert-butoxycarbonyl)-1H-indol-3-yl]prop-2-enoate **13***

To a solution of compound **12** (100 mg, 0.23 mmol) in isopropanol (6 mL) were successively added iron powder (79 mg, 1.41 mmol), ammonium chloride (5 mg, 0.09 mmol) and water (0.6 mL). The mixture was vigorously stirred at 60 °C for 4 h. The reaction mixture was filtered through a short pad of Celite and was washed with water and EtOAc. The product was extracted with EtOAc and the combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, from 1:4 to 1:3) to give **13** (64 mg, 0.16 mmol, 69%) as a green-yellow solid. Mp = 47–48 °C; IR (ATR): 1720, 1437, 1370, 1150 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 1.63 (s, 9H, 3CH_3 Boc), 3.83 (s, 3H, CH_3), 5.18–5.19 (br s, 2H, NH_2), 6.64 (d, 1H, J = 8.0 Hz), 7.10 (d, 1H, J = 8.0 Hz), 7.44 (d, 1H, J = 8.0 Hz), 8.48 (s, 1H), 8.82 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 27.5 (3CH_3 Boc), 53.3 (CH_3 ester), 84.7 (C Boc), 108.8 (CH), 110.7 (C), 111.3 (CH), 114.7 (C), 116.8 (C), 126.0 (CH), 127.4 (CH), 134.2 (CH), 135.5 (C), 148.6 (C=O Boc), 160.0 (C), 163.1 (C=O ester); HRMS (ESI+) calcd for $C_{17}H_{20}{}^{79}BrN_2O_4$ $(M + H)^+$ 395.0606, found 395.0604.

*4.1.12. Methyl 2-bromo-3-[4-azido-1-(tert-butoxycarbonyl)-1H-indol-3-yl]prop-2-enoate **14***

To a solution of compound **13** (423 mg, 1.07 mmol) in AcOH (15 mL) at 0 °C was slowly added a cold solution of sodium nitrite (81 mg, 1.18 mmol) in water (3 mL). The mixture was stirred at 0 °C for 10 min and a cold solution of sodium azide (76 mg, 1.18 mmol) in water (3 mL) was slowly added. This solution was then stirred at 0 °C for 1.5 h. A saturated aqueous NaCl solution was added and the

product was extracted with CH_2Cl_2. The combined organic fractions were dried over $MgSO_4$ and evaporated under reduced pressure (cold water bath to avoid possible explosion). Residue was purified by column chromatography (CH_2Cl_2) to give **14** (439 mg, 1.04 mmol, 97%) as a pale brown powder. Mp = 150–151 °C; IR (ATR): 2128, 1732, 1700, 1435, 1372, 1152, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 1.69 (s, 9H, $3CH_3$ Boc), 3.94 (s, 3H, CH_3), 7.09 (dd, 1H, J = 0.8 Hz, J = 8.0 Hz), 7.37 (t, 1H, J = 8.0 Hz), 8.00 (d, 1H, J = 8.0 Hz), 8.81 (s, 1H), 9.15 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 28.1 ($3CH_3$ Boc), 53.5 (CH_3 ester), 85.3 (C Boc), 111.0 (C), 111.9 (CH), 112.8 (CH), 120.6 (C), 125.8 (CH), 128.7 (CH), 133.3 (C), 133.8 (CH), 137.8 (C), 142.6 (C), 146.9 (C=O Boc), 163.9 (C=O ester); HRMS (ESI+) calcd for $C_{17}H_{17}{}^{79}BrN_4NaO_4$ $(M + Na)^+$ 443.0331, found 443.0340.

4.1.13. Methyl 3-{4-azido-1-(tert-butoxycarbonyl)-1H-indol-3-yl}-2-[1-(triisopropylsilyl)-1H-pyrrol-3-yl]prop-2-enoate **15**

To a solution of compounds **14** (420 mg, 0.99 mmol) and **5** (485 mg, 1.39 mmol) in 1,4-dioxane (15 mL) at room temperature under argon were added water (5 mL) and K_2CO_3 (547 mg, 3.96 mmol). The solution was degassed with argon for 20 min, tetrakis(triphenylphosphine)palladium(0) (114 mg, 0.10 mmol) was added. Reaction mixture was heated overnight at 70 °C. Water and EtOAc were added and the mixture was filtered through a short pad of Celite which was next washed several times with EtOAc. The filtrate was washed with a saturated aqueous NaCl solution and the organic fraction was dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (Et_2O/pentane, 1:6) to give **15** (338 mg, 0.60 mmol, 60%) as a pale yellow solid. Mp = 45–46 °C; IR (ATR): 2943, 2866, 2127, 1731, 1700, 1535, 1435, 1371, 1151, 779 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 1.07 (t, 18H, J = 7.6 Hz, $6CH_3$ iPr), 1.42 (sept, 3H, J = 7.6 Hz, 3CH iPr), 1.56 (s, 9H, $3CH_3$ Boc), 3.82 (s, 3H, CH_3), 6.30 (d, 1H, J = 1.6 Hz), 6.69 (d, 1H, J = 1.6 Hz), 6.80 (d, 1H, J = 1.6 Hz), 7.05 (dd, 1H, J = 8.0 Hz, J = 0.8 Hz), 7.30 (t, 1H, J = 8.0 Hz), 7.41 (d, 1H, J = 1.2 Hz), 7.91 (d, 1H, J = 8.0 Hz), 8.35 (d, 1H, J = 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 11.6 (3CH iPr), 17.8 ($6CH_3$ iPr), 28.0 ($3CH_3$ Boc), 52.1 (CH_3 ester), 84.2 (C Boc), 111.5 (CH), 111.8 (CH), 112.4 (CH), 115.8 (C), 119.6 (2C), 121.5 (C), 124.0 (CH), 124.1 (CH), 125.1 (CH), 126.4 (C), 126.7 (CH), 130.9 (CH), 136.4 (C), 148.9 (C=O Boc), 169.0 (C=O ester); HRMS (ESI+) calcd for $C_{30}H_{42}N_5O_4Si$ $(M + H)^+$ 564.3006, found 564.2990.

4.1.14. Methyl 3-{4-amino-1-(tert-butoxycarbonyl)-1H-indol-3-yl}-2-[1-(triisopropylsilyl)-1H-pyrrol-3-yl]prop-2-enoate **16**

To a solution of **15** (128 mg, 0.23 mmol) in MeOH/THF 2:1 (3 mL) at 0 °C under argon was added anhydrous $SnCl_2$ (215 mg, 1.13 mmol). The suspension was stirred at room temperature for 45 min and finally refluxed for 2 h. After cooling to room temperature, a 1 N aqueous NaOH solution was added and the product was extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, 1:3) to give **16** (75 mg, 0.14 mmol, 61%) as an orange yellow powder. Mp = 92–93 °C; IR (ATR): 2945, 2866, 1739, 1691, 1478, 1426, 1370, 1154 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 0.95 (d, 18H, J = 8.0 Hz, $6CH_3$ iPr), 1.33 (sept, 3H, J = 8.0 Hz, 3CH iPr), 1.52 (s, 9H, $3CH_3$ Boc), 3.74 (s, 3H, CH_3), 5.03 (br s, 2H, NH_2), 6.21–6.22 (m, 1H), 6.50 (dd, 1H, J = 8.0 Hz, J = 1.2 Hz), 6.62 (t, 1H, J = 2.0 Hz), 6.78 (t, 1H, J = 2.0 Hz), 7.01 (t, 1H, J = 8.0 Hz), 7.13 (d, 1H, J = 1.2 Hz), 7.33 (dd, 1H, J = 8.0 Hz, J = 0.8 Hz), 7.82 (d, 1H, J = 2.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 10.6 (3CH iPr), 17.4 ($6CH_3$ iPr), 27.4 ($3CH_3$ Boc), 51.8 (CH_3 ester), 83.4 (C Boc), 103.9 (CH), 109.0 (CH), 111.6 (CH), 116.4 (C), 116.9 (C), 118.7 (C), 122.3 (CH), 123.8 (CH), 124.1 (CH), 125.5 (CH), 128.1 (C), 129.4 (CH), 135.8 (C), 142.6 (C), 148.6 (C=O Boc), 167.7 (C=O ester); HRMS (ESI+) calcd for $C_{30}H_{44}N_3O_4Si$ $(M + H)^+$ 538.3101, found 538.3117.

4.1.15. Methyl 3-{4-amino-1-(tert-butoxycarbonyl)-1H-indol-3-yl}-2-(1H-pyrrol-3-yl)prop-2-enoate **17**

To a solution of compound **16** (76 mg, 0.14 mmol) in THF (3 mL) at 0 °C under argon was slowly added a 1 M solution of TBAF in THF (1.41 mL, 1.41 mmol). The solution was stirred at 0 °C for 15 min. A saturated aqueous NaCl solution was added and the product was extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, 2:3) to give **17** (48 mg, 0.12 mmol, 89%) as a orange yellow. Mp = 41–42 °C; IR (ATR): 3381, 1732, 1698, 1614, 1492, 1437, 1370, 1153 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 1.54 (s, 9H, $3CH_3$ Boc), 3.72 (s, 3H, CH_3), 5.04 (br s, 2H, NH_2), 5.91–5.93 (m, 1H), 6.52 (d, 1H, J = 8.0 Hz, H_5), 6.71–6.72 (m, 1H), 6.76–6.78 (m, 1H), 7.01 (t, 1H, J = 8.0 Hz), 7.08 (s, 1H), 7.35 (d, 1H, J = 8.0 Hz), 7.92 (d, 1H, J = 1.2 Hz), 10.92 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 27.5 ($3CH_3$ Boc), 51.7 (CH_3 ester), 83.6 (C Boc), 104.1 (CH), 107.8 (CH), 109.6 (CH), 116.1 (2C), 116.9 (C), 117.4 (CH), 118.1 (CH), 123.5 (CH), 125.4 (CH), 127.5 (C), 129.7 (CH), 135.6 (C), 142.7 (C), 148.6 (C=O Boc), 168.1 (C=O ester); HRMS (ESI+) calcd for $C_{21}H_{24}N_3O_4$ $(M + H)^+$ 382.1767, found 382.1776.

4.1.16. Methyl 6-amino-1,10-dihydropyrrolo[2,3-a]carbazole-4-carboxylate **19**

To a solution of compound **17** (72 mg, 0.19 mmol) in a mixture of MeOH/water 3:1 was added K_2CO_3 (77 mg, 0.56 mmol) at room temperature. The reaction mixture was stirred under reflux for 25 min. Saturated NH_4Cl solution and EtOAc were added and the product was extracted at pH 7–8. Organic layers were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, 1:1) and directly engaged in the next step.

To a solution of this compound (36 mg, 0.13 mmol) in freshly distilled CH_3CN (150 mL) in a Pyrex reactor was added one crystal of iodine. The solution was degassed for 45 min with argon and irradiated for 20 min. The solvent was removed under reduced pressure. Residue was purified by column chromatography (MeOH/CH_2Cl_2, 1:99) to give **19** (21 mg, 0.08 mmol, 41% from **17**) as a light brown solid. Mp = 217–218 °C; IR (ATR): 3381, 1678, 1646, 1514, 1439, 1388, 1151 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 3.92 (s, 3H, CH_3), 5.40 (br s, 2H, NH_2), 6.52 (d, 1H, J = 7.2 Hz, H_7), 6.91 (d, 1H, J = 8.0 Hz, H_9), 7.09 (t, 1H, J = 7.8 Hz, H_8), 7.11 (dd, 1H, J = 2.1 Hz, J = 2.9 Hz, H_3), 7.50 (t, 1H, J = 2.4 Hz, H_2), 8.58 (s, 1H, H_5), 10.95 (br s, 1H, NH_1), 11.13 (br s, 1H, NH_{10}); ^{13}C NMR (100 MHz, DMSO-d_6): 51.2 (CH_3), 100.4 (CH), 103.8 (CH), 106.0 (CH), 111.0 (C), 111.9 (C), 116.0 (C), 118.5 (CH), 121.3 (C), 124.6 (C), 125.1 (CH), 125.4 (CH), 128.9 (C), 140.2 (C), 143.4 (C), 166.8 (C=O); HRMS (ESI+) calcd for $C_{16}H_{14}N_3O_2$ $(M + H)^+$ 280.1086, found 280.1085.

4.1.17. 6-Amino-1,10-dihydropyrrolo[2,3-a]carbazole-4-carboxylic acid **20**

Compound **19** (9 mg, 0.03 mmol) was dissolved in MeOH (1.6 mL) and THF (0.4 mL). A 1 N aqueous NaOH solution (1.6 mL) was added and the mixture was refluxed for 5 h. After cooling to room temperature, a saturated aqueous NH_4Cl solution was added to adjust the pH to 8–9, and the product was extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, 2:1) to give **20** (5 mg, 0.02 mmol, 60%) as a dark green solid. Mp = 224–225 °C; IR (ATR): 3391, 1738, 1647, 1559, 1464, 1377, 1154 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 5.37 (br s, 2H, NH_2), 6.51 (dd, 1H, J = 1.2 Hz, J = 8.0 Hz), 6.90 (dd, 1H, J = 1.2 Hz, J = 8.0 Hz), 7.08 (t, 1H, J = 8.0 Hz), 7.12–7.14 (m, 1H), 7.46–7.48 (m, 1H), 8.55 (s, 1H), 10.96 (br s, 1H), 11.16 (br s, 1H), 12.20

(br s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6): 100.2 (CH), 104.0 (CH), 105.8 (CH), 111.1 (C), 113.2* (C), 115.9 (C), 118.5 (CH), 121.2 (C), 124.6 (CH), 124.8 (C), 125.1 (CH), 128.6 (C), 140.1 (C), 143.3 (C), 168.4 (C=O), *chemical shift measured from a ^{1}H–^{13}C HMBC experiment; HRMS (ESI+) calcd for $C_{15}H_{12}N_3O_2$ $(M + H)^+$ 266.0930, found 266.0923.

4.1.18. 1-(tert-Butoxycarbonyl)-1H-indole-3-carbaldehyde **21**

To a solution of 1*H*-indole-3-carbaldehyde (500 mg, 3.44 mmol) in anhydrous acetonitrile (12 mL) at room temperature was added DMAP (84 mg, 0.69 mmol) and then Boc_2O (1.13 g, 5.17 mmol). The mixture was stirred at room temperature overnight and then solvent was removed under reduced pressure. Residue was purified by column chromatography (EtOAc/cyclohexane, 1:1) to give **21** (840 mg, 3.42 mmol, 99%) as a beige solid. Mp = 116–117 °C; IR (ATR): 1741, 1676, 1451, 1397, 1134 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.66 (s, 9H, $3CH_3$ Boc), 7.38–7.47 (m, 2H), 8.14 (2d, 2H, J = 7.6 Hz), 8.67 (s, 1H), 10.08 (s, 1H, CHO); ^{13}C NMR (100 MHz, DMSO-d_6): 27.4 ($3CH_3$ Boc), 85.4 (C Boc), 114.9 (CH), 120.6 (C), 121.3 (CH), 124.4 (CH), 125.5 (C), 125.8 (CH), 135.2 (C), 138.2 (CH), 148.2 (C=O Boc), 187.0 (C=O aldehyde); HRMS (ESI+) calcd for $C_{14}H_{15}NNaO_3$ $(M + Na)^+$ 268.0950, found 268.0963.

4.1.19. Methyl 3-[1-(tert-butoxycarbonyl)-1H-indol-3-yl]-2-[1-(triisopropylsilyl)-1H-pyrrol-3-yl]prop-2-enoate **23**

To a solution of compounds **22** (350 mg, 0.92 mmol) and **5** (549 mg, 1.57 mmol) in 1,4-dioxane (15 mL) at room temperature under argon were added water (5 mL) and K_2CO_3 (509 mg, 3.68 mmol). The solution was degassed with argon for 20 min, tetrakis(triphenylphosphine)palladium(0) (106 mg, 0.10 mmol) was added. Reaction mixture was heated overnight at 70 °C. Water and EtOAc were added and the mixture was filtered through a short pad of Celite which was next washed several times with EtOAc. The filtrate was washed with a saturated aqueous NaCl solution and the organic fraction was dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (CH_2Cl_2/cyclohexane, 1:2, 1:1, 2:1, and pure CH_2Cl_2) to give **23** (370 mg, 0.71 mmol, 77%) as pale yellow solid. Mp = 112–113 °C; IR (ATR): 1741, 1708, 1456, 1370, 1217 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 1.06 (d, 18H, J = 7.2 Hz, $6CH_3$ iPr), 1.38 (sept, 3H, J = 7.2 Hz, 3CH iPr), 1.61 (s, 9H, $3CH_3$ Boc), 3.82 (s, 3H, CH_3), 6.33 (s, 1H), 6.75 (d, 2H, J = 6.0 Hz), 7.17 (t, 1H, J = 6.8 Hz), 7.28 (t, 1H, J = 6.8 Hz), 7.48 (d, 1H, J = 8.0 Hz), 7.56 (s, 1H), 7.86 (s, 1H), 8.08 (d, 1H, J = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 11.5 (3CH iPr), 17.7 ($6CH_3$ iPr), 28.0 ($3CH_3$ Boc), 52.1 (CH_3 ester), 83.8 (C Boc), 108.0 (C), 111.8 (CH), 115.0 (CH), 116.5 (C), 119.6 (C), 119.8 (CH), 122.8 (CH), 123.9 (CH), 124.5 (CH), 124.6 (CH), 126.7 (CH), 126.8 (C), 128.8 (CH), 129.7 (C), 149.3 (C=O Boc), 169.0 (C=O ester); HRMS (ESI+) calcd for $C_{30}H_{42}N_2NaO_4Si$ $(M + Na)^+$ 545.2812, found 545.2833.

4.1.20. Methyl 3-[1-(tert-butoxycarbonyl)-1H-indol-3-yl]-2-(1H-pyrrol-3-yl)prop-2-enoate **24**

To a solution of compound **23** (147 mg, 0.28 mmol) in THF (4 mL) at 0 °C under argon was slowly added a 1 M solution of TBAF in THF (2.81 mL, 2.81 mmol). The solution was stirred at 0 °C for 10 min and then refluxed for 2.5 h. A saturated aqueous NaCl solution was added and the product was extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, 1:2) to give **24** (95 mg, 0.26 mmol, 92%) as a pale yellow solid. Mp = 135–136 °C; IR (ATR): 3248, 1742, 1684, 1399, 1372, 1154 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 1.62 (s, 9H, $3CH_3$ Boc), 3.85 (s, 3H, CH_3), 6.24 (s, 1H), 7.06 (s, 1H), 7.26–7.28 (m, 3H), 7.37 (d, 2H, J = 7.2 Hz), 7.80 (d, 1H, J = 6.4 Hz), 8.23 (s, 1H), 8.47 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): 28.0 ($3CH_3$ Boc), 52.1 (CH_3 ester), 83.8 (C Boc), 111.3 (CH), 112.1 (C), 113.6 (CH), 118.7 (CH), 119.1 (CH), 120.0 (C), 120.4 (CH), 120.9 (CH), 122.3 (C), 122.9 (CH), 126.6 (CH), 127.6 (C), 133.2 (CH), 135.2 (C), 148.7 (C=O Boc), 168.6 (C=O ester); HRMS (ESI+) calcd for $C_{21}H_{23}N_2O_4$ $(M + H)^+$ 367.1658, found 367.1662.

4.1.21. Methyl 3-(1H-indol-3-yl)-2-(1H-pyrrol-3-yl)prop-2-enoate **25**

To a solution of compound **24** (257 mg, 0.70 mmol) in MeOH (9 mL) at room temperature were added water (3 mL) and then K_2CO_3 (291 mg, 2.10 mmol). The solution was stirred at 70 °C for 50 min. After cooling to room temperature, a 1 N aqueous HCl solution (5 mL) was added and the product was extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane 1:1) to give **25** (143 mg, 0.54 mmol, 76%) as a yellow powder. Mp = 172–173 °C; IR (ATR): 3375, 1691, 1516, 1457, 1438, 1071 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 3.68 (s, 3H, CH_3), 5.97 (dd, 1H, J = 2.4 Hz, J = 4.0 Hz), 6.67 (dd, 1H, J = 2.0 Hz, J = 4.0 Hz), 6.85 (dd, 1H, J = 2.4 Hz, J = 4.4 Hz), 6.90 (d, 1H, J = 2.4 Hz), 7.05 (ddd, 1H, J = 1.2 Hz, J = 7.2 Hz, J = 8.4 Hz), 7.12 (ddd, 1H, J = 1.2 Hz, J = 7.2 Hz, J = 8.4 Hz), 7.36 (d, 1H, J = 8.4 Hz), 7.51 (d, 1H, J = 7.6 Hz), 7.97 (s, 1H), 10.88 (br s, 1H, NH), 11.42 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 51.4 (CH_3), 108.2 (CH), 110.9 (C), 111.7 (CH), 116.7 (CH), 117.4 (C), 117.9 (CH), 118.0 (CH), 119.9 (CH), 120.9 (C), 121.8 (CH), 127.0 (C), 127.2 (CH), 131.5 (CH), 135.4 (C), 168.4 (C=O); HRMS (ESI+) calcd for $C_{16}H_{15}N_2O_2$ $(M + H)^+$ 267.1134, found 267.1137.

4.1.22. 3-(1H-Indol-3-yl)-2-(1H-pyrrol-3-yl)prop-2-enoic acid **26**

If the previous experiment was performed for a longer period (1.5–2 h), some hydrolysis of ester **25** was observed and **26** was isolated in 12–15% yield as a yellow powder. Mp = 191–192 °C; IR (ATR): 3376, 1653, 1507, 1458, 1417, 1072 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 5.97 (d, 1H, J = 1.2 Hz), 6.65 (d, 1H, J = 2.4 Hz), 6.82 (d, 1H, J = 2.0 Hz), 6.93 (d, 1H, J = 2.4 Hz), 7.04 (dd, 1H, J = 0.4 Hz, J = 7.6 Hz), 7.11 (dd, 1H, J = 0.4 Hz, J = 7.6 Hz), 7.35 (d, 1H, J = 8.0 Hz), 7.51 (d, 1H, J = 8.0 Hz), 7.93 (s, 1H), 10.83 (br s, 1H, NH), 11.36 (br s, 1H, NH), 11.93 (br s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6): 108.3 (CH), 111.0 (C), 111.7 (CH), 116.8 (CH), 118.0 (CH), 118.7 (CH), 119.8 (CH), 121.7 (CH), 122.0 (C), 124.4 (C), 126.8 (CH), 127.1 (C), 130.7 (CH), 135.4 (C), 169.4 (C=O); HRMS (ESI+) calcd for $C_{15}H_{12}N_2NaO_2$ $(M + Na)^+$ 275.0796, found 275.0797.

4.1.23. Methyl 1,10-dihydropyrrolo[2,3-a]carbazole-4-carboxylate **27** and methyl 6-(2-aminophenyl)-1H-indole-4-carboxylate **28**

To a solution of compound **25** (44 mg, 0.17 mmol) in freshly distilled CH_3CN (150 mL) in a Pyrex reactor was added one crystal of iodine. The solution was degassed for 45 min with argon and irradiated for 20 min. The solvent was removed under reduced pressure and residue was purified by column chromatography (MeOH/CH_2Cl_2, 1:99 to 5:95) to give **27** (26 mg, 0.10 mmol, 59%) as black powder and **28** (13 mg, 0.05 mmol, 29%) as a brown-pink solid.

Compound **27**: Mp = 246–247 °C; IR (ATR): 3324, 2919, 1644, 1557, 1509, 1437, 1156 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): 3.92 (s, 3H, CH_3), 7.15 (t, 1H, J = 2.5 Hz, H_3), 7.21 (t, 1H, J = 7.4 Hz, H_7), 7.37 (t, 1H, J = 7.5 Hz, H_8), 7.53 (t, 1H, J = 2.7 Hz, H_2), 7.66 (d, 1H, J = 8.0 Hz, H_9), 8.16 (d, 1H, J = 7.6 Hz, H_6), 8.56 (s, 1H, H_5), 11.13 (br s, 1H, NH_1), 11.31 (br s, 1H, NH_{10}); ^{13}C NMR (100 MHz, DMSO-d_6): 51.2 (CH_3), 104.0 (CH), 111.5 (CH), 112.6 (C), 115.8 (C), 116.9 (CH), 119.4 (CH), 119.6 (CH), 121.5 (C), 123.9 (C), 124.3 (CH), 125.3 (CH), 125.4 (C), 129.6 (C), 138.8 (C), 167.5 (C=O ester); HRMS (ESI+) calcd for $C_{16}H_{12}N_2NaO_2$ $(M + Na)^+$ 287.0796, found 287.0800.

Compound **28**: Mp = 70–71 °C; IR (ATR): 3359, 2925, 1694, 1559, 1495, 1437, 1171 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 3.89 (s, 3H, CH_3), 4.77 (br s, 2H, NH_2), 6.65 (t, 1H, J = 7.6 Hz), 6.77 (d, 1H, J = 8.0 Hz), 6.95 (s, 1H), 7.02–7.07 (m, 2H), 7.56 (s, 1H), 7.70 (s, 1H), 7.77 (s, 1H), 11.48 (br s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 51.5 (CH_3), 101.9 (CH), 115.0 (CH), 116.5 (CH), 116.6 (CH), 120.3 (C), 123.2 (CH), 125.8 (C), 127.9 (CH), 128.0 (CH), 130.1 (C), 131.6 (CH), 137.1 (2C), 145.1 (C), 167.1 (C=O); HRMS (ESI+) calcd for $C_{16}H_{15}N_2O_2$ $(M + H)^+$ 267.1134, found 267.1135.

4.1.24. 1,10-Dihydropyrrolo[2,3-a]carbazole-4-carboxylic acid **29**

To a solution of compound **27** (32 mg, 0.12 mmol) in MeOH (8 mL) and THF (2 mL) was added at room temperature a 1 N aqueous NaOH solution (6 mL) and the mixture was refluxed for 2.5 h. After cooling to room temperature, the mixture was acidified with a 1 N aqueous HCl solution and the product was extracted with EtOAc. The combined organic fractions were extracted with a 1 N aqueous NaOH solution (3 × 20 mL), and the aqueous phase was acidified to pH 4 with a 1 N aqueous HCl solution and extracted with EtOAc. The combined organic fractions were dried over $MgSO_4$ and evaporated. Residue was purified by column chromatography (EtOAc/cyclohexane, 1:1) to give **29** (23 mg, 0.09 mmol, 76%) as a beige powder. Mp = 234–235 °C; IR (ATR): 3401, 2925, 1653, 1558, 1506, 1457, 1165 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 7.15 (t, 1H, J = 2.8 Hz), 7.21 (t, 1H, J = 7.2 Hz), 7.36 (t, 1H, J = 7.2 Hz), 7.49 (t, 1H, J = 2.8 Hz), 7.64 (d, 1H, J = 8.0 Hz), 8.14 (d, 1H, J = 8.0 Hz), 8.52 (s, 1H), 11.05 (br s, 1H, NH), 11.24 (br s, 1H, NH), 12.26 (br s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6): 104.3 (CH), 111.5 (CH), 113.7 (C), 115.7 (C), 117.0 (CH), 119.3 (CH), 119.5 (CH), 121.5 (C), 124.0 (C), 124.2 (CH), 125.0 (CH), 125.7 (C), 129.4 (C), 138.8 (C), 168.8 (C=O); HRMS (ESI+) calcd for $C_{15}H_{10}N_2NaO_2$ $(M + Na)^+$ 273.0640, found 273.0653.

4.2. Kinase assays

4.2.1. In vitro kinase inhibition assays

The procedures for the in vitro protein kinase assays and for the expression and activation of the protein kinases have been described previously [25].

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 (His_6)-tagged proteins in Sf21 (*Spodoptera frugiperda* 21) insect cells. GST fusion proteins were purified by affinity chromatography on glutathione–Sepharose, and His_6-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. 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 inhibition profile of the tested compounds was expressed as the percentage of the residual kinase activity for an inhibitor concentration of 10 μM. The IC_{50} values of inhibitors were determined after carrying out assays at 10 different concentrations of each compound.

4.3. Molecular modeling experiments

Modeller9V10 software [32] from the module included in UCSF Chimera molecular modeling system [26,33] was used to generate the 3D model of Pim-3. The model was generated using two program options taking into account the water molecules of the model and introducing a refinement on the possible loops. Before docking experiments, the Pim-3 model obtained by this method was processed under Sybylx2.0 software [34], using the biopolymer module, to add properly all hydrogen atoms and to verify that none of the residues were subjected to steric clashes. An energy minimization calculation was then performed under Tripos force field, with Gasteiger-Hückel charges, and finally water molecules were removed from the model. For docking experiments in Sybylx2.0, the threshold and bloat values were respectively set to 0.05 and 9. The surflex module was used with GEOM X options allowing H and heavy atom movement.

4.4. Antiproliferative activities

4.4.1. Cell cultures

Stock cell cultures were maintained as monolayers in 75-cm^2 culture flasks in Glutamax Eagle's minimum essential medium (MEM) with Earle's salts supplemented with 10% fetal calf serum, 5 mL 100 mM sodium pyruvate, 5 mL of 100× non-essential amino acids and 2 mg gentamicin base. Cells were grown at 37 °C in a humidified incubator under an atmosphere containing 5% CO_2.

4.4.2. Survival assays

Cells were plated at a density of 5 × 10^3 cells in 150 μL culture medium in each well of 96-well microplates and were allowed to adhere for 16 h before treatment with tested drug. A stock solution 20 mM of each tested drug was prepared in DMSO and kept at −20 °C until use. Then 50 μL of each tested solution were added to the cultures. A 48 h continuous drug exposure protocol was used. The antiproliferative effect of the tested drug was assessed by the resazurin reduction test.

4.4.3. Resazurin reduction test

Plates were rinsed with 200 μL PBS at 37 °C and emptied by overturning on absorbent toweling. Then 150 μL of a 25 μg/mL solution of resazurin in MEM without phenol red was added to each well. Plates were incubated for 1 h at 37 °C in a humidified atmosphere containing 5% CO_2. Fluorescence was then measured on an automated 96-well plate reader (Fluoroscan Ascent FL, Labsystem) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Under the conditions used, fluorescence was proportional to the number of living cells in the well. The IC_{50}, defined as the drug concentration required to inhibit cell proliferation by 50%, was calculated from the curve of concentration-dependent survival percentage, defined as fluorescence in experimental wells compared with fluorescence in control wells, after subtraction of the blank values.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.08.029.

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