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Synthesis, kinase inhibition and anti-leukemic activities of diversely substituted indolopyrazolocarbazoles

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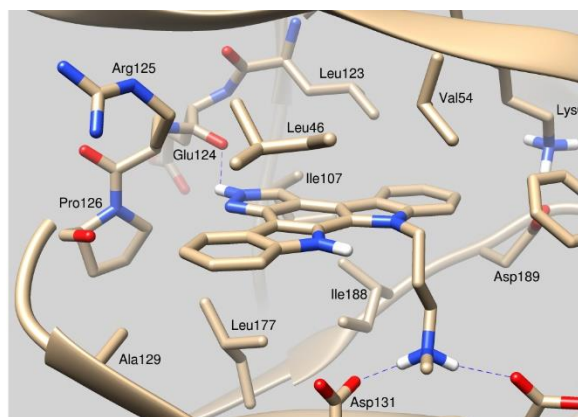
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Abstract

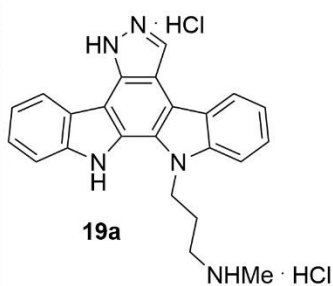
Pyrazole analogues of the staurosporine aglycone K252c, in which the lactam ring was replaced by a pyrazole moiety, were synthesized. In this series, one or the other nitrogen atoms of the indolocarbazole scaffold was substituted by aminoalkyl chains, aiming at improving protein kinase inhibition as well as cellular potency toward acute myeloid leukemia (AML) cell lines. Compound **19a**, substituted at the N12-position by a 3-(methylamino)propyl group, showed high cellular activity in the low micromolar range toward three AML cell lines (MOLM-13, OCI-AML3 and MV4-11) with selectivity over non-cancerous cells (NRK, H9c2). **19a** is also a highly potent inhibitor of the three Pim kinase isoforms, Pim-3 being the most inhibited with an IC₅₀ value in the nanomolar range. A selectivity screening toward a panel of 50 protein kinases showed that **19a** also potently inhibited PRK2 and to a lower extent AMPK, MARK3, GSK3 β and JAK3. Our results enhance the understanding of the structural characteristics of indolopyrazolocarbazoles essential for potent protein kinase inhibition with therapeutic potential against AML.

Keywords: Indolopyrazolocarbazoles; kinase inhibition; Anti-leukemic activity

Graphical abstract



Docking model of **19a** bound to Pim-3 ATP-binding pocket



EC_{50} MOLM-13 = 0.21 μ M
 EC_{50} OCI-AML3 = 0.64 μ M
 EC_{50} MV4-11 = 0.45 μ M

EC_{50} NRK = 4.0 μ M
 EC_{50} H9c2 = 5.3 μ M

IC_{50} Pim-1 = 45 nM
 IC_{50} Pim-2 = 316 nM
 IC_{50} Pim-3 = 7.8 nM

1. Introduction

As part of our ongoing studies dedicated to the identification of new anticancer drugs, we previously reported the synthesis and biological activities of pyrazole analogues of the staurosporine aglycon **K252c** (Fig. 1). [1,2]

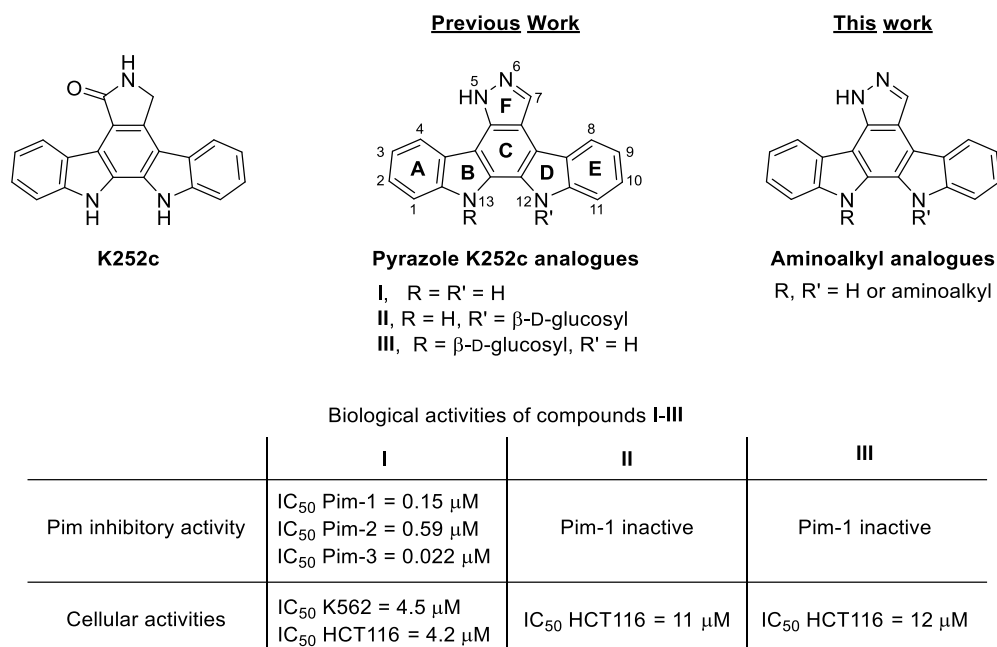


Figure 1. Structures and biological activities of **K252c** and pyrazole **K252c** analogues **I–III** previously described, and structure of new aminoalkyl derivatives (This work).

The evaluation of biological properties of compound **I** (Fig. 1), demonstrated that, contrarily to **K252c**, this analogue bearing a pyrazole ring was not active toward isoforms α and γ of PKC (protein kinase C). On the other hand, **I** was a potent Pim (Proviral Integration site for Moloney murine leukemia virus) protein kinase inhibitor, with IC₅₀ values in the sub-micromolar range for the three Pim isoforms (0.15 μM for Pim-1, 0.59 μM for Pim-2 and 0.022 μM for Pim-3). Due to the known functions of Pim kinases in the cell cycle progression and apoptosis regulation [3] as well as their over-expression in various human cancers such as acute myeloid leukemia and colon carcinoma [4–6], the antiproliferative activity of compound **I** was studied. Pyrazole **K252c** analogue **I** exhibited micromolar cytotoxic potencies toward leukemia K562 and colon carcinoma HCT116 cell lines. All these results demonstrated the potential of this scaffold for the development of new Pim kinase inhibitors with anticancer activities. However, one of the drawbacks of compound **I** was its poor solubility in water. Therefore, a structure-activity relationship (SAR) study was undertaken in this indolopyrazolocarbazole series to address this issue. Thus, we first prepared derivatives **II** and **III** bearing β-D-glucosyl moieties at the N12- or N13-position (Fig. 1)

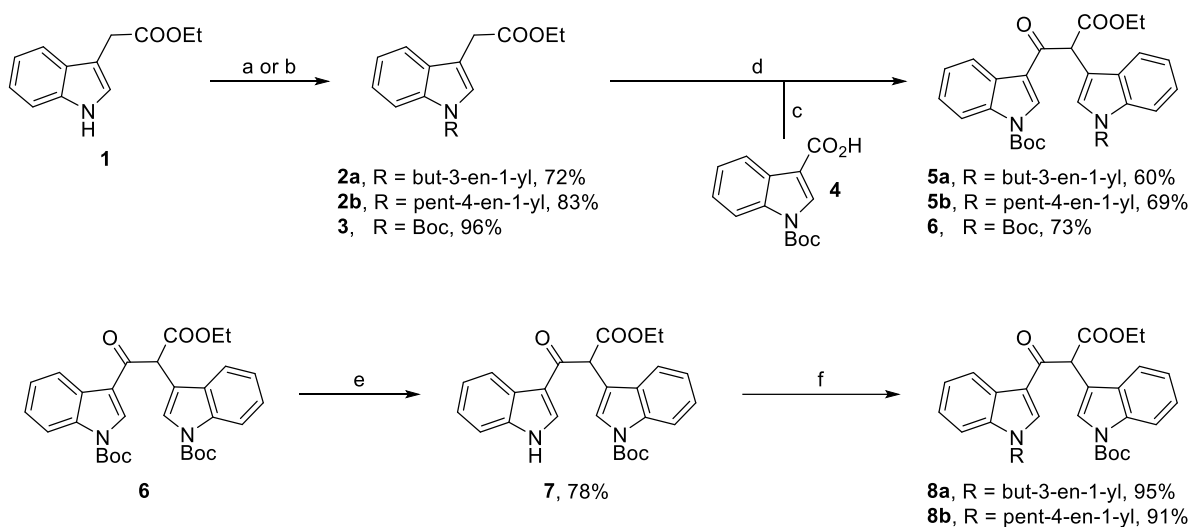
[2]. According to the binding mode of compound **I** in Pim-1 and Pim-3 [1], substituents at these positions should be oriented toward the outside of the binding pocket. Unfortunately, compounds **II** and **III** exhibited slightly decreased antiproliferative activities together with a loss of their Pim kinase inhibitory potency. This indicated that, in case of these analogues, cellular antiproliferative activities were mediated by other targets than Pim kinases. The loss of Pim inhibition could be due to steric hindrance or high polarity caused by the glucosyl moiety, that precluded an optimal molecular interaction of **II** and **III** within Pim ATP-binding site. Our goal aiming at improving water solubility and Pim inhibitory potency could also be reached by the introduction, at the N12- or N13-position, of more flexible aminoalkyl substituents showing various chain lengths and amine substitution (Fig. 1). Chain length and nature of the amino group were based on the structure of some existing Pim inhibitors and their binding mode to Pim-1 ATP-pocket. For instance, non-selective protein kinase inhibitor staurosporine features a methylamino group that interacts with Asp128 of Pim-1, spaced from the indolocarbazole moiety by three carbon atoms (PDB ID: 1YHS [7]). Bisindolylmaleimide BIM-1 has a dimethylaminopropyl group (PDB ID: 1XWS [8]), whereas LY-333531 has a dimethylamino group spaced from one of the indoles by a four-carbon chain (PDB ID: 2J2I [9]). In addition, we also previously showed that the introduction of a dimethylaminobutyl chain to Pim-selective 1,10-dihydropyrrolo[2,3-*a*]carbazole-3-carbaldehyde led to enhanced cellular potency [10]. Therefore, in this work, we describe the synthesis of new analogues, most being substituted at the N12- or N13-position by various 3-aminopropyl and 4-aminobutyl chains, as well as their *in vitro* protein kinases inhibition and cytotoxicity toward three acute myeloid leukemia (AML) (MOLM-13, OCI-AML3 and MV4-11) and two non-cancerous (NRK, H9c2) cell lines.

2. Chemistry

The heterocyclic scaffold of the new K252c analogues was constructed by applying the same synthetic strategy as for the synthesis of compounds **I–III** (Fig. 1), starting from *N*-substituted ethyl 2-(indol-3-yl)acetates **2–3** and Boc-protected indole-3-carboxylic acid **4** (Scheme 1) [1,2]. As our goal was the introduction of aminoalkyl side chains at N12- and N13-positions of the final indolopyrazolocarbazole moiety, we considered that the amino groups would advantageously be introduced by reductive amination from an aldehyde precursor. The latter could be prepared from an alkene function *via* oxidative cleavage. Therefore, we have intended the *N*-substitution of indole moieties by alkenyl chains bearing a terminal alkene, that is, but-3-en-1-yl or pent-4-en-1-yl substituents.

First, the synthesis of N12-substituted indolopyrazolocarbazoles started from ethyl indole-3-carboxylate **1** (Scheme 1). Alkenyl chains were introduced from 4-bromobut-1-ene and 5-

bromopent-1-ene, cesium carbonate and tetrabutylammonium iodide in acetonitrile [11], to give **2a** and **2b**. Claisen-type condensation with activated carboxylic acid **4** led to ketoesters **5a** and **5b**, as precursors of N12-substituted indolopyrazolocarbazoles. Precursors of their N13-substituted counterparts needed the synthesis of ketoester **6**, easily prepared from **3** and **4** [1]. Compound **6** was then first regioselectively mono-deprotected to give **7**, before alkylation with 4-bromobut-1-ene and 5-bromopent-1-ene to produce **8a** and **8b** (Scheme1).

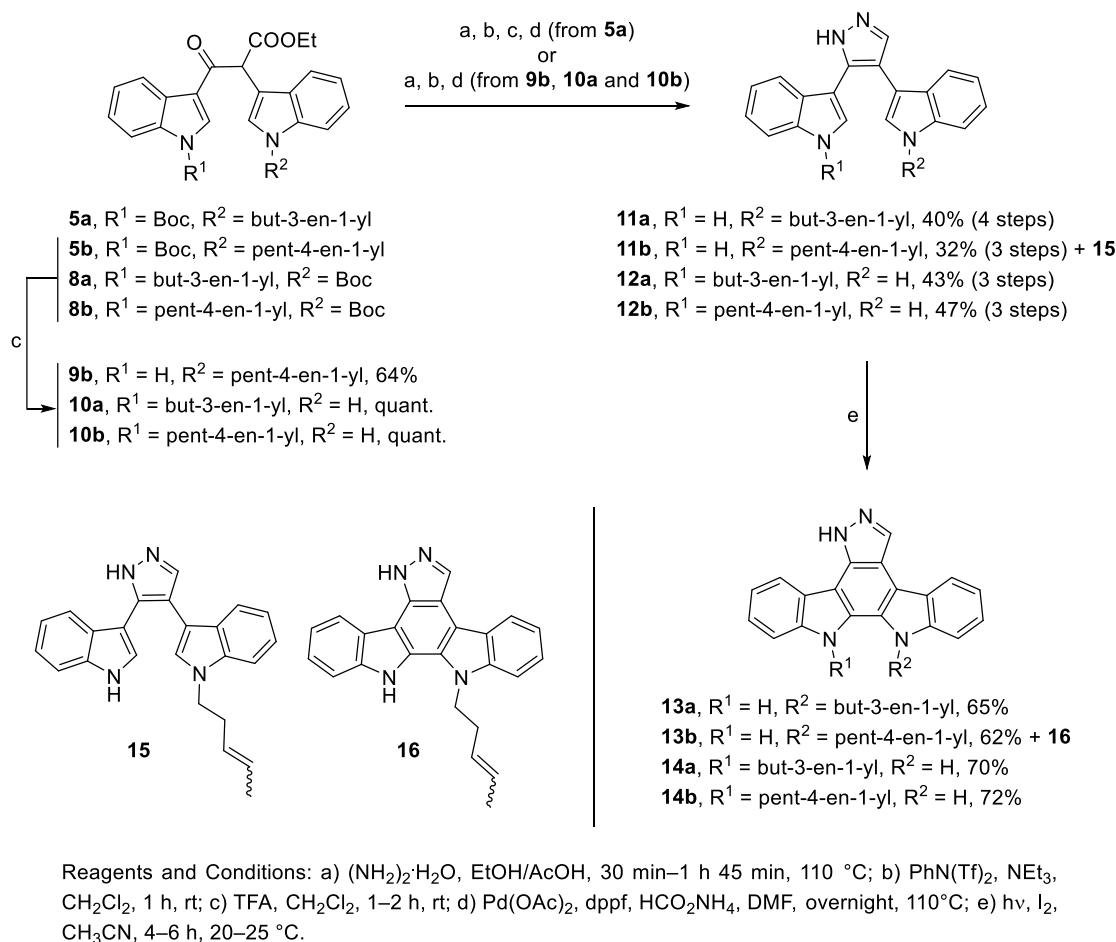


Reagents and Conditions: a) 4-bromobut-1-ene or 5-bromopent-1-ene, Cs₂CO₃, TBAI, CH₃CN, H₂O, 1.5–12 h, reflux; b) Boc₂O, DMAP, CH₃CN, 20 min, rt; c) COCl₂, DMF CH₂Cl₂, 30 min–1.5 h, 0 °C to rt; d) LDA, THF, –78 °C, 1 h, then to rt; e) (NH₂)₂H₂O, EtOH, 15 min, 60 °C; f) 4-bromobut-1-ene or 5-bromopent-1-ene, K₂CO₃, CH₃CN, 2 h, reflux.

Scheme 1. Synthesis of ketoesters **5a**, **5b**, **8a** and **8b**.

As previously reported [1,2], ketoester **5** and **8** were then converted in four steps to bis(indol-3-yl)pyrazoles **11a/b** and **12a/b**, via synthetic transformations including pyrazolone ring formation, triflation, Boc and triflate cleavage, leading to the pyrazole heterocycle (Scheme 2) [1,2]. In the case of the preparation of **11a** from **5a**, pyrazolone formation and triflation were carried out before Boc cleavage. However, this step sequence was unfavorable from **8a** and **8b** due to solubility reasons. Therefore, for the preparation of **11b** and **12a/b**, the Boc group was removed first from **5b**, **8a** and **8b** leading to intermediates **9b**, **10a** and **10b**. It was also paid attention to perform pyrazolone ring synthesis under argon atmosphere. Indeed, we found that in the presence of air, substantial alkene reduction took place, probably due to the presence in the reaction mixture of diimide, generated from hydrazine and oxygen [12]. Finally, in the particular case of compound **11b**, alkene isomerization occurred during the palladium-catalyzed reductive cleavage. We detected by NMR the presence of ca. 26% of (*Z,E*)-**15**, which remained inseparable from **11b** by chromatography. This isomerization was not detected for **11a**, **12a** and **12b**. Subsequent photo-

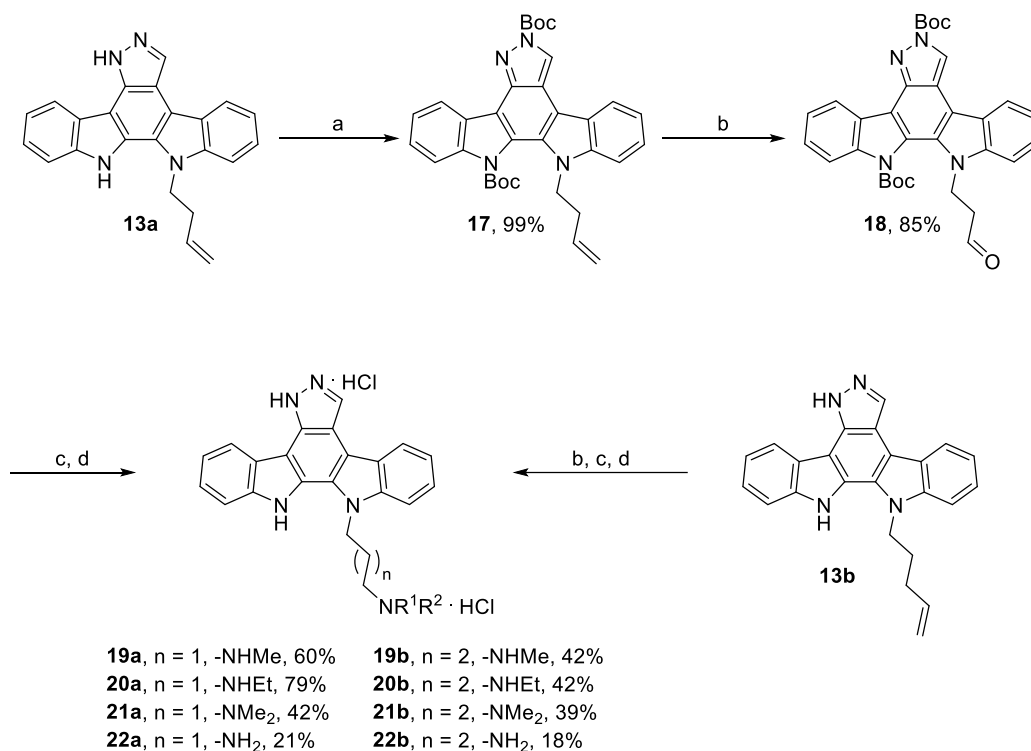
induced cyclization/oxidation of **11a/b** and **12a/b** produced N12-substituted indolopyrazolocarbazoles **13a/b** and N13-substituted regioisomers **14a/b**. In the case of **11b**, this step was carried out in the presence of side-products **15** to give **13b** containing ca. 33% of side-products (*Z,E*)-**16**.



Scheme 2. Synthesis of N12-substituted indolopyrazolocarbazoles **13a**, **13b**, and N13-substituted indolopyrazolocarbazoles **14a** and **14b**. Structures of side-products **15** and **16**.

As depicted in Scheme 3, access to aminopropyl groups at N12-position was carried out in three steps from **13a**. First, **13a** was Boc-protected, both at indole and pyrazole moieties, leading to compound **17**. The pyrazole Boc group was introduced at the N6- rather than the N5-position, probably due to steric hindrance of the surrounding indole part. Compound **17** was then subjected to alkene oxidative cleavage in the presence of potassium osmate and sodium periodate to give **18** [13]. Reductive amination was then performed from intermediate aldehyde by imine formation in methanol prior to addition of sodium borohydride. Final Boc cleavage and dihydrochloride salt preparation led to compounds **19a–22a**. The reaction performed well with methylamine (**19a**) and ethylamine (**20a**), and was poorly effective with dimethyl amine (**21a**) and ammonia (**22a**). Attempts with diethylamine remained unsuccessful. Similar oxidative cleavage/reductive

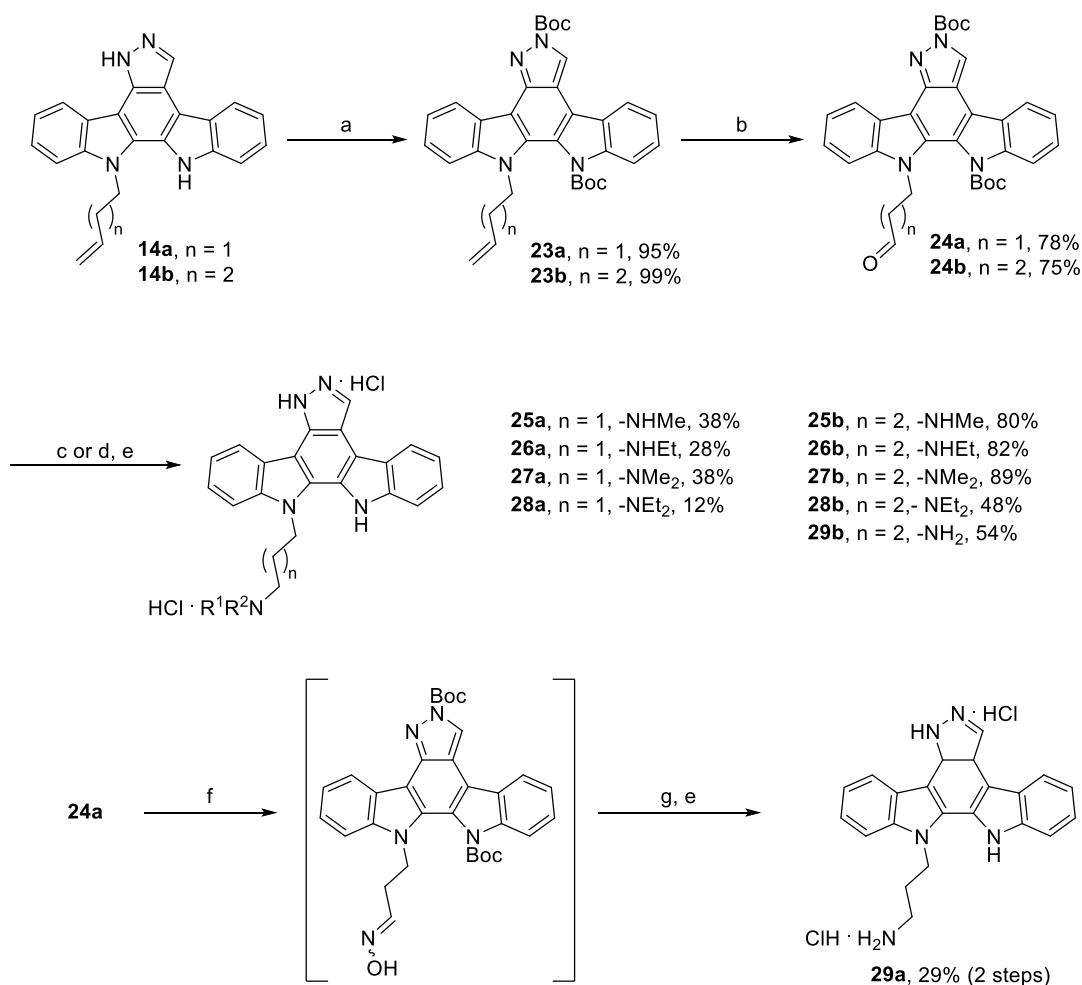
amination/dihydrochloride salt formation sequence was applied from **13b** (containing **16**), finally leading to pure products **19b–22b**.



Reagents and Conditions: a) Boc₂O, DMAP, CH₃CN, overnight, rt; b) K₂OsO₄·2H₂O, NaIO₄, THF/H₂O, 2 h–overnight, rt; c) NHR₁R₂, MeOH, 2 h–overnight, rt then NaBH₄, 1–1.5h, rt; d) HCl 4 M, rt.

Scheme 3. Synthesis of N12-substituted products **19a–22a** and **19b–22b**.

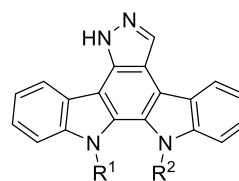
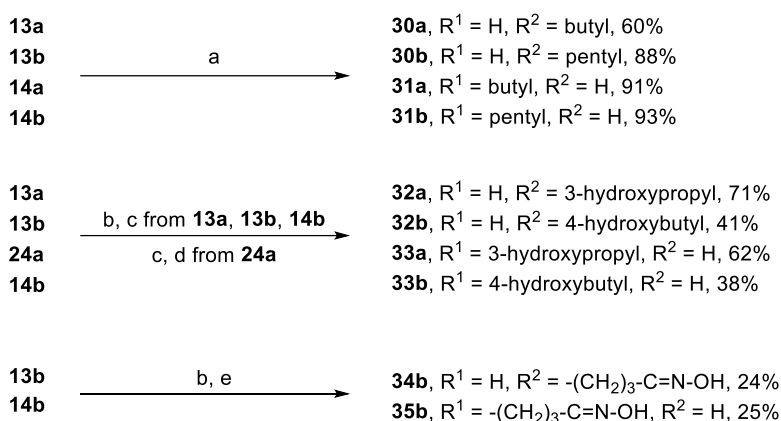
The synthesis of N13-substituted analogues was performed analogously from **14a** and **14b** (Scheme 4). If the reaction sequence worked acceptably for the preparation for **25b–29b**, even in the case of the diethylamino group, the reductive amination yields dropped with the aminopropyl chains, making this pathway impractical for the synthesis of compound **29a** with ammonia. Therefore, **29a** was obtained from aldehyde **24a**, *via* oxime formation and subsequent Zn/HCl reduction [14,15].



Reagents and Conditions: a) Boc₂O, DMAP, CH₃CN, overnight, rt; b) K₂OsO₄·2H₂O, NaIO₄, THF/H₂O, 2–4 h, rt; c) NHR₁R₂, MeOH, 2h–overnight, rt, then NaBH₄, 1 h, rt; d) NHR₁R₂, EtOH, AcOH, overnight, rt, then NaBH₄, 30 min–2 h, 0 °C–rt; e) HCl 4 M, rt; f) NH₂OH·HCl, NEt₃, EtOH, 2 h, rt; g) Zn, aq. HCl, THF, 24 h, reflux.

Scheme 4. Synthesis of N13-substituted products **25a–29a** and **25b–29b**.

In order to evaluate the effect of the introduced amino groups on biological activity, the synthesis of analogues with butyl, pentyl, hydroxypropyl, hydroxybutyl and hydroxyiminobutyl chains was undertaken (Scheme 5). From compounds **13a/b** and **14a/b**, catalytic transfer hydrogenation of the alkene function performed well to give butyl (**30a**, **31a**) and pentyl (**30b**, **31b**) analogues at N12- and N13-positions, respectively. Three hydroxyalkyl-substituted indolopyrazolocarbazoles **32a**, **32b** and **33b** were also obtained from compounds **13a**, **13b** and **14b**, respectively, *via* oxidative cleavage of the alkene function followed by reduction with sodium borohydride. Alternatively, last N13-hydroxypropyl analogue **33a** was prepared from isolated aldehyde **24a**, after carbonyl reduction and Boc cleavage. Finally, oximes **34b** and **35b** were synthesized in two steps from **13b** and **14b**, as mixtures of *Z/E* diastereoisomers.



Reagents and Conditions: a) 10% Pd/C, HCO₂NH₄, MeOH, 6 h, reflux; b) K₂OsO₄·2H₂O, NaIO₄, THF/H₂O, overnight, rt; c) NaBH₄, THF/MeOH, 1–1.5 h, rt; d) K₂CO₃, MeOH/H₂O, 48 h, reflux; e) NH₂OH·HCl, NaOAc, MeOH/H₂O, 2 h, rt.

Scheme 5. Synthesis of alkyl, hydroxyalkyl and hydroxyiminobutyl analogues **30a–33a** and **30b–35b**.

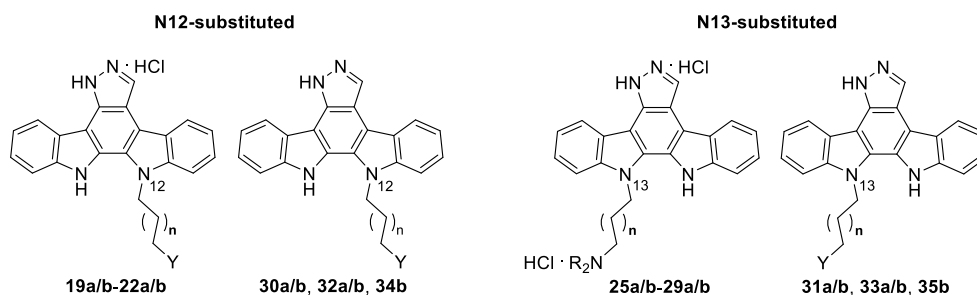
3. Biological evaluation

3.1. Pim-3 kinase inhibition potency

Pyrazole K252c analogue **I** exhibited high Pim-3 kinase inhibitory activity, with an IC₅₀ value of 22 nM [1]. In order to make a comparison with new analogues substituted at the N12- or N13-position with aminoalkyl (**19a/b–22a/b**, **25a/b–29a/b**), alkyl (**30a/b**, **31a/b**), hydroxyalkyl (**32a/b**, **33a/b**) and hydroxyiminoalkyl (**34b** and **35b**) chains, these compounds were evaluated toward Pim-3 at 1 μM concentration (Table 1). These assays were performed at the International Centre for Kinase Profiling (ICKP) in Dundee, Scotland, using previously reported procedure [16]. At 1 μM, compound **I** strongly inhibited Pim-3, with a kinase residual activity of 2.5% (data not shown). All analogues substituted at the N12-position, showed high activity in the same range (~1–5% Pim-3 residual activity at 1 μM), except for pentyl derivative **30b** (Table 1). In contrast, substitution at the N13-position seemed generally less favorable, even if the 1–5% range of Pim-3 residual activity (1 μM concentration) was reached for compounds **25a/b**, **26a/b**, **27b**, **33a** and **35b** (Table 1). According to these screening results, IC₅₀ values were determined only for compounds leading to 5% or less Pim-3 residual activity (Table 1). The tendency in favor of the N12-substitution was confirmed, and potency of compound **I** (IC₅₀ Pim-3 = 22 nM) was equaled or improved, in particular for amino- (**22a/22b**) methylamino- (**19a/b**) and ethylaminoalkyl (**20a/b**) chains. Comparison with alkyl, hydroxyalkyl and hydroxyiminoalkyl chains was clearly in favor of the primary and secondary aminoalkyl substituents. In addition, in both N12- and N13-substituted series, the Pim-3 potency was higher for the shorter alkyl chain (n = 1, Table 1). Overall, the best Pim-3 inhibitors were compounds **19a** (3-(methylamino)propyl, IC₅₀ = 7.8 nM),

20a (3-(ethylamino)propyl, $IC_{50} = 1.2$ nM), **20b** (4-(ethylamino)butyl, $IC_{50} = 6.9$ nM) and **22a** (3-aminopropyl, $IC_{50} = 4.6$ nM).

Table 1. Pim-3 kinase activities and EC_{50} values toward the AML cell line MOLM-13 for N12-substituted analogues (**19a/b–22a/b**, **30a/b**, **32a/b** and **34b**) and N13-substituted analogues (**25a/b–29a/b**, **31a/b**, **33a/b**, and **35b**).



N12-substituted

Compound	n	Y	Pim-3 inhibitory potency		MOLM-13
			% res. act.	IC_{50} (nM)	cytotoxic activity
			EC_{50} (μ M)		
19a	1	NHMe·HCl	1.21 ± 0.05	7.8 ± 1.4	$0.21 \pm 0.05^*$
19b	2	NHMe·HCl	2.4 ± 0.3	10.7 ± 4.3	$0.62 \pm 0.22^*$
20a	1	NHEt·HCl	2.19 ± 0.04	1.2 ± 0.6	$0.35 \pm 0.06^*$
20b	2	NHEt·HCl	1.3 ± 0.3	6.9 ± 2.2	1.5 ± 0.61
21a	1	NMe ₂ ·HCl	2.0 ± 0.5	14.4 ± 6.9	1.3 ± 1.0
21b	2	NMe ₂ ·HCl	4.9 ± 0.7	20.6 ± 2.5	3.2 ± 1.0
22a	1	NH ₂ ·HCl	0.9 ± 0.7	4.6 ± 0.4	$0.27 \pm 0.05^*$
22b	2	NH ₂ ·HCl	3.0 ± 1.3	11.6 ± 1.9	$0.48 \pm 0.16^*$
30a	1	CH ₃	4.6 ± 0.3	27.6 ± 0.3	1.6 ± 1.0
30b	2	CH ₃	9.2 ± 2.6	nd	> 5
32a	1	OH	3.2 ± 0.2	11.4 ± 2.0	$0.65 \pm 0.05^*$
32b	2	OH	5.4 ± 1.1	18.3 ± 0.2	4.4 ± 2.7
34b	1	C=NOH	5.3 ± 0.6	32.9 ± 0.2	> 5

N13-substituted

Compound	n	Y	Pim-3 inhibitory potency		MOLM-13
			% res. act.	IC_{50} (nM)	cytotoxic activity
			EC_{50} (μ M)		
25a	1	NHMe·HCl	3.4 ± 0.9	14.6 ± 2.7	0.83 ± 0.7
25b	2	NHMe·HCl	3.8 ± 1.3	20.0 ± 3.8	3.1 ± 0.69
26a	1	NHEt·HCl	4.2 ± 0.3	24.8 ± 3.0	2.7 ± 4.7
26b	2	NHEt·HCl	3.7 ± 1.3	34.7 ± 1.3	2.9 ± 1.9
27a	1	NMe ₂ ·HCl	12.1 ± 1.9	nd	3.8 ± 1.9
27b	2	NMe ₂ ·HCl	5.3 ± 2.0	65.1 ± 6.2	3.1 ± 1.8
28a		NEt ₂ ·HCl	32.5 ± 1.7	nd	> 4
28b		NEt ₂ ·HCl	13.6 ± 0.2	nd	4.3 ± 2.3
29a	1	NH ₂ ·HCl	16.6 ± 2.0	nd	> 40
29b	2	NH ₂ ·HCl	10.1 ± 1.6	nd	2.7 ± 0.48

31a	1	CH ₃	12.2 ± 1.5	nd	> 5
31b	2	CH ₃	16.4 ± 5.4	nd	15
33a	1	OH	5.0 ± 2.4	27.8 ± 3.1	1.6 ± 1.3
33b	2	OH	11.6 ± 1.2	nd	4.1 ± 3.4
35b	1	C=NOH	5.7 ± 1.3	39.2 ± 0.5	> 3

Pim-3: results are expressed as percentages of enzyme residual activities at 1 μ M compound concentration, and IC₅₀ values (mean of duplicate assays \pm SD); IC₅₀ values were determined when Pim-3 residual activity was found \leq 5%; nd: not determined. MOLM-13: the cells were treated with different concentrations of the analogues for 24 h and EC₅₀ values (mean \pm SE) were determined by non-linear regression as described in the methods section. The regression data were performed on data from two separate experiments, except from *, which are from 4–6 separate experiments.

3.2. Cytotoxicity

In order to assess the cellular potency of these compounds, all analogues were first tested for their ability to induce cell death in the AML cell line MOLM-13. According to previous experience with Pim kinase inhibitors [17], 24 hours of incubation is best suited to reveal difference in potency, and is also sufficient to induce cell death in AML patient blasts [5]. All analogues tested had cytotoxic effect to some extent, but with large variations in the potency (Table 1). The most potent analogues had EC₅₀ values below 0.5 μ M, such as compounds **19a**, **20a**, **22a** and **22b**. The least active analogue was **29a**, with an EC₅₀ value of above 40 μ M. The same trend found for Pim inhibition was observed in these results, namely better cytotoxic activity with a substitution at the N12-position with 3-aminopropyl (**22a**), 3-(methylamino)propyl (**19a**), 3-(ethylamino)propyl (**19b**) or 3-hydroxypropyl groups (**32a**). Compared to the non-substituted compound **I** (EC₅₀ MOLM-13 = 1.7 \pm 3.2 μ M), the cytotoxic potency was increased up to 8-fold for these analogues. The introduction of aminoalkyl chains at the N12-position was therefore in favor of the cytotoxic potency, but this gain in activity was strongly dependent of the nature of the amino group and the length of the alkyl chain.

Six compounds (**19a/b**, **20a**, **22a/b** and **32a**) were selected based on their low EC₅₀ values towards MOLM-13 cells and their ability to inhibit Pim-3 kinase (Table 1). The cytotoxic potential of these analogues was tested toward two additional AML cell lines (OCI-AML3 and MV4-11) as well as two cell lines derived from normal kidney (NRK) and heart (H9c2). The results are presented in Table 2. For a given cell line, the compounds exhibited potencies in the same order of magnitude. Taken together, we found that all analogues had around ten times lower EC₅₀ values for the AML cells compared to the kidney or heart cells, demonstrating promising selectivity for AML over non-cancerous cells. Moreover, the three different AML cell lines, representing different subclasses of AML, did not differ significantly in their response to the Pim inhibitors, but a general trend was that OCI-AML3 was more resistant than MOLM-13 and MV4-11, with around three times higher EC₅₀ values. This could be due to the complex karyotype of OCI-AML3 [18], which

is associated with poor prognosis and therapy resistance. The MV4-11 cell line, which harbors mutated FLT3 in both alleles, is also a cell line representing poor prognosis. However, these cells were the most sensitive towards the Pim kinase inhibitors, supporting our notion that inhibition of Pim could be beneficial in AML with mutated FLT3 [5]. We did observe that dose-response curves obtained from these cells were less steep compared to the other cells, which could indicate growth inhibition rather than cytotoxicity.

Table 2. EC₅₀ values on two AML cell lines (OCI-AML3 and MV4-11) and two cell lines derived from normal tissue (NRK and H9c2) and Pim-1/2 kinase activities for selected analogues **19a/b**, **20a**, **22a/b** and **32a**. Cytotoxicity assays: EC₅₀ values (mean ± SD) were obtained by non-linear regression from 4–6 separate experiments, as described in the methods section. Pim-1 and Pim-2: results are expressed as percentages of enzyme residual activities at 1 μM compound concentration, and IC₅₀ values (mean of duplicate assays ± SD); IC₅₀ values were determined when residual activity was ≤ 20% at 1 μM concentration; nd: not determined.

Compound	19a	19b	20a	22a	22b	32a
Cytotoxic activity (EC₅₀, μM)						
Cell lines						
OCI-AML3	0.64 ± 0.06	2.3 ± 0.27	1.0 ± 0.09	0.68 ± 0.14	1.8 ± 0.28	1.2 ± 0.12
MV4-11	0.45 ± 0.11	0.53 ± 0.53	0.41 ± 0.10	0.14 ± 0.14	0.30 ± 0.32	0.67 ± 0.13
NRK	4.0 ± 0.41	5.9 ± 0.44	5.1 ± 0.56	4.2 ± 0.56	5.8 ± 0.62	15 ± 2.6
H9c2	5.3 ± 0.61	4.5 ± 1.3	4.2 ± 0.35	4.1 ± 0.40	3.8 ± 0.32	9.4 ± 0.97
Pim inhibitory potency						
Pim-1						
% res. act.	4.6 ± 0.4	4.1 ± 0.7	7.6 ± 0.2	7.6 ± 0.1	1.9 ± 0.5	4.1 ± 0.1
IC ₅₀ (nM)	45 ± 14	30 ± 11	47 ± 4	26.2 ± 0.1	16.4 ± 0.6	51 ± 8
Pim-2						
% res. act.	21.3 ± 1.1	67 ± 11	43 ± 13	40 ± 14	53 ± 17	50 ± 1
IC ₅₀ (nM)	316 ± 118	nd	nd	nd	nd	nd

The selected analogues were also evaluated for their ability to inhibit Pim-1 and Pim-2 (Table 2). It was found previously [1] that compound **I** is a potent inhibitor of Pim-3, and to a lesser extent of Pim-1 and Pim-2 (Figure 1). Here we found that the selected compounds were also efficient Pim-1 inhibitors with improved IC₅₀ values found in the 15–50 nM range. Not surprisingly, Pim-

2 was more difficult to target. However, compound **19a** still inhibits this isoform with an IC_{50} value below $0.5 \mu\text{M}$, thus indicating that **19a** is the foremost Pim inhibitor within the series.

The results of cytotoxicity and Pim inhibition assays of compounds **19a/b**, **20a**, **22a/b** and **32a** can be compared to the effect of Pan-Pim inhibitor AZD1208. AZD1208 potently inhibits Pim kinases in the low nanomolar range (IC_{50} at K_m ATP concentration: Pim-1 = 0.4 nM , Pim-2 = 5.0 nM , Pim-3 = 1.9 nM) [19,20]. When we tested AZD1208 on the five cell lines, it exhibited EC_{50} (\pm SD) values of $72 \pm 20 \mu\text{M}$ and $60 \pm 3 \mu\text{M}$, toward NRK and H9c2 cells, respectively, whereas the EC_{50} values for AML cell lines were $35 \pm 2 \mu\text{M}$ (MOLM-13), $37 \pm 2 \mu\text{M}$ (OCI-AML3) and $52 \pm 6 \mu\text{M}$ (MV4-11). While AZD1208 showed equal potency towards the three AML cell lines, it showed almost the same cytotoxic activity towards the normal cell lines. Thus, despite high activity towards Pim kinases, AZD1208 has both lower cytotoxic potency and selectivity compared to the indolopyrazolocarbazole analogues listed in Table 2.

3.3. Protein kinase selectivity profile of compound **19a**

We further assessed the selectivity of compound **19a** toward other protein kinases. Therefore, **19a** has been assayed at $0.5 \mu\text{M}$ on a panel of 50 protein kinases, that provides a representative sampling of the human kinome (Express Screen, ICKP, Dundee, Scotland). The testing concentration of $0.5 \mu\text{M}$ is in the range of EC_{50} values found for the AML cell lines tested, and at this concentration, Pim1-3 are inhibited $>50\%$. Pim-1 was part of the panel and showed $11 \pm 3\%$ residual activity (Figure 2 and Table S1). Kinase activity in the presence of **19a** was reduced for twenty other enzymes (mean residual activity $< 50\%$ at $0.5 \mu\text{M}$) with only three that showed a percentage of residual activity \pm SD inferior to the one of Pim-1 (PRK2, AMPK and MARK3) or in the same range (GSK3 β , JAK3). Thus, IC_{50} values were determined with **19a** for these five protein kinases. The results are indicated in Table 3 together with IC_{50} values for Pim1-3. Four of these protein kinases (PRK2, AMPK, MARK3 and JAK3) were affected at least as much as Pim-1 by **19a**. Pim-3 remained the most inhibited kinase ($IC_{50} = 7.8 \text{ nM}$), PRK2 being affected in the same range ($IC_{50} = 9 \text{ nM}$). Interestingly, these kinases were reported to be involved in cancer biology or were identified as potential therapeutic targets for the treatment of malignancies [21–29].

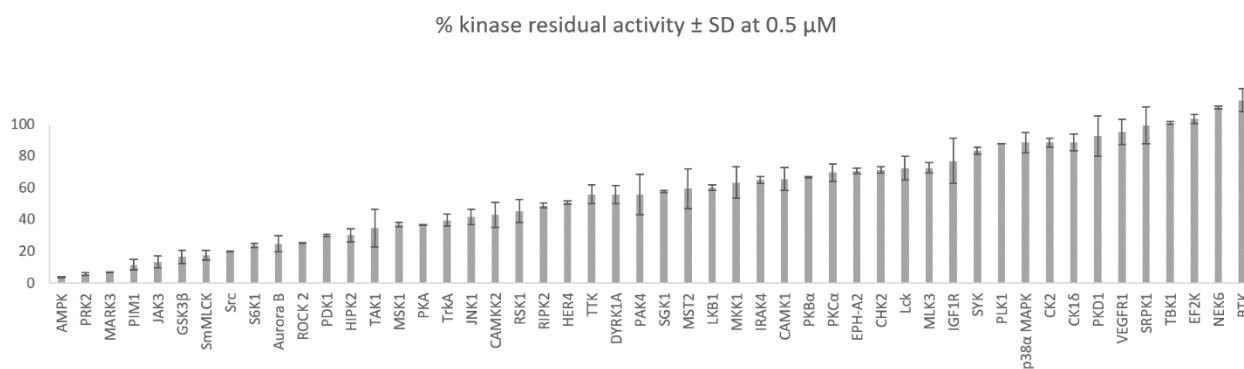


Figure 2. Histogram representing percentage of kinase residual activities (mean of duplicate assays \pm SD) at 0.5 μ M **19a** concentration toward a panel of 50 protein kinases (Table S1).

Table 3. IC₅₀ values (mean of duplicate assays \pm SD) of **19a** toward Pim-1, Pim-2, Pim-3, PRK2, AMPK, MARK3, GSK3 β , and JAK3.

Protein kinase	IC ₅₀ \pm SD (nM)
Pim-1	45 \pm 14
Pim-2	316 \pm 118
Pim-3	7.8 \pm 1.4
PRK2	9.0 \pm 1.8
AMPK	14.4 \pm 0.2
MARK3	24 \pm 1
GSK3 β	128 \pm 12
JAK3	41 \pm 3

4. Molecular modelling

We next studied the binding mode of compound **19a** within the ATP-binding pocket of Pim-3. In our previous study reporting the synthesis of compound **I**, a docking study showed that the indolopyrazolocarbazole scaffold bound the ATP-site of Pim-1 and Pim-3 by establishing hydrophobic interactions as well as a hydrogen bond with the oxygen atom of the Glu124 backbone carbonyl [1]. The two pyrazole tautomers were then considered, showing a more favorable ATP-mimetic binding mode for the 6*H*-tautomer. In addition, it was found that the indolocarbazole could adopt two orientations, flipped by 180°, the difference being the relative positions of

pyrazole N5 and C7 atoms. Here, we performed a docking study in Pim-3 ATP-binding pocket considering the 6*H*-tautomer of **19a**. We found a similar ATP-mimetic binding mode to compound **I**, with a hydrogen bond (2.0 Å) between the hydrogen at the N6-position and Glu124 (Figure 3A). However, one orientation of the heterocyclic scaffold seemed to be privileged, cycle E, rather than cycle A (Figure 1), being oriented toward the direction of Lys67 side chain. The docking model also showed a salt bridge between the amino group of **19a** and Asp131/Glu174. This interaction could explain the high Pim inhibitory potency of the new analogues whereas similar interaction was found between staurosporine and Asp128 (Figure 3B). The best Pim inhibitory potency of N12-substituted analogues compared to their N13 counterparts could also be explained by the results of our docking study. Indeed, in the model depicted in Figure 3A, N5 atom of **19a** is oriented toward a more hydrophilic area in the surrounding of Pro126 than the C7 atom which is directed to the more hydrophobic Leu123 region. Regarding N13-substituted analogues, retention of similar interactions with Asp131/Glu174 would imply a 180° flip of the indolocarbazole, and then a reverse and less favorable orientation of N5/C7 atoms.

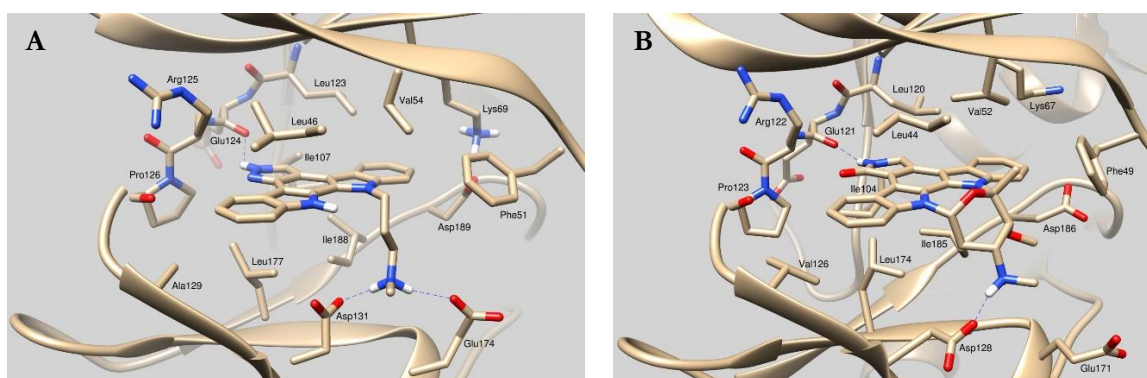


Figure 3. A) Docking model of compound **19a** within Pim-3 ATP-binding pocket; B) Binding mode of staurosporine in Pim-1 ATP-binding pocket (PDB ID: 1YHS). The hydrogen bonds and salt bridges are shown by a dashed line. The images were produced using UCSF Chimera [30].

5. Conclusion

In summary, we synthesized novel pyrazole analogues of staurosporine aglycone K252c, introducing aminoalkyl, hydroxyalkyl, alkyl or hydroxyimino chains at the N12- or N13-position of the indolopyrazolocarbazole scaffold. We found that the substitution of the N12-position

produced highly potent Pim-3 inhibitors, predominantly when 3-(methylamino)propyl (**19a**), 3-(ethylamino)propyl (**20a**), 4-(ethylamino)butyl (**20b**) and 3-aminopropyl (**22a**) chains were introduced. Particularly, compound **19a** exhibited pan-Pim inhibition. Study of the binding mode of **19a** by molecular modeling experiments showed that **19a** could act as an ATP-mimetic inhibitor and that its 3-(methylamino)propyl chain favors the binding to the protein.

In addition, a screening of compound **19a** toward a panel of 50 protein kinases showed that **19a** is a multi-target protein kinase inhibitor, with nanomolar potency toward Pim-3 and PRK2. In the order of decreasing sensitivity to **19a**, AMPK, MARK3, JAK3 and Pim-1 were also inhibited with IC₅₀ values ranging from 14.4 nM to 45 nM.

All the new analogues were also evaluated toward the AML cell line MOLM-13, revealing high cytotoxicity for some derivatives. More particularly, compounds **19a/b**, **20a**, **22a/b** and **32a** were selected to be further evaluated toward two additional AML cell lines (OCI-AML3 and MV4-11) and two non-cancerous cells (NRK, H9c2) demonstrating high activity against AML cells, with a selectivity over their non-cancerous counterparts, which was superior to that of the commercially available pan-Pim inhibitor AZD1208.

In conclusion, these indolopyrazolocarbazole derivatives are promising multi-target protein kinase inhibitors for acute myeloid leukemia therapy.

6. Experimental section

6.1 Chemistry

6.1.1. General.

Starting materials were obtained from commercial suppliers and used without further purification. IR spectra were recorded on a Perkin-Elmer Spectrum 65 FT-IR spectrometer ($\bar{\nu}$ in cm⁻¹). NMR spectra, performed on a Bruker AVANCE 400 III HD (¹H: 400 MHz, ¹³C: 101 MHz) or Bruker AVANCE 500 III HD (¹H: 500 MHz, ¹³C: 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), quadruplet (q), quintuplet (quint), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), triplet of doublets (td), doublet of doublet of triplets (ddt), multiplet (m), broad signal (br s). Coupling constants are expressed in Hertz. In ¹³C NMR (jmod experiments), some carbon signals were weak or not observed, probably due to an equilibrium between pyrazole tautomers. In most cases, the corresponding ¹H–¹³C correlations were observed in ¹H–¹³C HSQC or HMBC experiments. High resolution mass spectra were determined on a Thermo Scientific Q Exactive Q-Orbitrap apparatus (UCA Partner, Université Clermont

Auvergne, Clermont-Ferrand, France). Chromatographic purifications were performed by column chromatography using 40–63 μm silica gel or by flash column chromatography using puriFlash® XS 520 Plus with IR-50SI-F0004, IR-50SI-F0012, IR-50SI-F0025 or IR-50SI-F0040 columns. Reactions were monitored by TLC using fluorescent silica gel plates (60 F254 from Macherey Nagel). Melting points were measured on a Stuart SMP3 apparatus and are uncorrected.

The purity of compounds **19a–22a**, **19b–22b**, **25a–29a**, **25b–29b**, **30a–32a**, **30b–35b** was established by HPLC analysis using a VWR Hitachi chromatograph with DAD detector and a Macherey Nagel Nucleodur gravity column (4.6 mm \times 250 mm, 5 μM). Flow rate was 0.5 mL/min and analysis were performed at 25 °C. Detection wavelength is indicated for each compound. Solvents were (A) water/0.1% trifluoroacetic acid (B) acetonitrile (C) water. Method A: gradient was 95:5 A/B for 5 min, then 95:5 A/B to 5:95 A/B in 20 min and then 5:95 A/B for 10 min. Method B: gradient was 95:5 C/B for 5 min, then 95:5 C/B to 5:95 C/B in 20 min and then 5:95 C/B for 10 min.

6.1.2. General Procedure A: reductive amination

To a solution of aldehyde in MeOH (amount for 0.022 M starting material concentration) and 4 Å molecular sieves was added the corresponding amine. The mixture was stirred for several hours at room temperature and then cooled to 0 °C. NaBH₄ (5.0 eq.) was added at 0 °C and the mixture was stirred at room temperature for 1 h before concentration under reduced pressure. The residue was solubilized in methanol (1 mL) and 4 M aqueous HCl solution (10 mL) was added. The solution was stirred for 1 h at room temperature and concentrated under reduced pressure. The obtained crude salt was solubilized in MeOH and 1 mL of triethylamine was added. After evaporation, the crude was purified by column chromatography. The free amine was solubilized in CH₂Cl₂/MeOH (9:1) washed with a saturated aqueous solution of Na₂CO₃, dried over MgSO₄ and concentrated under reduced pressure. 4 M aqueous HCl was added to the residue, and the salt was dried under reduced pressure and acetonitrile co-evaporation. The solid was washed with dichloromethane to give the corresponding dihydrochloride salt.

6.1.3. Ethyl 2-(1-(but-3-en-1-yl)-1*H*-indol-3-yl)acetate (**2a**)

To a solution of **1** (500 mg, 2.46 mmol) in acetonitrile (40 mL) were added 4-bromobut-1-ene (1.74 mL, 17.1 mmol, 7 eq.), Cs₂CO₃ (5.61 g, 17.2 mmol, 7 eq.), TBAI (908 mg, 2.46 mmol, 1 eq.) and water (18 μL , 1.0 mmol, 0.4 eq.). The mixture was refluxed for 12 h. After evaporation, the residue was directly purified by column chromatography (SiO₂, cyclohexane/EtOAc 9:1) to give **2a** (454 mg, 1.76 mmol, 72%) as a brown oil. R_f = 0.6 (cyclohexane/EtOAc 8:2); IR (ATR)

2980, 2929, 1730, 1139, 736 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.61 (d, $J = 7.9$, 1H), 7.32 (d, $J = 8.2$, 1H), 7.22 (t, $J = 7.6$, 1H), 7.15–7.09 (m, 2H), 5.79 (ddt, $J = 17.1$, 10.2, 6.8, 1H), 5.12–5.04 (m, 2H), 4.20–4.13 (m, 4H), 3.75 (s, 2H), 2.58 (q, $J = 7.1$, 2H), 1.26 (t, $J = 7.1$, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.2 (C), 136.2 (C), 134.7 (CH), 127.9 (C), 126.7 (CH), 121.7 (CH), 119.20 (CH), 119.21 (CH), 117.4 (CH_2), 109.5 (CH), 107.1 (C), 60.8 (CH_2), 46.0 (CH_2), 34.6 (CH_2), 31.4 (CH_2), 14.3 (CH_3); HRMS (ESI+) m/z calcd for $\text{C}_{16}\text{H}_{20}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$ 258.1489, found 258.1485.

6.1.4. Ethyl 2-(1-(pent-4-en-1-yl)-1*H*-indol-3-yl)acetate (**2b**)

To a solution of **1** (5.39 g, 26.5 mmol) in acetonitrile (540 mL) were added 5-bromopent-1-ene (15.7 mL, 132.5 mmol, 5 eq.), Cs_2CO_3 (43.2 g, 132.6 mmol, 5 eq.), TBAI (9.79 g, 26.5 mmol, 1 eq.) and water (191 μL , 10.6 mmol, 0.4 eq.). The mixture was refluxed for 1 h 30 min then was filtered through a pad of Celite and concentrated under reduced pressure. The residue was directly purified by column chromatography (SiO_2 , cyclohexane/EtOAc 97.5:2.5 to 95:5) to give **2b** (5.98 g, 22.04 mmol, 83%) as a yellow oil. $R_f = 0.44$ (cyclohexane/EtOAc 9:1); IR (ATR) 2980, 2932, 1730, 1139, 736 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.50 (d, $J = 7.9$, 1H), 7.42 (d, $J = 8.2$, 1H), 7.28 (s, 1H), 7.13 (ddd, $J = 8.2$, 7.1, 1.1, 1H), 7.01 (ddd, $J = 7.9$, 7.0, 0.9, 1H), 5.82 (ddt, $J = 17.1$, 10.3, 6.5, 1H), 5.05–4.95 (m, 2H), 4.13 (t, $J = 7.0$, 2H), 4.07 (q, $J = 7.1$, 2H), 3.72 (s, 2H), 2.02–1.95 (m, 2H), 1.82 (quint, $J = 7.1$, 2H), 1.18 (t, $J = 7.1$, 3H); ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 171.4 (C), 137.8 (CH), 135.8 (C), 127.4 (C), 127.3 (CH), 121.1 (CH), 118.8 (CH), 118.6 (CH), 115.3 (CH_2), 109.7 (CH), 106.5 (C), 60.1 (CH_2), 44.7 (CH_2), 30.7 (CH_2), 30.3 (CH_2), 28.9 (CH_2), 14.1 (CH_3); HRMS (ESI+) m/z calcd for $\text{C}_{17}\text{H}_{22}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$ 272.1645, found 272.1637.

6.1.5. *Tert*-butyl 3-{2-[1-(but-3-en-1-yl)-1*H*-indol-3-yl]-3-ethoxy-3-oxopropanoyl}-1*H*-indole-1-carboxylate (**5a**)

A suspension of **4** (1.52 g, 5.82 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 $^\circ\text{C}$. A catalytic amount of DMF (27 μL , 0.35 mmol, 6 mol%) was added. Oxalyl chloride (995 μL , 11.8 mmol, 2 eq.) was added dropwise and the obtained mixture was stirred for 30 min while it was allowed to reach room temperature. The white suspension was concentrated under reduced pressure to give the acyl chloride (1.63 g) which was used for the next step without further purification.

BuLi (1.5 M in hexane, 5.42 mL, 8.13 mmol, 2.1 eq.) was added to a solution of diisopropylamine (1.36 mL, 9.7 mmol, 2.5 eq.) in THF (14.8 mL) under argon at 0 $^\circ\text{C}$. The solution was cooled to –78 $^\circ\text{C}$ and a solution of indole **2a** (1.0 g, 3.89 mmol, 1.0 eq.) in THF (4.2 mL) was slowly added. After 1 h, a suspension of the acyl chloride (1.63 g) in THF (10 mL) was slowly added, and the mixture was allowed to reach room temperature. After completion of the reaction, the solvents

were evaporated under reduced pressure and the crude product was dissolved in EtOAc, washed with a saturated aqueous NaHCO₃ solution, dried over MgSO₄ and concentrated under reduced pressure. The product was purified by column chromatography (cyclohexane/EtOAc 95:5 to 90:10) to give **5a** (1.16 g, 2.32 mmol, 60%) as a yellowish foam. *R*_f = 0.22 (cyclohexane/EtOAc 9:1); Mp 121 °C; IR (ATR) 2979, 2941, 1736, 1149, 1134, 1096, 738 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.75 (s, 1H), 8.22 (d, *J* = 7.5, 1H), 8.04 (d, *J* = 8.1, 1H), 7.76 (d, *J* = 7.9, 1H), 7.44 (d, *J* = 8.2, 1H), 7.42 (s, 1H), 7.42–7.32 (m, 2H), 7.12 (ddd, *J* = 8.2, 7.1, 1.1, 1H), 7.02 (ddd, *J* = 8.0, 7.0, 1.0, 1H), 6.23 (s, 1H), 5.72 (ddt, *J* = 17.1, 10.2, 6.8, 1H), 4.97–4.90 (m, 1H), 4.89–4.84 (m, 1H), 4.21 (t, *J* = 7.1, 2H), 4.16 (q, *J* = 7.1, 2H), 2.51–2.42 (m, 2H), 1.64 (s, 9H), 1.19 (t, *J* = 7.1, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 189.5 (C), 169.3 (C), 148.9 (C), 136.1 (C), 135.5 (C), 134.5 (CH), 132.8 (CH), 128.1 (CH), 127.9 (C), 127.3 (C), 125.7 (CH), 124.6 (CH), 122.9 (CH), 121.9 (CH), 119.9 (CH), 118.9 (C), 118.6 (CH), 117.6 (CH₂), 115.0 (CH), 109.8 (CH), 107.0 (C), 85.5 (C), 61.8 (CH₂), 53.7 (CH), 46.2 (CH₂), 34.6 (CH₂), 28.2 (3CH₃), 14.3 (CH₃); HRMS (ESI+) *m/z* calcd for C₃₀H₃₃N₂O₅ (M + H)⁺ 501.2384, found 501.2378.

6.1.6. *Tert*-butyl 3-{3-ethoxy-3-oxo-2-[1-(pent-4-en-1-yl)-*1H*-indol-3-yl]propanoyl}-*1H*-indole-1-carboxylate (**5b**)

A suspension of **4** (5.72 g, 21.9 mmol) in CH₂Cl₂ (57 mL) was cooled to 0 °C. A catalytic amount of DMF (92 μL, 1.19 mmol, 5 mol%) was added. Oxalyl chloride (3.73 mL, 44 mmol, 2 eq.) was added dropwise and the obtained mixture was stirred for 1 h 30 min while it was allowed to reach room temperature. The white suspension was concentrated under reduced pressure to give the acyl chloride (6.02 g) which was used for the next step without further purification.

BuLi (2.25 M in hexane, 16.7 mL, 37.6 mmol, 2.15 eq.) was added to a solution of diisopropylamine (6.14 mL, 44 mmol, 2.5 eq.) in THF (66 mL) under argon at 0 °C. The solution was cooled to -78 °C and a solution of indole **2b** (4.76 g, 17.5 mmol, 1.0 eq.) in THF (20 mL) was slowly added. After 1 h, the acyl chloride (6.02 g) was added portionwise, and the mixture was allowed to reach room temperature. After completion of the reaction, solvents were evaporated under reduced pressure and the crude product was dissolved in EtOAc, washed with a saturated aqueous Na₂CO₃ solution, brine, dried over MgSO₄ and concentrated under reduced pressure. The product was purified by column chromatography (cyclohexane/EtOAc 95:5 to 90:10) to give **5b** (6.22 g, 12.1 mmol, 69%) as an orange wax. *R*_f = 0.22 (cyclohexane/EtOAc 9:1); IR (ATR) 2980, 2930, 1740, 1450, 1368, 1236, 1133, 1099, 738 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.77 (s, 1H), 8.21 (d, *J* = 7.7, 1H), 8.04 (d, *J* = 8.2, 1H), 7.77 (d, *J* = 8.0, 1H), 7.44–7.32 (m, 4H), 7.12 (t, *J* = 7.6, 1H), 7.02 (t, *J* = 7.5, 1H), 6.25 (s, 1H), 5.75 (ddt, *J* = 16.9, 10.4, 6.4, 1H), 4.96–4.89 (m,

2H), 4.22–4.10 (m, 4H), 1.96–1.88 (m, 2H), 1.78 (quint, $J = 7.1$, 2H), 1.63 (s, 9H), 1.19 (t, $J = 7.1$, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 190.1 (C), 169.2 (C), 148.3 (C), 137.7 (CH), 135.7 (C), 134.8 (C), 134.3 (CH), 128.6 (CH), 127.1 (C), 126.9 (C), 125.6 (CH), 124.4 (CH), 121.8 (CH), 121.2 (CH), 119.7 (CH), 119.0 (CH), 117.6 (C), 115.2 (CH₂), 114.8 (CH), 109.9 (CH), 106.9 (C), 85.5 (C), 60.8 (CH₂), 52.9 (CH), 44.9 (CH₂), 30.2 (CH₂), 28.8 (CH₂), 27.5 (3CH₃), 14.1 (CH₃); HRMS (ESI+) m/z calcd for C₃₁H₃₅N₂O₅ (M + H)⁺ 515.2540, found 515.2535.

6.1.7. Di-*tert*-butyl 3,3'-(3-ethoxy-1,3-dioxopropane-1,2-diyl)di(1*H*-indole-1-carboxylate) (**6**)

A suspension of **4** (5.0 g, 19.1 mmol) in CH₂Cl₂ (50 mL) was cooled to 0° C. A catalytic amount of DMF (80 μL , 1.03 mmol, 5 mol%) was added. Oxalyl chloride (3.26 mL, 38.3 mmol, 2.0 eq.) was added dropwise and the obtained mixture was stirred for 30 min while it was allowed to reach room temperature. The white suspension was concentrated under reduced pressure to give the acyl chloride (5.19 g) which was used for the next step without further purification.

BuLi (1.9 M in hexane, 17.3 mL, 32.9 mmol, 2.15 eq.) was added to a solution of diisopropylamine (5.35 mL, 38.2 mmol, 2.5 eq.) in THF (58 mL) under argon at 0 °C. The solution was cooled to –78 °C and a solution of indole **3** (4.65 g, 15.3 mmol, 1.0 eq.) in THF (17 mL) was slowly added. After 1 h, a suspension of the acyl chloride (5.19 g) in THF (43 mL) was slowly added, and the mixture was allowed to reach room temperature. After completion of the reaction, the solvents were evaporated under reduced pressure and the crude product was dissolved in EtOAc, washed with a saturated aqueous NaHCO₃ solution, brine, dried over MgSO₄ and concentrated under reduced pressure. The product was purified by column chromatography (cyclohexane/EtOAc, 95:5 to 90:10) to give **6** (6.13 g, 11.2 mmol, 73%) as a yellow solid. $R_f = 0.35$ (EtOAc/cyclohexane 1:9); Mp 75–77 °C; IR (ATR) 1738, 1370, 1152 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 8.91 (s, 1H), 8.21 (d, $J = 7.6$, 1H), 8.07 (d, $J = 8.2$, 1H), 8.03 (d, $J = 8.3$, 1H), 7.81 (s, 1H), 7.80 (d, $J = 7.7$, 1H), 7.45–7.29 (m, 3H), 7.24 (t, $J = 7.5$, 1H), 6.39 (s, 1H), 4.19 (q, $J = 7.1$, 2H), 1.66 (s, 9H), 1.62 (s, 9H), 1.19 (t, $J = 7.1$, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 189.3 (C), 168.5 (C), 148.8 (C), 148.3 (C), 135.0 (CH), 134.9 (C), 134.6 (C), 129.1 (C), 126.9 (C), 125.8 (CH), 125.5 (CH), 124.5 (2CH), 122.6 (CH), 121.8 (CH), 120.7 (CH), 117.7 (C), 114.9 (CH), 114.6 (CH), 114.1 (C), 85.6 (C), 84.1 (C), 61.2 (CH₂), 52.5 (CH), 27.6 (3CH₃), 27.5 (3CH₃), 14.0 (CH₃); HRMS (ESI+) m/z calcd for C₃₁H₃₅N₂O₇ (M + H)⁺ 547.2444, found 547.2433.

6.1.8. *Tert*-butyl 3-(1-ethoxy-3-(1*H*-indol-3-yl)-1,3-dioxopropan-2-yl)-1*H*-indole-1-carboxylate (**7**)

To a solution of bis-indolyl **6** (500 mg, 0.915 mmol) and in EtOH (9 mL) was added hydrazine monohydrate (453 μ L, 9.34 mmol, 10 eq.). The mixture was stirred at 60 °C for 15 min. The solution was diluted with water and extracted three times with EtOAc, combined organic extracts were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, cyclohexane/EtOAc 7:3) to give **7** (320 mg, 0.717 mmol, 78%) as a yellow solid. R_f = 0.30 (cyclohexane/EtOAc 7:3); for characterization data, see Ref. [2].

6.1.9. *Tert*-butyl 3-{1-[1-(but-3-en-1-yl)-1*H*-indol-3-yl]-3-ethoxy-1,3-dioxopropan-2-yl}-1*H*-indole-1-carboxylate (**8a**)

To a solution of **7** (498 mg, 1.12 mmol) in acetonitrile (15 mL) were added K₂CO₃ (772 mg, 5.59 mmol, 5 eq.) and 4-bromobut-1-ene (227 μ L, 2.24 mmol, 2 eq.). The mixture was refluxed for 2 h and then was filtered through a pad of Celite which was then washed with ethyl acetate. After evaporation under reduced pressure, the crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 9:1 to 8:2) to give **8a** (528 mg, 1.05 mmol, 95%) as a yellow solid. R_f = 0.44 (cyclohexane/EtOAc 2:8); Mp 62 °C; IR (ATR) 2984, 2936, 1728, 1644, 1525, 1451, 1366, 1253, 1145, 1081, 1016, 742 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.70 (s, 1H), 8.15 (d, J = 7.8, 1H), 8.02 (d, J = 8.2, 1H), 7.84–7.78 (m, 2H), 7.61 (d, J = 8.0, 1H), 7.34–7.19 (m, 4H), 6.04 (s, 1H), 5.74 (ddt, J = 17.1, 10.2, 6.9, 1H), 4.94–4.88 (m, 1H), 4.87 (d, J = 10.3, 1H), 4.40–4.25 (m, 2H), 4.23–4.10 (m, 2H), 2.65–2.50 (m, 2H), 1.62 (s, 9H), 1.18 (t, J = 7.1, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 187.1 (C), 168.7 (C), 148.9 (C), 138.2 (CH), 136.7 (C), 134.6 (CH), 134.5 (C), 129.3 (C), 126.0 (C), 125.3 (CH), 124.4 (CH), 123.3 (CH), 122.52 (CH), 122.47 (CH), 121.4 (CH), 120.7 (CH), 117.5 (CH₂), 114.8 (C), 114.5 (CH), 113.8 (C), 111.0 (CH), 84.0 (C), 61.0 (CH₂), 52.0 (CH), 45.6 (CH₂), 33.4 (CH₂), 27.6 (3CH₃), 14.0 (CH₃); HRMS (ESI+) m/z calcd for C₃₀H₃₃N₂O₅ (M + H)⁺ 501.2384, found 501.2374.

6.1.10. *Tert*-butyl 3-{1-ethoxy-1,3-dioxo-3-[1-(pent-4-en-1-yl)-1*H*-indol-3-yl]propan-2-yl}-1*H*-indole-1-carboxylate (**8b**)

To a solution of **7** (626 mg, 1.40 mmol) in acetonitrile (19 mL) were added K₂CO₃ (971 mg, 7.03 mmol, 5 eq.) and 5-bromopent-1-ene (498 μ L, 4.20 mmol, 3 eq.). The mixture was refluxed for 2 h and then was filtered through a pad of Celite which was then washed with ethyl acetate. After evaporation under reduced pressure, the crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 9:1 to 8:2) to give **8b** (655 mg, 1.27 mmol, 91%) as a light orange solid. R_f = 0.35 (cyclohexane/EtOAc 2:8); Mp 51–52 °C; IR (ATR) 2976, 2936, 1728, 1644, 1525, 1451, 1364, 1145, 1081, 1016, 741 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.71 (s, 1H), 8.17 (d, J = 7.4,

1H), 8.02 (d, $J = 8.1$, 1H), 7.80 (d, $J = 6.8$, 1H), 7.80 (s, 1H), 7.59 (d, $J = 7.9$, 1H), 7.33–7.19 (m, 4H), 6.06 (s, 1H), 5.78 (ddt, $J = 16.9$, 10.4, 6.3, 1H), 5.00–4.92 (m, 2H), 4.32–4.18 (m, 2H), 4.23–4.10 (m, 2H), 2.01–1.84 (m, 4H), 1.61 (s, 9H), 1.17 (t, $J = 7.1$, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 187.1 (C), 168.7 (C), 148.9 (C), 138.2 (CH), 137.3 (CH), 136.7 (C), 134.5 (C), 129.3 (C), 126.1 (C), 125.2 (CH), 124.4 (CH), 123.3 (CH), 122.6 (CH), 122.5 (CH), 121.5 (CH), 120.7 (CH), 115.5 (CH₂), 114.8 (C), 114.6 (CH), 113.9 (C), 111.0 (CH), 84.0 (C), 61.0 (CH₂), 52.0 (CH), 45.8 (CH₂), 30.1 (CH₂), 28.3 (CH₂), 27.6 (3CH₃), 14.0 (CH₃); HRMS (ESI+) m/z calcd for C₃₁H₃₅N₂O₅ (M + H)⁺ 501.2540, found 515.2533.

6.1.11. Ethyl 3-(*1H*-indol-3-yl)-3-oxo-2-[1-(pent-4-en-1-yl)-*1H*-indol-3-yl]propanoate (**9b**)

To a solution of **5b** (6.17 g, 12.0 mmol) in CH₂Cl₂ (70 mL) was added TFA (70 mL, 914 mmol, 76 eq.). The mixture was stirred for 2 h at room temperature. After evaporation under reduced pressure, the residue was solubilized in EtOAc, washed with a saturated aqueous Na₂CO₃ solution, water and brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The product was purified by column chromatography (cyclohexane/EtOAc 25:75 to 20:80) to give **9b** (3.18 g, 7.67 mmol, 64%) as a pale yellow solid. $R_f = 0.33$ (cyclohexane/EtOAc 3:7); Mp 155–156 °C; IR (ATR) 3197, 2984, 2930, 1734, 1611, 1417, 1147, 736 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 12.01 (br s, 1H), 8.51 (d, $J = 3.1$, 1H), 8.17–8.13 (m, 1H), 7.76 (d, $J = 7.9$, 1H), 7.42 (s, 1H), 7.45–7.42 (m, 1H), 7.40 (d, $J = 8.2$, 1H), 7.22–7.14 (m, 2H), 7.10 (ddd, $J = 8.2$, 7.0, 1.2, 1H), 7.00 (ddd, $J = 8.0$, 7.0, 0.9, 1H), 6.00 (s, 1H), 5.79 (ddt, $J = 17.1$, 10.4, 6.5, 1H), 5.00–4.92 (m, 2H), 4.20–4.07 (m, 4H), 1.99–1.92 (m, 2H), 1.79 (quint, $J = 7.1$, 2H), 1.17 (t, $J = 7.1$, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 188.7 (C), 169.6 (C), 137.7 (CH), 136.6 (C), 135.6 (C), 134.9 (CH), 128.3 (CH), 127.0 (C), 125.7 (C), 123.1 (CH), 122.0 (CH), 121.3 (CH), 121.1 (CH), 119.9 (CH), 118.7 (CH), 115.3 (CH₂), 114.9 (C), 112.2 (CH), 109.7 (CH), 107.9 (C), 60.5 (CH₂), 52.4 (CH), 44.8 (CH₂), 30.3 (CH₂), 28.8 (CH₂), 14.1 (CH₃); HRMS (ESI+) m/z calcd for C₂₆H₂₇N₂O₃ (M + H)⁺ 415.2016, found 415.2008.

6.1.12. Ethyl 3-[1-(but-3-en-1-yl)-*1H*-indol-3-yl]-2-(*1H*-indol-3-yl)-3-oxopropanoate (**10a**)

To a solution of **8a** (322 mg, 0.643 mmol) in CH₂Cl₂ (4 mL) was added TFA (3.85 mL, 50.3 mmol, 78 eq.). The mixture was stirred for 1 h at room temperature. After evaporation under reduced pressure, the residue was solubilized in EtOAc, washed with a saturated aqueous Na₂CO₃ solution, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. Evaporation give **10a** (258 mg, 0.644 mmol, quantitative) as an orange solid. $R_f = 0.22$ (cyclohexane/EtOAc 3:7); Mp 87 °C; IR (ATR) 3450–3250, 1733, 1644, 1523, 1449, 1382, 1176, 740 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 11.04 (br s, 1H), 8.59 (s, 1H), 8.15 (d, $J = 7.6$, 1H), 7.72 (d, $J = 7.9$, 1H), 7.58 (d, J

= 8.0, 1H), 7.40 (d, $J = 2.1$, 1H), 7.32 (d, $J = 8.0$, 1H), 7.27–7.17 (m, 2H), 7.04 (ddd, $J = 8.2$, 7.2, 1.4, 1H), 6.97 (ddd, $J = 8.1$, 7.0, 1.1, 1H), 5.92 (s, 1H), 5.73 (ddt, $J = 17.1$, 10.3, 6.8, 1H), 4.97–4.87 (m, 2H), 4.35–4.23 (m, 2H), 4.21–4.07 (m, 2H), 2.60–2.46 (m, 2H), 1.18 (t, $J = 7.1$, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 188.3 (C), 169.6 (C), 137.6 (CH), 136.5 (C), 136.0 (C), 134.5 (CH), 126.5 (C), 126.2 (C), 125.0 (CH), 123.1 (CH), 122.3 (CH), 121.5 (CH), 121.0 (CH), 119.6 (CH), 118.5 (CH), 117.6 (CH₂), 113.9 (C), 111.3 (CH), 110.8 (CH), 108.4 (C), 60.5 (CH₂), 52.7 (CH), 45.5 (CH₂), 33.5 (CH₂), 14.1 (CH₃); HRMS (ESI+) m/z calcd for C₂₅H₂₅N₂O₃ (M + H)⁺ 401.1860, found 401.1850.

6.1.13. Ethyl 2-(1*H*-indol-3-yl)-3-oxo-3-[1-(pent-4-en-1-yl)-1*H*-indol-3-yl]propanoate (**10b**)

To a solution of **8b** (639 mg, 1.24 mmol) in CH₂Cl₂ (7.5 mL) was added TFA (7.4 mL, 96.6 mmol, 78 eq.). The mixture was stirred for 1 h at room temperature. After evaporation under reduced pressure, the residue was solubilized in EtOAc, washed with a saturated aqueous Na₂CO₃ solution, water and brine, dried over MgSO₄, filtered and concentrated under reduced pressure. Evaporation give **10b** (520 mg, 1.25 mmol, quantitative) as an orange solid. $R_f = 0.29$ (cyclohexane/EtOAc 3:7); Mp 69 °C; IR (ATR) 3354, 2980, 2932, 1733, 1635, 1523, 1383, 1175, 740 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 11.04 (br d, $J = 2.1$, 1H), 8.60 (s, 1H), 8.17 (d, $J = 7.6$, 1H), 7.74 (d, $J = 7.9$, 1H), 7.56 (d, $J = 8.0$, 1H), 7.41 (d, $J = 2.6$, 1H), 7.32 (dt, $J = 8.1$, 0.9, 1H), 7.27–7.18 (m, 2H), 7.04 (ddd, $J = 8.2$, 7.0, 1.3, 1H), 7.04 (ddd, $J = 8.0$, 7.0, 1.1, 1H), 5.95 (s, 1H), 5.79 (ddt, $J = 17.0$, 10.4, 6.4, 1H), 5.01–4.94 (m, 2H), 4.22 (t, $J = 6.9$, 2H), 4.21–4.08 (m, 2H), 2.00–1.93 (m, 2H), 1.91–1.82 (m, 2H), 1.18 (t, $J = 7.1$, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 188.3 (C), 169.6 (C), 137.5 (CH), 137.4 (CH), 136.5 (C), 136.0 (C), 126.5 (C), 126.2 (C), 125.0 (CH), 123.1 (CH), 122.3 (CH), 121.5 (CH), 121.0 (CH), 119.6 (CH), 118.6 (CH), 115.5 (CH₂), 114.0 (C), 111.4 (CH), 110.8 (CH), 108.4 (C), 60.5 (CH₂), 52.6 (CH), 45.7 (CH₂), 30.1 (CH₂), 28.4 (CH₂), 14.1 (CH₃); HRMS (ESI+) m/z calcd for C₂₆H₂₇N₂O₃ (M + H)⁺ 415.2016, found 415.2003.

6.1.14. 3-[5-(1*H*-Indol-3-yl)-1*H*-pyrazol-4-yl]-1-(but-3-en-1-yl)-1*H*-indole (**11a**)

To a screw cap tube under argon with **5a** (1.16 g, 2.32 mmol) was added a solution of hydrazine monohydrate (5.21 mL, 107 mmol, 46 eq.) in EtOH/AcOH 1:1 (13.3 mL) previously degassed with argon for 15 minutes in an ice bath. The tube was sealed and stirred for 30 minutes in an oil bath heated at 110 °C. The mixture was cooled to room temperature with an ice bath and then diluted with water and extracted three times with EtOAc. Combined organic extracts were washed with 1 M aqueous HCl solution, water, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. Evaporation provided pyrazolone product (1.087 g) which was directly use for the next step without further purification.

To a solution of intermediate (1.087 g) in CH₂Cl₂ (32.5 mL) under argon was added PhNTf₂ (1.665 g, 4.66 mmol, 2 eq.), then the mixture was cooled in an ice bath for 10 minutes before addition of Et₃N (3.88 mL, 28 mmol, 12 eq.). The mixture was stirred at room temperature for 1 h. Evaporation under reduced pressure provided triflated product (3.67 g) which was directly use for the next step without further purification.

To a solution of intermediate (3.67 g) in CH₂Cl₂ (13.8 mL) was added TFA (13.8 mL, 180 mmol, 78 eq.). The mixture was stirred for 1 h at room temperature. After evaporation under reduced pressure, the residue was solubilized in EtOAc, washed with a saturated aqueous Na₂CO₃ solution, brine, dried with MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography (SiO₂, cyclohexane/EtOAc 9:1 to 7:3) to give deprotected product (685 mg) as brown wax, which was directly use for the next step without further purification.

A screw cap tube under argon was charged with intermediate (685 mg), Pd(OAc)₂ (46 mg, 0.205 mmol, 0.09 eq.), DPPF (295 mg, 0.53 mmol, 0.23 eq.) and NH₄HCO₂ (518 mg, 8.21 mmol, 3.5 eq.), then DMF (26 mL) previously degassed with argon for 15 minutes was added. The tube was sealed and stirred overnight in an oil bath heated at 110 °C. The mixture was filtered through a pad of Celite which was then washed with EtOAc. The solution was washed with water and then with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 7:3 to 5:5) to give **11a** (330 mg, 0.94 mmol, 40% in four steps) as a brown solid. *R*_f = 0.24 (cyclohexane/EtOAc 5:5); Mp 98–100 °C; IR (ATR) 3397, 2921, 1455, 1336, 1242, 1096, 1067, 904, 736 cm⁻¹; ¹H NMR (400 MHz, CD₃CN) δ 11.08 (br s, 1H), 9.33 (br s, 1H), 7.76 (s, 1H), 7.63 (br s, 1H), 7.44–7.40 (m, 2H), 7.37 (d, *J* = 7.9, 1H), 7.22 (br s, 1H), 7.18–7.11 (m, 2H), 7.11 (s, 1H), 6.98 (dd, *J* = 8.1, 7.0, 1.0, 1H), 6.95 (ddd, *J* = 8.0, 7.0, 1.0, 1H), 5.74 (ddt, *J* = 17.1, 10.1, 6.9, 1H), 5.01–4.92 (m, 2H), 4.17 (t, *J* = 6.9, 2H), 2.49 (qt, *J* = 6.9, 1.3, 2H); ¹³C NMR (101 MHz, CD₃CN) δ 140.6^a (C), 137.2 (C), 137.1 (C), 136.3 (CH), 135.4^b (CH), 128.5 (C), 127.6 (CH), 126.9 (C), 125.0 (CH), 122.9 (CH), 122.4 (CH), 121.6 (CH), 120.8 (CH), 120.6 (CH), 120.0 (CH), 117.5 (CH₂), 112.8 (C), 112.4 (CH), 110.7 (CH), 108.5^a (C), 108.3 (C), 46.3 (CH₂), 35.3 (CH₂), ^aestimated value from HMBC experiment, ^bestimated value from HSQC experiment; HRMS (ESI+) *m/z* calcd for C₂₃H₂₁N₄ (M + H)⁺ 353.1751, found 353.1755.

6.1.15. 3-(5-(1*H*-Indol-3-yl)-1*H*-pyrazol-4-yl)-1-(pent-4-en-1-yl)-1*H*-indole (**11b**)

To a screw cap tube under argon with **9b** (1.00 g, 2.41 mmol) was added a solution of hydrazine monohydrate (5.53 mL, 114 mmol, 47 eq.) in EtOH/AcOH 1:1 (14 mL) previously degassed with

argon for 15 minutes in an ice bath. The tube was sealed and stirred for 1 h in an oil bath heated at 110 °C. The mixture was cooled to room temperature with an ice bath and then diluted with water and extracted three times with EtOAc. Combined organic extracts were washed with brine, dried over MgSO₄, filtered and concentrated under reduced. Evaporation provided crude pyrazolone product (2.03 g) which was directly use for the next step without further purification.

To a solution of intermediate (2.03 g) in dry CH₂Cl₂ (34 mL) under argon was added PhNTf₂ (1.76 g, 4.93 mmol, 2 eq.), then the mixture was cooled in an ice bath for 10 minutes before addition of Et₃N (4.13 mL, 29.6 mmol, 12 eq.). The mixture was stirred at room temperature for 1 h. After evaporation under reduced pressure, the crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 9:1 to 8:2) to give triflated product (1.54 g) as brown wax, which was directly use for the next step without further purification.

A screw cap tube under argon was charged with intermediate (1.54 g), Pd(OAc)₂ (83 mg, 0.37 mmol, 0.15 eq.), DPPF (534 mg, 0.96 mmol, 0.4 eq.) and NH₄HCO₂ (933 mg, 14.8 mmol, 6 eq.), then DMF (31 mL) previously degassed with argon for 15 minutes was added. The tube was sealed and stirred overnight in an oil bath heated at 110 °C. The mixture was filtered through a pad of Celite which was then washed with EtOAc. The solution was washed two times with water, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 7:3 to 6:4) to give **11b** (385 mg, containing 26% of **15**), 0.78 mmol of **11b**, 32% in three steps) as a beige solid. *R*_f = 0.33 (cyclohexane/acetone 6:4); IR (ATR) 3393, 2930, 1456, 1335, 1096, 904, 737 cm⁻¹; ¹H NMR (400 MHz, CD₃CN) δ 11.14 (br s, 1H), 9.36 (br s, 1H), 7.78 (s, 1H), 7.58 (br s, 1H), 7.45–7.36 (m, 3H), 7.25 (d, *J* = 2.4, 1H), 7.15 (ddd, *J* = 8.3, 7.1, 1.2, 1H), 7.12 (ddd, *J* = 8.2, 7.0, 1.2, 1H), 7.08 (s, 1H), 6.98–6.93 (m, 2H), 5.84–5.73 (m, 1H), 4.98–4.92 (m, 2H), 4.09 (t, *J* = 6.9, 2H), 1.98–1.91 (m, 2H), 1.81 (quint, *J* = 7.1, 2H); ¹³C NMR (101 MHz, CD₃CN) δ 140.6^a (C), 138.9 (CH), 137.18 (C), 137.17 (C), 135.6^b (CH), 128.5 (C), 127.6 (CH), 126.8 (C), 125.0 (CH), 122.9 (CH), 122.3 (CH), 121.5 (CH), 120.8 (CH), 120.6 (CH), 120.0 (CH), 115.7 (CH₂), 113.0 (C), 112.4 (CH), 110.7 (CH), 108.7^a (C), 108.4 (C), 46.1 (CH₂), 31.4 (CH₂), 30.0 (CH₂), ^aestimated value from HMBC experiment, ^bestimated value from HSQC experiment; HRMS (ESI+) *m/z* calcd for C₂₄H₂₃N₄ (M + H)⁺ 367.1917, found 367.1910.

6.1.16. 3-[4-(1*H*-Indol-3-yl)-1*H*-pyrazol-5-yl]-1-(but-3-en-1-yl)-1*H*-indole (**12a**)

To a screw cap tube under argon with **10a** (1.00 g, 2.50 mmol) was added a solution of hydrazine monohydrate (5.6 mL, 115 mmol, 46 eq.) in EtOH/AcOH 1:1 (14 mL) previously degassed with argon for 15 minutes in an ice bath. The tube was sealed and stirred for 1 h in an oil bath heated at

110 °C. The mixture was cooled to room temperature with an ice bath and then diluted with water and extracted three times with EtOAc. Combined organic extracts were washed with 1 M aqueous HCl solution, water, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. Evaporation provided pyrazolone product (1.14 g) which was directly use for the next step without further purification.

To a solution of intermediate (1.14 g) in anhydrous CH₂Cl₂ (36 mL) under argon was added PhNTf₂ (1.77 g, 5.0 mmol, 2 eq.), then the mixture was cooled in an ice bath for 10 minutes before addition of Et₃N (4.3 mL, 31 mmol, 12 eq.). The mixture was stirred at room temperature for 1 h. After evaporation under reduced pressure, the crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 9:1 to 8:2) to give triflated product (1.40 g) as brown wax, which was directly use for the next step without further purification.

A screw cap tube under argon was charged with intermediate (1.40 g), Pd(OAc)₂ (84 mg, 0.374 mmol, 0.15 eq.), DPPF (541 mg, 0.976 mmol, 0.39 eq.) and NH₄HCO₂ (945 mg, 15.0 mmol, 6 eq.), then DMF (32 mL) previously degassed with argon for 15 minutes was added. The tube was sealed and stirred overnight in an oil bath heated at 110 °C. The mixture was filtered through a pad of Celite which was then washed with EtOAc. The solution was washed two times with water, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 7:3 to 6:4) to give **12a** (377 mg, 1.07 mmol, 43% in three steps) as a white solid. *R*_f = 0.35 (CH₂Cl₂/MeOH 99:1); Mp 92 °C; IR (ATR) 3403, 2932, 1455, 1334, 1239, 1095, 907, 737 cm⁻¹; ¹H NMR (400 MHz, CD₃CN) δ 11.11 (br s, 1H), 9.28 (br s, 1H), 7.78 (s, 1H), 7.66 (d, *J* = 7.9, 1H), 7.44–7.39 (m, 2H), 7.34 (d, *J* = 8.0, 1H), 7.20–7.14 (m, 3H), 7.12 (ddd, *J* = 8.2, 7.1, 1.1, 1H), 7.00 (ddd, *J* = 8.0, 7.1, 0.9, 1H), 6.94 (ddd, *J* = 8.0, 7.0, 1.0, 1H), 5.67 (ddt, *J* = 17.1, 10.3, 6.9, 1H), 4.96–4.89 (m, 2H), 4.11 (t, *J* = 6.9, 2H), 2.44 (qt, *J* = 6.9, 1.2, 2H); ¹³C NMR (101 MHz, CD₃CN) δ 140.8^a (C), 137.4 (C), 137.0 (C), 136.1 (CH), 135.1^b (CH), 128.5 (CH), 127.8 (C), 127.5 (C), 124.0 (CH), 122.7 (CH), 122.5 (CH), 121.8 (CH), 120.6 (CH), 120.5 (CH), 120.2 (CH), 117.6 (CH₂), 113.1 (C), 112.4 (CH), 110.8 (CH), 109.2 (C), 107.7^a (C), 46.4 (CH₂), 35.1 (CH₂), ^aestimated value from HMBC experiment, ^bestimated value from HSQC experiment; HRMS (ESI+) *m/z* calcd for C₂₃H₂₁N₄ (M + H)⁺ 353.1761, found 353.1755.

6.1.17. 3-(4-(1*H*-Indol-3-yl)-1*H*-pyrazol-5-yl)-1-(pent-4-en-1-yl)-1*H*-indole (**12b**)

To a screw cap tube under argon with **10b** (500 mg, 1.21 mmol) was added a solution of hydrazine monohydrate (2.7 mL, 56 mmol, 46 eq.) in EtOH/AcOH 1:1 (7 mL) previously degassed with argon for 15 minutes in an ice bath. The tube was sealed and stirred for 1 h 45 min in an oil bath

heated at 110 °C. The mixture was cooled to room temperature with an ice bath and then diluted with water and extracted three times with EtOAc. Combined organic extracts were washed with 1 M aqueous HCl solution, water, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. Evaporation provided crude pyrazolone product (993 mg) which was directly use for the next step without further purification.

To a solution of intermediate (993 mg) in dry CH₂Cl₂ (17 mL) under argon was added PhNTf₂ (857 mg, 2.40 mmol, 2 eq.), then the mixture was cooled in an ice bath for 10 minutes before addition of Et₃N (2.1 mL, 15 mmol, 12.5 eq.). The mixture was stirred at room temperature for 1 h. After evaporation under reduced pressure, the crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 9:1 to 8:2) to give triflated product (719 mg) as brown wax, which was directly use for the next step without further purification.

A screw cap tube under argon was charged with intermediate (719 mg), Pd(OAc)₂ (40 mg, 0.18 mmol, 0.15 eq.), DPPF (262 mg, 0.47 mmol, 0.39 eq.) and NH₄HCO₂ (457 mg, 7.25 mmol, 6 eq.), then DMF (15.5 mL) previously degassed with argon for 15 minutes was added. The tube was sealed and stirred overnight in an oil bath heated at 110 °C. The mixture was filtered through a pad of Celite which was then washed with EtOAc. The solution was washed two times with water, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 7:3 to 6:4) to give **12b** (206 mg, 0.562 mmol, 47% in three steps) as a beige solid. *R*_f = 0.16 (cyclohexane/EtOAc 6:4); Mp 81 °C; IR (ATR) 3403, 2928, 1455, 1334, 1096, 907, 737 cm⁻¹; ¹H NMR (400 MHz, CD₃CN) δ 11.06 (br s, 1H), 9.28 (br s, 1H), 7.77 (s, 1H), 7.68 (d, *J* = 7.9, 1H), 7.43–7.38 (m, 2H), 7.31 (d, *J* = 8.0, 1H), 7.19–7.14 (m, 2H), 7.15 (s, 1H), 7.10 (ddd, *J* = 8.2, 7.0, 1.2, 1H), 7.00 (ddd, *J* = 8.0, 7.0, 1.0, 1H), 6.91 (ddd, *J* = 8.0, 7.0, 1.0, 1H), 5.75 (ddt, *J* = 17.0, 10.5, 6.5, 1H), 4.95–4.88 (m, 2H), 4.04 (t, *J* = 6.8, 2H), 1.92–1.85 (m, 2H), 1.79–1.71 (m, 2H); ¹³C NMR (101 MHz, CD₃CN) δ 140.0^a (C), 138.8 (CH), 137.4 (C), 137.0 (C), 135.3^b (CH), 128.5 (CH), 127.8 (C), 127.5 (C), 124.0 (CH), 122.7 (CH), 122.5 (CH), 121.8 (CH), 120.6 (CH), 120.5 (CH), 120.1 (CH), 115.7 (CH₂), 113.1 (C), 112.4 (CH), 110.7 (CH), 109.3 (C), 107.6^b (C), 46.1 (CH₂), 31.3 (CH₂), 29.8 (CH₂), ^aestimated value from HMBC experiment, ^bweak signal; HRMS (ESI+) *m/z* calcd for C₂₄H₂₃N₄ (M + H)⁺ 367.1917, found 367.1912.

6.1.18. 12-(But-3-en-1-yl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole (**13a**)

The reaction was performed in parallel in two Pyrex round bottom flasks. Each flask (250 mL) was charged with **11a** (50 mg, 0.142 mmol) and CH₃CN (83 mL). The solution was degassed with argon for 15 min, iodine (3.5 mg, 0.014 mmol, 0.1 eq.) was added and the mixture was irradiated

for 4 h at 20–25 °C with two Ultra-Vitalux UV-A lamps (Osram). Saturated aqueous Na₂S₂O₃ solution (3 mL) was added. The contents of the two flasks were combined and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc, washed with water and dried over MgSO₄. After evaporation the crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 7:3) to give **13a** (65 mg, 0.185 mmol, 65%) as a beige solid. *R*_f = 0.31 (CH₂Cl₂/MeOH 98:2); Mp > 130 °C (decomposition); IR (ATR) 3441, 3156, 3050, 2920, 1610, 1460, 1324, 920, 736 cm⁻¹; ¹H NMR (400 MHz, CD₃CN) δ 11.81 (br s, 1H), 10.06 (s, 1H), 8.63 (s, 1H), 8.43 (d, *J* = 7.8, 1H), 8.33 (d, *J* = 7.8, 1H), 7.82 (d, *J* = 8.1, 1H), 7.69 (d, *J* = 8.3, 1H), 7.51–7.46 (m, 2H), 7.46 (ddd, *J* = 8.0, 6.9, 1.1, 1H), 7.34 (ddd, *J* = 7.9, 7.0, 1.0, 1H), 5.91 (ddt, *J* = 17.1, 10.1, 7.0, 1H), 4.96 (dq, *J* = 17.1, 1.6, 1H), 4.89 (t, *J* = 7.0, 1H), 4.89–4.85 (m, 1H), 2.75 (qt, *J* = 7.0, 1.2, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.0 (C), 138.6 (C), 135.0 (CH), 132.0 (CH), 131.2 (C), 125.7 (C), 123.9 (2CH), 123.3 (C), 122.8 (C), 121.5 (C), 120.8 (CH), 120.6 (CH), 119.9 (CH), 119.3 (CH), 117.4 (CH₂), 111.9 (CH), 111.3 (C), 110.9 (C), 110.1 (CH), 104.9 (C), 43.5 (CH₂), 35.0 (CH₂); HRMS (ESI+) *m/z* calcd for C₂₃H₁₉N₄ (M + H)⁺ 351.1604, found 351.1595.

6.1.19. 12-(Pent-4-en-1-yl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole (**13b**)

Compound **13b** was prepared using the same procedure described for **13a**, from **11b** (2 x 50 mg, containing 26% of **15**, 2 x 0.101 mmol of **11b**) and CH₃CN (2 x 83 mL) and 6 h of irradiation, to give **13b** (68 mg, containing 33% of **16**, 0.125 mmol of **13b**, 62%) as a beige solid; *R*_f = 0.53 (cyclohexane/EtOAc 5:5); IR (ATR) 3431, 3059, 2926, 1461, 1325, 922, 736 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.71 (s, 1H), 11.66 (s, 1H), 8.75 (s, 1H), 8.67 (d, *J* = 7.6, 1H), 8.41 (d, *J* = 7.4, 1H), 7.79 (d, *J* = 8.1, 1H), 7.75 (d, *J* = 8.3, 1H), 7.49–7.41 (m, 2H), 7.36–7.26 (m, 2H), 5.76 (ddt, *J* = 16.9, 10.4, 6.2, 1H), 5.02–4.86 (m, 4H), 2.08–1.99 (m, 2H), 1.98–1.88 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.0 (C), 138.6 (C), 137.9 (CH), 131.9^a (CH), 130.8^b (C), 125.6 (C), 123.8 (2CH), 123.3^c (C), 122.7 (C), 121.6 (C), 120.8 (CH), 120.6 (CH), 119.8 (CH), 119.3 (CH), 115.0 (CH₂), 111.8 (CH), 111.2 (C), 110.8 (C), 109.9 (CH), 104.9^b (C), 43.5 (CH₂), 30.2 (CH₂), 29.7 (CH₂), ^aestimated value from HSQC experiment, ^bestimated value from HMBC experiment, ^cweak signal; HRMS (ESI+) *m/z* calcd for C₂₄H₂₁N₄ (M + H)⁺ 365.1761, found 365.1760.

6.1.20. 13-(But-3-en-1-yl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole (**14a**)

Compound **14a** was prepared using the same procedure described for **13a**, from **12a** (2 x 50 mg, 2 x 0.142 mmol) and CH₃CN (2x 83 mL), to give **14a** (70 mg, 0.200 mmol, 70%) as a beige solid. *R*_f = 0.44 (cyclohexane/EtOAc 6:4); Mp > 135 °C (decomposition); IR (ATR) 3400, 3052, 2923, 1463, 1399, 1324, 1247, 921, 839, 735 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.69 (s, 1H),

11.49 (s, 1H), 8.75 (s, 1H), 8.66 (d, $J = 7.4$, 1H), 8.40 (d, $J = 7.6$, 1H), 7.79 (d, $J = 8.3$, 1H), 7.75 (d, $J = 8.1$, 1H), 7.50–7.40 (m, 2H), 7.37–7.27 (m, 2H), 5.85 (ddt, $J = 17.1, 10.2, 7.0$, 1H), 5.04 (t, $J = 6.6$, 2H), 4.87 (d, $J = 17.0$, 1H), 4.79 (d, $J = 10.1$, 1H), 2.69–2.61 (m, 2H); ^{13}C NMR (101 MHz, DMSO- d_6 /CD $_3$ CN 5:1) δ 139.2 (C), 138.6 (C), 135.0 (CH), 132.0^a (CH), 131.5^b (C), 127.2 (C), 123.9 (CH), 123.7 (CH), 122.8 (C), 122.0^c (C), 121.8^c (C), 120.9 (CH), 120.5 (CH), 119.8 (CH), 119.4 (CH), 117.3 (CH $_2$), 112.0 (CH), 111.8^c (C), 111.4 (C), 110.0 (CH), 43.6 (CH $_2$), 34.9 (CH $_2$), ^aestimated value from HSQC experiment, ^b estimated value from HMBC experiment, ^cweak signal, one C was not observed; HRMS (ESI+) m/z calcd for C $_{23}$ H $_{19}$ N $_4$ (M + H)⁺ 351.1604, found 351.1597.

6.1.21. 13-(Pent-4-en-1-yl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole (**14b**)

Compound **14b** was prepared using the same procedure described for **13a**, from **12b** (2 x 50 mg, 2 x 0.136 mmol) and CH $_3$ CN (2x 83 mL) and 5 h of irradiation, to give **14b** (72 mg, 0.198 mmol, 72%) as a beige solid. $R_f = 0.39$ (cyclohexane/EtOAc 6:4); Mp > 201 °C (decomposition); IR (ATR) 3435, 3056, 2912, 1323, 917, 733 cm $^{-1}$; ^1H NMR (400 MHz, DMSO- d_6) δ 13.70 (s, 1H), 11.46 (s, 1H), 8.75 (s, 1H), 8.68 (d, $J = 7.5$, 1H), 8.40 (d, $J = 7.8$, 1H), 7.79 (d, $J = 8.5$, 1H), 7.76 (d, $J = 8.2$, 1H), 7.48 (t, $J = 7.5$, 1H), 7.43 (t, $J = 7.6$, 1H), 7.35 (t, $J = 7.3$, 1H), 7.30 (t, $J = 7.4$, 1H), 5.77 (ddt, $J = 17.0, 10.4, 6.3$, 1H), 4.98 (t, $J = 6.4$, 2H), 4.95–4.86 (m, 2H), 2.10–2.02 (m, 2H), 2.01–1.91 (m, 2H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 139.0 (C), 138.6 (C), 137.9 (CH), 132.0 (CH), 131.1^a (C), 127.0 (C), 123.9 (CH), 123.7 (CH), 122.7 (C), 121.8 (C), 121.6 (C), 120.9 (CH), 120.6 (CH), 119.8 (CH), 119.4 (CH), 115.0 (CH $_2$), 112.0 (CH), 111.7 (C), 111.3 (C), 109.9 (CH), 43.6 (CH $_2$), 30.2 (CH $_2$), 29.7 (CH $_2$), ^aweak signal, experiment one C was not observed; HRMS (ESI+) m/z calcd for C $_{24}$ H $_{21}$ N $_4$ (M + H)⁺ 365.1761, found 365.1754.

6.1.22. Di-*tert*-butyl 12-(but-3-en-1-yl)-6*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole-6,13(12*H*)-dicarboxylate (**17**)

To a solution of **13a** (250 mg, 0.713 mmol) in acetonitrile (30 mL) were added Boc $_2$ O (467 mg, 2.14 mmol, 3 eq.) and DMAP (35 mg, 0.286 mmol, 0.4 eq.). The solution was stirred overnight at room temperature. After evaporation under reduced pressure, the crude was purified by column chromatography (SiO $_2$, cyclohexane/EtOAc 9:1) to give **17** (387 mg, 0.703 mmol, 99%) as a yellow fluorescent solid. $R_f = 0.23$ (cyclohexane/acetone 95:5); Mp 125 °C; IR (ATR) 2984, 2932, 2851, 1741, 1248, 1137, 962, 842, 743 cm $^{-1}$; ^1H NMR (400 MHz, DMSO- d_6) δ 9.48 (s, 1H), 8.48 (d, $J = 7.8$, 1H), 8.46–8.41 (m, 1H), 8.20–8.15 (m, 1H), 7.87 (d, $J = 8.3$, 1H), 7.61–7.56 (m, 2H), 7.48 (t, $J = 7.6$, 1H), 7.35 (t, $J = 7.4$, 1H), 5.36 (ddt, $J = 17.0, 10.2, 6.9$, 1H), 4.68–4.60 (m, 4H), 2.16 (q, $J = 6.8$, 2H), 1.75 (s, 9H), 1.64 (s, 9H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 151.3 (C),

147.8 (C), 144.0 (C), 140.0 (C), 138.8 (C), 134.2 (CH), 129.8 (C), 128.9 (C), 126.4 (CH), 125.7 (C), 124.7 (CH), 124.5 (CH), 123.8 (C), 122.6 (CH), 121.5 (CH), 121.2 (CH), 120.6 (CH), 117.1 (CH₂), 115.4 (CH), 115.1 (C), 113.6 (C), 111.9 (C), 111.8 (CH), 85.8 (C), 85.6 (C), 45.8 (CH₂), 33.0 (CH₂), 27.6 (3CH₃), 27.5 (3CH₃); HRMS (ESI+) *m/z* calcd for C₃₃H₃₅N₄O₄ (M + H)⁺ 551.2653, found 551.2640.

6.1.23. Di-*tert*-butyl 12-(3-oxopropyl)-6*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole-6,13(12*H*)-dicarboxylate (**18**)

To a solution of **15** (20 mg, 0.036 mmol) in THF/H₂O 1:1 (2 mL) were added K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (39 mg, 0.182 mmol, 5 eq.). The mixture was protected from light and stirred for 2 h at room temperature. The mixture was filtered and the solid was washed with CH₂Cl₂. The filtrate was washed with a saturated aqueous solution of Na₂S₂O₃, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 8:2) to give **18** (17 mg, 0.031 mmol, 85%) as a yellow solid. *R*_f = 0.26 (cyclohexane/EtOAc 8:2); Mp > 120 °C (decomposition); IR (ATR) 2924, 2847, 1768, 1729, 1717, 1286, 1140, 963, 844, 741 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.49 (s, 1H), 9.40 (s, 1H), 8.49 (d, *J* = 7.8, 1H), 8.46–8.41 (m, 1H), 8.20–8.14 (m, 1H), 7.84 (d, *J* = 8.3, 1H), 7.61–7.56 (m, 2H), 7.48 (t, *J* = 7.6, 1H), 7.36 (t, *J* = 7.4, 1H), 4.84 (t, *J* = 7.0, 2H), 2.65 (t, *J* = 6.8, 2H), 1.75 (s, 9H), 1.67 (s, 9H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 201.1 (CH), 151.2 (C), 147.8 (C), 144.0 (C), 139.7 (C), 138.8 (C), 129.6 (C), 128.7 (C), 126.4 (CH), 125.7 (C), 124.7 (CH), 124.6 (CH), 123.9 (C), 122.6 (CH), 121.5 (CH), 121.2 (CH), 120.8 (CH), 115.5 (CH), 115.0 (C), 113.8 (C), 112.1 (C), 111.8 (CH), 85.8 (C), 85.6 (C), 42.1 (CH₂), 40.3 (CH₂), 27.6 (3CH₃), 27.5 (3CH₃); HRMS (ESI+) *m/z* calcd for C₃₃H₃₇N₄O₆ (M + CH₃OH + H)⁺ 585.2708, found 585.2704.

6.1.24. 3-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)-*N*-methylpropan-1-amine dihydrochloride (**19a**)

Compound **19a** was prepared according to general procedure A, starting from **18** (75 mg, 0.136 mmol) and methylamine 33% in ethanol (388 μL, 3.12 mmol, 23 eq.). The mixture was stirred for 2 h before addition of NaBH₄. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99:1 to 97:3, then 95:5 + 0.5% Et₃N). The dihydrochloride salt was washed with CH₂Cl₂ to give **19a** (36 mg, 0.082 mmol, 60%) as a pale yellow solid. *R*_f (free amine) = 0.26 (CH₂Cl₂/MeOH 9:1); Mp > 160 °C (decomposition); IR (ATR) 3600–3100, 3055, 3870–2500, 1618, 1462, 1328, 732 cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.99 (s, 1H), 8.84 (s, 1H), 8.78–8.70 (br s, 2H), 8.67 (d, *J* = 7.8, 1H), 8.43 (d, *J* = 7.7, 1H), 7.88–7.84 (m, 2H), 7.48 (ddd, *J* = 8.2, 7.1, 1.2, 1H), 7.45 (ddd, *J* = 8.2, 7.0, 1.2, 1H), 7.34 (ddd, *J* = 8.0, 7.0, 1.0, 1H), 7.33 (ddd, *J* = 7.9,

7.0, 0.8, 1H), 5.10 (t, $J = 7.0$, 2H), 3.02–2.95 (m, 2H), 2.45 (t, $J = 5.4$, 3H), 2.20 (quint, $J = 7.4$, 2H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 138.9 (C), 138.8 (C), 132.1 (C), 129.9 (CH), 126.3 (C), 124.2 (CH), 124.0 (CH), 123.6 (C), 122.6 (C), 121.4 (C), 120.9 (CH), 120.7 (CH), 120.1 (CH), 119.8 (CH), 112.3 (CH), 110.9 (C), 110.6 (C), 110.1 (CH), 104.7 (C), 45.7 (CH₂), 41.6 (CH₂), 32.3 (CH₃), 27.1 (CH₂); HRMS (ESI+) m/z calcd for C₂₃H₂₂N₅ (M + H)⁺ 368.1870, found 368.1865; HPLC purity \geq 96%, Method A: $t_{\text{R}} = 20.95$ min, $\lambda = 240$ nm.

6.1.25. 4-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)-*N*-methylbutan-1-amine dihydrochloride (**19b**)

To a solution of **13b** (100 mg, containing 33% of **16**, 0.184 mmol of **13b**) in THF/H₂O 2:1 (15 mL) were added K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (294 mg, 1.37 mmol, 7.5 eq.). The mixture was protected from light and stirred overnight at room temperature. After addition of a saturated aqueous solution of Na₂S₂O₃, the mixture was extracted 3 times with CH₂Cl₂, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was solubilized in MeOH (12.5 mL) and methylamine 33% in ethanol (682 μL , 5.48 mmol, 30 eq.) was added. The solution was stirred at room temperature for 2 h, then cooled in an ice bath before addition of NaBH₄ (52 mg, 1.37 mmol, 7.5 eq.). The solution was stirred for 1 h 30 min at room temperature and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 95:5 + 0.5% Et₃N). 4 M aqueous HCl was added to the free amine, and the dihydrochloride salt was dried under reduced pressure and acetonitrile co-evaporation, and finally washed with dichloromethane to give **19b** (35 mg, 0.077 mmol, 42%) as a beige solid. R_f (free amine) = 0.26 (CH₂Cl₂/MeOH 9:1 + 0.5% Et₃N); Mp > 160 °C (decomposition); IR (ATR) 3600–3110, 2920, 2880–2400, 1614, 1462, 1330, 1240, 739 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 11.86 (s, 1H), 8.82 (s, 1H), 8.66 (d, $J = 7.8$, 1H), 8.42 (d, $J = 7.8$, 1H), 8.37 (br s, 2H), 7.83–7.79 (m, 2H), 7.50–7.41 (m, 2H), 7.37–7.29 (m, 2H), 5.02 (t, $J = 6.4$, 2H), 2.81–2.73 (m, 2H), 2.36 (t, $J = 5.3$, 3H), 1.93–1.84 (m, 2H), 1.62–1.53 (m, 2H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 139.0 (C), 138.7 (C), 132.1 (C), 129.9 (CH), 126.5 (C), 124.0 (CH), 123.9 (CH), 123.8 (C), 122.6 (C), 121.4 (C), 120.8 (CH), 120.7 (CH), 120.1 (CH), 119.6 (CH), 112.2 (CH), 110.9 (C), 110.5 (C), 110.2 (CH), 104.7 (C), 47.9 (CH₂), 43.5 (CH₂), 32.3 (CH₃), 27.7 (CH₂), 22.8 (CH₂); HRMS (ESI+) m/z calcd for C₂₄H₂₄N₅ (M + H)⁺ 382.2026, found 382.2015; HPLC purity \geq 95%, Method A: $t_{\text{R}} = 21.13$ min, $\lambda = 240$ nm.

6.1.26. 3-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)-*N*-ethylpropan-1-amine dihydrochloride (**20a**)

Compound **20a** was prepared according to general procedure A, starting from **18** (75 mg, 0.136 mmol) and ethylamine (2 M in methanol, 1.36 mL, 2.72 mmol, 20 eq.). The mixture was stirred for 2 h before addition of NaBH₄. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99:1 + 0.5% Et₃N to 98:2 + 0.5% Et₃N). The dihydrochloride salt was washed with CH₂Cl₂ to give **20a** (49 mg, 0.108 mmol, 79%) as a pale yellow solid. *R_f* (free amine) = 0.19 (CH₂Cl₂/MeOH 95:5); Mp > 160 °C (decomposition); IR (ATR) 3600–2500, 1606, 1461, 1328, 725 cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆) δ 12.02 (s, 1H), 8.85 (s, 1H), 8.74 (br s, 2H), 8.67 (d, *J* = 7.8, 1H), 8.43 (d, *J* = 7.8, 1H), 7.87 (d, *J* = 8.0, 1H), 7.86 (d, *J* = 8.2, 1H), 7.48 (ddd, *J* = 8.2, 7.1, 1.1, 1H), 7.45 (ddd, *J* = 8.2, 7.0, 1.2, 1H), 7.34 (ddd, *J* = 8.0, 6.9, 1.1, 1H), 7.33 (ddd, *J* = 7.9, 7.0, 0.9, 1H), 5.11 (t, *J* = 7.0, 2H), 3.04–2.96 (m, 2H), 2.88–2.80 (m, 2H), 2.20 (quint, *J* = 7.3, 2H), 1.11 (t, *J* = 7.2, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 138.9 (C), 138.8 (C), 132.1 (C), 129.7 (CH), 126.4 (C), 124.2 (CH), 124.0 (CH), 123.6 (C), 122.6 (C), 121.4 (C), 120.9 (CH), 120.7 (CH), 120.2 (CH), 119.8 (CH), 112.4 (CH), 110.8 (C), 110.5 (C), 110.1 (CH), 104.6 (C), 43.7 (CH₂), 41.8 (CH₂), 41.7 (CH₂), 27.2 (CH₂), 10.9 (CH₃); HRMS (ESI⁺) *m/z* calcd for C₂₄H₂₄N₅ (M + H)⁺ 382.2026, found 382.2021; HPLC purity ≥ 98%, Method A: *t_R* = 21.30 min, λ = 240 nm.

6.1.27. 4-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)-*N*-ethylbutan-1-amine dihydrochloride (**20b**)

To a solution of **13b** (100 mg, containing 33% of **16**, 0.184 mmol of **13b**) in THF/H₂O 2:1 (15 mL) were added K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (294 mg, 1.37 mmol, 7.5 eq.). The mixture was protected from light and stirred overnight at room temperature. After addition of a saturated aqueous solution of Na₂S₂O₃, the mixture was extracted 3 times with CH₂Cl₂, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was solubilized in MeOH (12.5 mL) and ethylamine (2 M in MeOH, 2.74 mL, 5.48 mmol, 30 eq.) was added. The solution was stirred at room temperature for 2 h, then cooled in an ice bath before addition of NaBH₄ (52 mg, 1.37 mmol, 7.5 eq.). The solution was stirred for 1 h 30 min at room temperature and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99:1 + 0.5% Et₃N to 97:3 + 0.5% Et₃N). 4 M aqueous HCl was added to the free amine, and the dihydrochloride salt was dried under reduced pressure and acetonitrile co-evaporation, and finally washed with dichloromethane to give **20b** (36 mg, 0.077 mmol, 42%) as a beige solid. *R_f* (free amine) = 0.23 (CH₂Cl₂/MeOH 95:5 + 0.5% Et₃N); Mp > 160 °C (decomposition); IR (ATR) 3600–3140, 2925, 2880–2450, 1616, 1463, 1329, 1242, 728 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.87 (s, 1H), 8.83 (s, 1H), 8.66 (d, *J* = 7.8, 1H), 8.42 (d, *J* = 7.8, 1H), 8.32 (br s, 2H), 7.83–7.80 (m, 2H), 7.47 (ddd, *J* = 8.3, 7.1, 1.2, 1H), 7.44 (ddd, *J* = 8.2, 7.0, 1.2, 1H), 7.36–7.29 (m, 2H), 5.03 (t, *J* = 6.4, 2H), 2.80–2.69 (m, 4H), 1.95–1.86 (m, 2H), 1.61–

1.51 (m, 2H), 1.03 (t, $J = 7.2$, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 139.0 (C), 138.7 (C), 132.1 (C), 129.7 (CH), 126.7 (C), 124.1 (CH), 124.0 (CH), 123.9 (C), 122.6 (C), 121.4 (C), 120.8 (CH), 120.7 (CH), 120.1 (CH), 119.6 (CH), 112.3 (CH), 110.8 (C), 110.4 (C), 110.3 (CH), 104.6 (C), 45.9 (CH₂), 43.6 (CH₂), 41.8 (CH₂), 27.8 (CH₂), 22.9 (CH₂), 10.8 (CH₃); HRMS (ESI+) m/z calcd for C₂₅H₂₆N₅ (M + H)⁺ 396.2183, found 396.2178; HPLC purity \geq 96%, Method A: $t_{\text{R}} = 21.51$ min, $\lambda = 240$ nm.

6.1.28. 3-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)-*N,N*-dimethylpropan-1-amine dihydrochloride (**21a**)

Compound **21a** was prepared according to general procedure A, starting from **18** (75 mg, 0.136 mmol) and dimethylamine (2 M in methanol, 1.36 mL, 2.72 mmol, 20 eq.). The mixture was stirred overnight before addition of NaBH₄. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99:1 to 97:3). The dihydrochloride salt was washed with CH₂Cl₂ to give **21a** (25 mg, 0.057 mmol, 42%) as a beige solid. R_f (free amine) = 0.46 (CH₂Cl₂/MeOH 95:5); Mp > 160 °C (decomposition); IR (ATR) 3600–3000, 2912, 2856, 2800–2540, 1614, 1462, 1330, 1241, 741 cm⁻¹; ^1H NMR (500 MHz, DMSO- d_6) δ 12.01 (s, 1H), 10.25 (br s, 1H), 8.84 (s, 1H), 8.67 (d, $J = 7.8$, 1H), 8.43 (d, $J = 7.8$, 1H), 7.88 (d, $J = 8.1$, 1H), 7.85 (d, $J = 8.3$, 1H), 7.48 (ddd, $J = 8.2$, 7.1, 1.1, 1H), 7.45 (ddd, $J = 8.2$, 7.0, 1.1, 1H), 7.36–7.32 (m, 2H), 5.09 (t, $J = 7.2$, 2H), 3.29–3.23 (m, 2H), 2.68 (d, $J = 4.9$, 6H), 2.23 (quint, $J = 7.4$, 2H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 138.8 (C), 138.7 (C), 132.1 (C), 129.9 (CH), 126.3 (C), 124.2 (CH), 123.9 (CH), 123.6 (C), 122.7 (C), 121.4 (C), 120.9 (CH), 120.7 (CH), 120.1 (CH), 119.8 (CH), 112.4 (CH), 110.9 (C), 110.6 (C), 110.1 (CH), 104.7 (C), 54.0 (CH₂), 42.0 (2CH₃), 41.6 (CH₂), 25.4 (CH₂); HRMS (ESI+) m/z calcd for C₂₄H₂₄N₅ (M + H)⁺ 382.2026, found 382.2021; HPLC purity \geq 95%, Method A: $t_{\text{R}} = 21.17$ min, $\lambda = 240$ nm.

6.1.29. 4-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)-*N,N*-dimethylbutan-1-amine dihydrochloride (**21b**)

To a solution of **13b** (90 mg, containing 33% of **16**, 0.165 mmol of **13b**) in THF/H₂O 2:1 (13.5 mL) were added K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (264 mg, 1.23 mmol, 7.5 eq.). The mixture was protected from light and stirred overnight at room temperature. After addition of a saturated aqueous solution of Na₂S₂O₃, the mixture was extracted 3 times with CH₂Cl₂, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was solubilized in MeOH (11 mL) and dimethylamine (2 M in MeOH, 2.47 mL, 4.94 mmol, 30 eq.) was added. The solution was stirred at room temperature for 2 h, then cooled in an ice bath before addition of NaBH₄ (47 mg, 1.24 mmol, 7.5 eq.). The solution was stirred for 1 h 30 min at room temperature and

concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 95:5 to 90:10). 4 M aqueous HCl was added to the free amine, and the dihydrochloride salt was dried under reduced pressure and acetonitrile co-evaporation, and finally washed with dichloromethane to give **21b** (30 mg, 0.064 mmol, 39%) as a beige solid. *R_f* (free amine) = 0.32 (CH₂Cl₂/MeOH 9:1); Mp > 160 °C (decomposition); IR (ATR) 3650–3090, 2937, 2990–2380, 1614, 1463, 1330, 1241, 742 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.91 (s, 1H), 9.78 (br s, 1H), 8.83 (s, 1H), 8.67 (d, *J* = 7.8, 1H), 8.42 (d, *J* = 7.8, 1H), 7.835 (d, *J* = 8.3, 1H), 7.826 (d, *J* = 8.1, 1H), 7.50–7.41 (m, 2H), 7.37–7.29 (m, 2H), 5.01 (t, *J* = 6.5, 2H), 3.02–2.94 (m, 2H), 2.61 (d, *J* = 4.8, 6H), 1.91–1.80 (m, 2H), 1.79–1.67 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.0 (C), 138.8 (C), 132.1 (C), 129.7 (CH), 126.6 (C), 124.1 (CH), 124.0 (CH), 123.8 (C), 122.6 (C), 121.4 (C), 120.9 (CH), 120.7 (CH), 120.1 (CH), 119.6 (CH), 112.3 (CH), 110.9 (C), 110.5 (C), 110.2 (CH), 104.6 (C), 56.1 (CH₂), 43.5 (CH₂), 41.9 (2CH₃), 27.7 (CH₂), 21.0 (CH₂); HRMS (ESI+) *m/z* calcd for C₂₅H₂₆N₅ (M + H)⁺ 396.2183, found 396.2178; HPLC purity ≥ 95%, Method A: *t_R* = 21.41 min, λ = 240 nm.

6.1.30. 3-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)propan-1-amine dihydrochloride (**22a**)

Compound **22a** was prepared according to general procedure A, starting from **18** (80 mg, 0.145 mmol) and NH₃ (7 M in MeOH, 1.5 mL, 10.5 mmol, 72 eq.). The mixture was stirred overnight before addition of NaBH₄. The crude was purified by column chromatography (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 99:1 to 96:4) The dihydrochloride salt was washed with CH₂Cl₂ to give **22a** (13 mg, 0.030 mmol, 21%) as a white solid. *R_f* (free amine) = 0.54 (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 9:1); Mp > 160 °C (decomposition); IR (ATR) 3680–3080, 2922, 2851, 1639, 1614, 1462, 1331, 740 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.96 (s, 1H), 8.82 (s, 1H), 8.66 (d, *J* = 7.8, 1H), 8.43 (d, *J* = 7.8, 1H), 7.85 (d, *J* = 8.3, 2H), 7.81 (br s, 3H), 7.51–7.42 (m, 2H), 7.34 (t, *J* = 7.3, 1H), 7.33 (t, *J* = 7.4, 1H), 5.09 (t, *J* = 6.6, 2H), 2.89–2.79 (m, 2H), 2.21–2.11 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 138.9 (C), 138.8 (C), 132.2 (C), 130.2 (CH), 126.0 (C), 124.1 (CH), 123.9 (CH), 123.5 (C), 122.7 (C), 121.4 (C), 120.9 (CH), 120.7 (CH), 120.0 (CH), 119.6 (CH), 112.3 (CH), 111.0 (C), 110.7 (C), 110.1 (CH), 104.9 (C), 41.6 (CH₂), 36.5 (CH₂), 28.7 (CH₂); HRMS (ESI+) *m/z* calcd for C₂₂H₂₀N₅ (M + H)⁺ 354.1713, found 354.1709; HPLC purity ≥ 96%, Method A: *t_R* = 20.72 min, λ = 240 nm.

6.1.31. 4-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)butan-1-amine dihydrochloride (**22b**)

To a solution of **13b** (110 mg, containing 33% of **16**, 0.202 mmol of **13b**) in THF/H₂O 2:1 (16.5 mL) were added K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (323 mg, 1.51 mmol, 7.5 eq.). The mixture was protected from light and stirred overnight at room temperature. After addition of a saturated aqueous solution of Na₂S₂O₃, the mixture was extracted 3 times with CH₂Cl₂, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was solubilized in MeOH (14 mL) and NH₃ (7 M in MeOH, 3 mL, 21 mmol, 104 eq.) was added. The solution was stirred at room temperature for 2 h, then cooled in an ice bath before addition of NaBH₄ (57 mg, 1.51 mmol, 7.5 eq.). The solution was stirred for 1 h 30 min at room temperature and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 95:5 + 0.5% Et₃N to 9:1 + 0.5% Et₃N). After evaporation, the dihydrochloride salt was prepared in MeOH with 4 M aqueous HCl, and the mixture was concentrated under reduced pressure before reverse flash chromatography (C18 4g, H₂O/MeOH 95:5 to 5:95). After evaporation, the dihydrochloride salt was prepared in MeOH with 4 M aqueous HCl, the product was dried under reduced pressure and acetonitrile co-evaporation, and finally washed with dichloromethane to give **22b** (16 mg, 0.036 mmol, 18%) as a beige solid. *R*_f (free amine) = 0.18 (CH₂Cl₂/MeOH 9:1 + 0.5% Et₃N); Mp > 160 °C (decomposition); IR (ATR) 3660–2400, 1600, 1462, 1331, 740 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.85 (s, 1H), 8.80 (s, 1H), 8.66 (d, *J* = 7.8, 1H), 8.41 (d, *J* = 7.8, 1H), 7.81 (d, *J* = 8.0, 1H), 7.80 (d, *J* = 8.2, 1H), 7.63 (br s, 3H), 7.50–7.41 (m, 2H), 7.36–7.29 (m, 2H), 5.02 (t, *J* = 6.4, 2H), 2.72–2.62 (m, 2H), 1.95–1.85 (m, 2H), 1.57–1.46 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.0 (C), 138.7 (C), 132.0^a (C), 130.0^a (CH), 126.3 (C), 124.0 (CH), 123.9 (CH), 123.8 (C), 122.6 (C), 121.5 (C), 120.8 (CH), 120.7 (CH), 120.0 (CH), 119.5 (CH), 112.2 (CH), 111.0 (C), 110.5 (C), 110.2 (CH), 104.8 (C), 43.6 (CH₂), 38.5 (CH₂), 27.7 (CH₂), 24.4 (CH₂), ^aweak signal; HRMS (ESI+) *m/z* calcd for C₂₃H₂₂N₅ (M + H)⁺ 368.1870, found 368.1861; HPLC purity ≥ 95%, Method A: *t*_R = 21.06 min, λ = 240 nm.

6.1.32. Di-*tert*-butyl 13-(but-3-en-1-yl)-6*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole-6,12(13*H*)-dicarboxylate (**23a**)

To a solution of **14a** (74 mg, 0.211 mmol) in acetonitrile (9 mL) were added Boc₂O (139 mg, 0.64 mmol, 3 eq.) and DMAP (10 mg, 0.082 mmol, 0.4 eq.). The solution was stirred overnight at room temperature. After evaporation under reduced pressure, the crude was purified by column chromatography (SiO₂, cyclohexane/ EtOAc 9:1) to give **23a** (110 mg, 0.200 mmol, 95%) as a yellow fluorescent solid. *R*_f = 0.14 (cyclohexane/EtOAc 9:1); Mp 128 °C; IR (ATR) 2984, 2932, 2847, 1733, 1250, 1136, 958, 841, 745 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.57 (s, 1H), 8.52 (d, *J* = 7.4, 1H), 8.39 (d, *J* = 7.7, 1H), 8.17 (d, *J* = 7.9, 1H), 7.88 (d, *J* = 8.2, 1H), 7.61–7.56 (m, 1H), 7.56–7.51 (m, 1H), 7.48 (ddd, *J* = 8.3, 7.0, 1.3, 1H), 7.39 (t, *J* = 7.4, 1H), 5.36 (ddt, *J* = 17.0,

10.2, 6.8, 1H), 4.69–4.59 (m, 4H), 2.21 (q, $J = 6.8$, 2H), 1.75 (s, 9H), 1.63 (s, 9H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 151.5 (C), 147.8 (C), 146.0 (C), 139.6 (C), 139.0 (C), 134.2 (CH), 132.4 (C), 126.5 (CH), 126.30 (C), 126.26 (C), 124.39 (CH), 124.37 (CH), 124.1 (CH), 123.3 (C), 121.3 (2CH), 121.1 (CH), 117.1 (CH₂), 116.5 (C), 115.6 (CH), 112.4 (C), 111.6 (CH), 109.3 (C), 85.7 (C), 85.4 (C), 45.8 (CH₂), 33.1 (CH₂), 27.6 (3CH₃), 27.5 (3CH₃); HRMS (ESI+) m/z calcd for C₃₃H₃₅N₄O₄ (M + H)⁺ 551.2653, found 551.2643.

6.1.33. Di-*tert*-butyl 13-(pent-4-en-1-yl)-6*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole-6,12(13*H*)-dicarboxylate (**23b**)

To a solution of **14b** (120 mg, 0.329 mmol) in acetonitrile (15 mL) were added Boc₂O (216 mg, 0.990 mmol, 3 eq.) and DMAP (16 mg, 0.131 mmol, 0.4 eq.). The solution was stirred overnight at room temperature. After evaporation under reduced pressure, the crude was purified by column chromatography (SiO₂, cyclohexane/ EtOAc 95:5 to 9:1) to give **23b** (185 mg, 0.328 mmol, 99%) as a yellow fluorescent solid. $R_f = 0.22$ (cyclohexane/EtOAc 9:1); Mp 111 °C; IR (ATR) 2980, 2928, 2847, 1733, 1249, 1136, 958, 841, 745 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 9.57 (s, 1H), 8.52 (d, $J = 7.5$, 1H), 8.40 (d, $J = 7.7$, 1H), 8.17 (d, $J = 8.0$, 1H), 7.85 (d, $J = 8.2$, 1H), 7.61–7.56 (m, 1H), 7.54 (t, $J = 7.4$, 1H), 7.48 (t, $J = 7.6$, 1H), 7.40 (t, $J = 7.4$, 1H), 5.54 (ddt, $J = 17.0$, 10.4, 6.2, 1H), 4.77 (d, $J = 10.2$, 1H), 4.71 (d, $J = 17.2$, 1H), 4.60 (t, $J = 6.5$, 2H), 1.75 (s, 9H), 1.67–1.50 (m, 4H), 1.63 (s, 9H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 151.3 (C), 147.8 (C), 146.0 (C), 139.6 (C), 139.0 (C), 137.0 (CH), 132.4 (C), 126.5 (CH), 126.3 (C), 126.2 (C), 124.38 (CH), 124.37 (CH), 124.1 (CH), 123.2 (C), 121.33 (CH), 121.31 (CH), 121.1 (CH), 116.5 (C), 115.6 (CH), 115.3 (CH₂), 112.4 (C), 111.7 (CH), 109.2 (C), 85.7 (C), 85.3 (C), 45.8 (CH₂), 30.0 (CH₂), 27.8 (CH₂), 27.6 (3CH₃), 27.5 (3CH₃); HRMS (ESI+) m/z calcd for C₃₄H₃₇N₄O₄ (M + H)⁺ 565.2809, found 565.2808.

6.1.34. Di-*tert*-butyl 13-(3-oxopropyl)-6*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole-6,12(13*H*)-dicarboxylate (**24a**)

Compound **24a** was prepared using the same procedure described for **18**, from **23a** (23 mg, 0.042 mmol) in THF/H₂O 1:1 (2 mL), K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (45 mg, 0.210 mmol, 5 eq.) to give **24a** (18 mg, 0.033 mmol, 78%) as a yellow solid. $R_f = 0.21$ (cyclohexane/EtOAc 8:2); Mp > 130 °C (decomposition); IR (ATR) 2983, 2928, 2850, 1764, 1730, 1718, 1296, 1231, 1135, 958, 840, 742 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 9.57 (s, 1H), 9.42 (s, 1H), 8.53 (d, $J = 7.5$, 1H), 8.39 (d, $J = 7.7$, 1H), 8.16 (d, $J = 8.1$, 1H), 7.86 (d, $J = 8.2$, 1H), 7.61–7.56 (m, 1H), 7.56–7.51 (m, 1H), 7.49 (ddd, $J = 8.3$, 7.0, 1.3, 1H), 7.41 (t, $J = 7.4$, 1H), 4.86 (t, $J = 7.1$, 2H), 2.71 (t, $J = 7.0$, 2H), 1.75 (s, 9H), 1.66 (s, 9H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 201.0 (CH), 151.4

(C), 147.8 (C), 145.9 (C), 139.3 (C), 139.0 (C), 132.2 (C), 126.5 (CH), 126.3 (C), 126.1 (C), 124.5 (CH), 124.4 (CH), 124.1 (CH), 123.4 (C), 121.34 (CH), 121.31 (CH), 121.29 (CH), 116.7 (C), 115.7 (CH), 112.5 (C), 111.7 (CH), 109.4 (C), 85.7 (C), 85.4 (C), 42.2 (CH₂), 40.3 (CH₂), 27.6 (3CH₃), 27.5 (3CH₃); HRMS (ESI+) m/z calcd for C₃₂H₃₃N₄O₅ (M + H)⁺ 553.2445, found 553.2430.

6.1.35. Di-*tert*-butyl 13-(4-oxobutyl)-6*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole-6,12(13*H*)-dicarboxylate (**24b**)

Compound **24b** was prepared using the same procedure described for **18**, from **23b** (40 mg, 0.071 mmol) in THF/H₂O 1:1 (4 mL), K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (76 mg, 0.355 mmol, 5 eq.), for 4 h. The crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 9:1 to 7:3) to give **24b** (30 mg, 0.053 mmol, 75%) as a pale-yellow solid. R_f = 0.33 (cyclohexane/EtOAc 8:2); Mp 160–161 °C; IR (ATR) 2980, 2928, 2855, 2722, 1769, 1728, 1286, 1228, 1134, 963, 841, 747 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) 9.59 (s, 1H), 9.30 (s, 1H), 8.54 (d, J = 7.5, 1H), 8.40 (d, J = 7.7, 1H), 8.16 (d, J = 8.0, 1H), 7.87 (d, J = 8.2, 1H), 7.61–7.56 (m, 1H), 7.54 (td, J = 7.4, 1.1, 1H), 7.49 (ddd, J = 8.2, 7.1, 1.1, 1H), 7.41 (t, J = 7.4, 1H), 4.60 (t, J = 6.8, 2H), 2.01 (t, J = 7.0, 2H), 1.75 (s, 9H), 1.75–1.66 (m, 2H), 1.63 (s, 9H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 202.1 (CH), 151.4 (C), 147.8 (C), 146.0 (C), 139.6 (C), 139.1 (C), 132.3 (C), 126.6 (CH), 126.3 (C), 126.1 (C), 124.5 (CH), 124.4 (CH), 124.2 (CH), 123.2 (C), 121.39 (CH), 121.37 (CH), 121.2 (CH), 116.7 (C), 115.7 (CH), 112.5 (C), 111.7 (CH), 109.3 (C), 85.7 (C), 85.4 (C), 45.6 (CH₂), 39.8 (CH₂), 27.6 (3CH₃), 27.5 (3CH₃), 21.7 (CH₂); HRMS (ESI+) m/z calcd for C₃₃H₃₅N₄O₅ (M + H)⁺ 567.2602, found 567.2599.

6.1.36. 3-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)-*N*-methylpropan-1-amine dihydrochloride (**25a**)

To a suspension of **24a** (50 mg, 0.090 mmol) in absolute ethanol (4 mL) under argon in an ice bath were added one drop of glacial acetic acid and then methylamine (33% in absolute ethanol 220 μ L, 1.77 mmol, 20 eq.). The solution was stirred overnight at room temperature. After cooling to the solution to 0 °C, NaBH₄ (14 mg, 0.37 mmol, 4 eq.) was added. After 30 minutes, a saturated aqueous solution of Na₂CO₃ was added and the mixture was extracted three times with CH₂Cl₂/MeOH (9:1). The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. The crude was purified by column chromatography (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 99:1 to 97:3 and then 95:5 + 0.5% Et₃N). The resulting yellow oil was solubilized in MeOH (1 mL) and 4 M aqueous HCl (10 mL) was added, the solution was stirred for 1 h at room temperature and concentrated under reduced pressure. The dihydrochloride salt was washed

with Et₂O to give **25a** (15 mg, 0.034 mmol, 38%) as a beige solid. *R*_f (free amine) = 0.14 (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 85:15); Mp > 160 °C (decomposition); IR (ATR) 3600–3100, 2658, 2890–2560, 1639, 1619, 1603, 1465, 1409, 1329, 1088, 740 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.01 (s, 1H), 9.06 (br s, 2H), 9.00 (s, 1H), 8.75 (d, *J* = 7.7, 1H), 8.44 (d, *J* = 7.7, 1H), 7.94–7.87 (m, 2H), 7.50 (t, *J* = 7.5, 1H), 7.43 (t, *J* = 7.5, 1H), 7.38 (t, *J* = 7.4, 1H), 7.31 (t, *J* = 7.4, 1H), 5.17 (t, *J* = 7.0, 2H), 3.10–3.00 (m, 2H), 2.46 (t, *J* = 5.1, 3H), 2.24 (quint, *J* = 7.0, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.2 (C), 138.4 (C), 132.0 (C), 130.0 (CH), 127.7 (C), 124.1 (CH), 124.0 (CH), 122.4 (C), 122.0 (C), 121.6 (C), 121.0 (CH), 120.5 (CH), 120.3 (CH), 119.6 (CH), 112.4 (CH), 111.5 (C), 111.1 (C), 110.0 (CH), 104.0 (C), 45.7 (CH₂), 41.7 (CH₂), 32.3 (CH₃), 27.1 (CH₂); HRMS (ESI+) *m/z* calcd for C₂₃H₂₂N₅ (M + H)⁺ 368.1870, found 368.1862; HPLC purity ≥ 99%, Method A: t_R = 21.07 min, λ = 240 nm.

6.1.37. 4-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)-*N*-methylbutan-1-amine dihydrochloride (**25b**)

Compound **25b** was prepared according to general procedure A, starting from **24b** (50 mg, 0.088 mmol) and methylamine 33% in ethanol (219 μL, 1.76 mmol, 20.0 eq.). The mixture was stirred for 2 h before addition of NaBH₄. The crude was purified by column chromatography (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 97:3 to 85:15). The dihydrochloride salt was washed with CH₂Cl₂ to give **25b** (32.2 mg, 0.071 mmol, 80%) as a pale yellow solid. *R*_f (free amine) = 0.16 (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 85:15); Mp > 160 °C (decomposition); IR (ATR) 3600–3100, 2960, 2890–2550, 1640, 1620, 1603, 1465, 1409, 1329, 1088, 740 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.66 (s, 1H), 8.81 (s, 1H), 8.67 (d, *J* = 7.6, 1H), 8.41 (d, *J* = 7.8, 1H), 8.36 (br s, 2H), 7.84 (d, *J* = 8.2, 1H), 7.78 (d, *J* = 8.1, 1H), 7.48 (t, *J* = 7.5, 1H), 7.43 (t, *J* = 7.6, 1H), 7.35 (t, *J* = 7.4, 1H), 7.30 (t, *J* = 7.4, 1H), 5.04 (t, *J* = 6.1, 2H), 2.82–2.73 (m, 2H), 2.36 (t, *J* = 5.1, 3H), 1.96–1.86 (m, 2H), 1.63–1.53 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.2 (C), 138.6 (C), 132.0^a (C), 130.1^a (CH), 127.8 (C), 124.04 (CH), 123.96 (CH), 122.5 (C), 122.2 (C), 121.7 (C), 121.0 (CH), 120.5 (CH), 120.1 (CH), 119.5 (CH), 112.4 (CH), 111.5 (C), 111.1 (C), 110.1 (CH), 103.9 (C), 47.9 (CH₂), 43.6 (CH₂), 32.3 (CH₃), 27.7 (CH₂), 22.8 (CH₂), ^aweak signal; HRMS (ESI+) *m/z* calcd for C₂₄H₂₄N₅ (M + H)⁺ 382.2026, found 382.2024; HPLC purity ≥ 99%, Method A: t_R = 21.26 min, λ = 240 nm.

6.1.38. 3-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)-*N*-ethylpropan-1-amine dihydrochloride (**26a**)

To a suspension of **24a** (47 mg, 0.085 mmol) in MeOH (4 mL) under argon in an ice bath were added one drop of glacial acetic acid and then ethylamine (2 M in MeOH, 423 μL, 0.846 mmol,

10 eq.). The solution was stirred at room temperature overnight. The solution was cooled to 0 °C and then NaBH₄ (13 mg, 0.34 mmol, 4 eq.) was added. After 30 minutes the ice bath was removed and the mixture was stirred for 2 h at room temperature. A saturated aqueous saturated solution of Na₂CO₃ was added and the mixture was extracted three times with CH₂Cl₂/MeOH (9:1). The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99:1 to 95:5). The yellow oil was solubilized in MeOH (1 mL) and 4 M aqueous HCl (10 mL) was added, the solution was stirred for 1 h at room temperature and concentrated under reduced pressure. The dihydrochloride salt was washed with Et₂O to give **26a** (11 mg, 0.024 mmol, 28%) as a beige solid. *R*_f (free amine) = 0.22 (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 9:1); Mp > 160 °C (decomposition); IR (ATR) 3600–3110, 2952, 2880–2450, 1639, 1620, 1603, 1463, 1409, 1329, 1085, 733 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.83 (s, 1H), 8.85 (s, 1H), 8.80 (br s, 2H), 8.69 (d, *J* = 7.7, 1H), 8.41 (d, *J* = 7.8, 1H), 7.90 (d, *J* = 8.3, 1H), 7.84 (d, *J* = 8.1, 1H), 7.50 (t, *J* = 7.6, 1H), 7.43 (t, *J* = 7.5, 1H), 7.37 (t, *J* = 7.4, 1H), 7.30 (t, *J* = 7.4, 1H), 5.13 (t, *J* = 6.8, 2H), 3.08–2.97 (m, 2H), 2.89–2.79 (m, 2H), 2.24 (quint, *J* = 7.2, 2H), 1.12 (t, *J* = 7.2, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.2 (C), 138.4 (C), 132.0^a (C), 130.2^a (CH), 127.5 (C), 124.02 (CH), 123.96 (CH), 122.4 (C), 121.9 (C), 121.6 (C), 121.0 (CH), 120.5 (CH), 120.2 (CH), 119.5 (CH), 112.3 (CH), 111.5 (C), 111.1 (C), 109.9 (CH), 104.1 (C), 43.7 (CH₂), 41.8 (CH₂), 41.7 (CH₂), 27.2 (CH₂), 10.9 (CH₃), ^aweak signal; HRMS (ESI+) *m/z* calcd for C₂₄H₂₄N₅ (M + H)⁺ 382.2026, found 382.2018; HPLC purity ≥ 95%, Method A: *t*_R = 21.39 min, λ = 240 nm.

6.1.39. 4-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)-*N*-ethylbutan-1-amine dihydrochloride (**26b**)

Compound **26b** was prepared according to general procedure A, starting from **24b** (50 mg, 0.088 mmol) and ethylamine (2 M in methanol, 880 μL, 1.76 mmol, 20 eq.). The mixture was stirred for 2 h before addition of NaBH₄. The crude was purified by column chromatography (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 97:3 to 9:1). The dihydrochloride salt was washed with CH₂Cl₂ to give **26b** (33.7 mg, 0.072 mmol, 82%) as a light grey solid. *R*_f (free amine) = 0.26 (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 9:1); Mp > 160 °C (decomposition); IR (ATR) 3600–3120, 2952, 2880–2400, 1639, 1620, 1602, 1329, 1138, 1085, 734 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.72 (s, 1H), 8.85 (s, 1H), 8.69 (d, *J* = 7.8, 1H), 8.47–8.34 (br s, 2H), 8.41 (d, *J* = 7.7, 1H), 7.84 (d, *J* = 8.3, 1H), 7.79 (d, *J* = 8.1, 1H), 7.49 (t, *J* = 7.6, 1H), 7.43 (t, *J* = 7.5, 1H), 7.36 (t, *J* = 7.4, 1H), 7.30 (t, *J* = 7.4, 1H), 5.05 (t, *J* = 6.5, 2H), 2.82–2.69 (m, 4H), 1.98–1.87 (m, 2H), 1.64–1.53 (m, 2H), 1.03 (t, *J* = 7.2, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.2 (C), 138.7 (C), 132.0^a (C), 130.0^a (CH), 128.0 (C), 124.1 (CH), 124.0 (CH), 122.5 (C), 122.3 (C), 121.6 (C), 121.1 (CH), 120.6 (CH), 120.2

(CH), 119.6 (CH), 112.4 (CH), 111.4 (C), 111.1 (C), 110.2 (CH), 103.9 (C), 46.0 (CH₂), 43.6 (CH₂), 41.9 (CH₂), 27.8 (CH₂), 23.0 (CH₂), 10.8 (CH₃), ^aweak signal; HRMS (ESI+) *m/z* calcd for C₂₅H₂₆N₅ (M + H)⁺ 396.2183, found 396.2180; HPLC purity ≥ 95%, Method A: t_R = 21.57 min, λ = 240 nm.

6.1.40. 3-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)-*N,N*-dimethylpropan-1-amine dihydrochloride (**27a**)

Compound **27a** was prepared according to general procedure A, starting from **24a** (55 mg, 0.100 mmol) and dimethylamine (2 M in methanol, 960 μL, 1.92 mmol, 19 eq.). The mixture was stirred overnight before addition of NaBH₄. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99:1 to 97:3). The dihydrochloride salt was washed with CH₂Cl₂ to give **27a** (17 mg, 0.037 mmol, 38%) as a beige solid. *R*_f (free amine) = 0.31 (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 95:5); Mp > 160 °C (decomposition); IR (ATR) 3600–3000, 3964, 2880–2450, 1639, 1620, 1608, 1464, 1329, 1154, 1084, 741 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.72 (s, 1H), 10.09 (br s, 1H), 8.79 (s, 1H), 8.68 (d, *J* = 7.8, 1H), 8.41 (d, *J* = 7.8, 1H), 7.87 (d, *J* = 8.3, 1H), 7.83 (d, *J* = 8.1, 1H), 7.50 (ddd, *J* = 8.2, 7.1, 1.1, 1H), 7.43 (ddd, *J* = 8.2, 7.0, 1.2, 1H), 7.37 (ddd, *J* = 7.9, 7.0, 0.8, 1H), 7.30 (ddd, *J* = 8.0, 7.0, 1.0, 1H), 5.09 (t, *J* = 7.1, 1H), 3.29–3.22 (m, 2H), 2.69 (d, *J* = 5.0, 6H), 2.31–2.21 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.2 (C), 138.3 (C), 132.0^a (C), 129.9^a (CH), 127.7 (C), 124.1 (2CH), 122.4 (C), 122.0 (C), 121.7 (C), 121.0 (CH), 120.5 (CH), 120.3 (CH), 119.6 (CH), 112.5 (CH), 111.5 (C), 111.1 (C), 110.0 (CH), 103.9 (C), 54.0 (CH₂), 42.1 (2CH₃), 41.7 (CH₂), 25.4 (CH₂), ^aweak signal; HRMS (ESI+) *m/z* calcd for C₂₄H₂₄N₅ (M + H)⁺ 382.2026, found 382.2019; HPLC purity ≥ 96%, Method A: t_R = 21.31 min, λ = 240 nm.

6.1.41. 4-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)-*N,N*-dimethylbutan-1-amine dihydrochloride (**27b**)

Compound **27b** was prepared according to general procedure A, starting from **24b** (46 mg, 0.081 mmol) and dimethylamine (2 M in methanol, 810 μL, 1.62 mmol, 20 eq.). The mixture was stirred overnight before addition of NaBH₄. The crude was purified by column chromatography (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 99:1 to 95:5). The dihydrochloride salt was washed with CH₂Cl₂ to give **27b** (34 mg, 0.073 mmol, 89%) as a beige solid. *R*_f (free amine) = 0.33 (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 95:5); Mp > 160 °C (decomposition); IR (ATR) 3600–3040, 2960, 2880–2420, 1639, 1619, 1604, 1464, 1329, 1145, 1086, 741 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.63 (s, 1H), 9.61 (br s, 1H), 8.80 (s, 1H), 8.67 (d, *J* = 7.6, 1H), 8.40 (d, *J* = 7.6, 1H), 7.85 (d, *J* = 8.3, 1H), 7.79 (d, *J* = 7.9, 1H), 7.49 (t, *J* = 7.5, 1H), 7.43 (t, *J* = 7.4, 1H), 7.36 (t, *J* = 7.1, 1H), 7.30 (t, *J* = 7.3, 1H), 5.07–4.98 (m, 2H), 3.04–2.94 (m, 2H), 2.61 (d, *J* = 4.2, 6H), 1.94–1.83 (m, 2H), 1.79–

1.67 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.2 (C), 138.6 (C), 132.0^a (C), 130.1^a (CH), 127.7 (C), 124.0 (CH), 123.9 (CH), 122.5 (C), 122.1 (C), 121.7 (C), 121.0 (CH), 120.5 (CH), 120.1 (CH), 119.5 (CH), 112.4 (CH), 111.5 (C), 111.1 (C), 110.0 (CH), 104.0 (C), 56.1 (CH₂), 43.6 (CH₂), 41.9 (2CH₃), 27.6 (CH₂), 21.0 (CH₂), ^aweak signal; HRMS (ESI+) *m/z* calcd for C₂₅H₂₆N₅ (M + H)⁺ 396.2183, found 396.2180; HPLC purity ≥ 95%, Method A: t_R = 21.40 min, λ = 240 nm.

6.1.42. 3-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)-*N,N*-diethylpropan-1-amine dihydrochloride (**28a**)

Compound **28a** was prepared according to general procedure A, starting from **24a** (65 mg, 0.118 mmol) and diethylamine (244 μL, 2.36 mmol, 20 eq.). The mixture was stirred for 2 h before addition of NaBH₄. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99.6:0.4 to 88:12). The dihydrochloride salt was washed with CH₂Cl₂ to give **28a** (7 mg, 0.0145 mmol, 12%) as a beige solid. *R*_f (free amine) = 0.39 (CH₂Cl₂/MeOH 95:5); Mp > 160 °C (decomposition); IR (ATR) 3670–3040, 2923, 2830–2500, 1637, 1622, 1604, 1464, 1408, 1329, 742 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.81 (s, 1H), 9.93 (br s, 1H), 8.82 (s, 1H), 8.68 (d, *J* = 7.8, 1H), 8.41 (d, *J* = 7.8, 1H), 7.91 (d, *J* = 8.2, 1H), 7.83 (d, *J* = 8.1, 1H), 7.50 (t, *J* = 7.6, 1H), 7.43 (t, *J* = 7.5, 1H), 7.37 (t, *J* = 7.4, 1H), 7.30 (t, *J* = 7.3, 1H), 5.11 (t, *J* = 7.2, 2H), 3.37–3.30 (m, 2H), 3.07–2.99 (m, 4H), 2.25–2.15 (m, 2H), 1.13 (t, *J* = 7.2, 6H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.3 (C), 138.3 (C), 132.0^a (C), 130.4^a (CH), 127.3 (C), 124.0 (CH), 123.9 (CH), 122.5 (C), 121.8 (C), 121.7 (C), 121.0 (CH), 120.5 (CH), 120.1 (CH), 119.4 (CH), 112.3 (CH), 111.6 (C), 111.2 (C), 109.9 (CH), 104.2 (C), 47.5 (CH₂), 46.4 (2CH₂), 41.6 (CH₂), 24.5 (CH₂), 8.5 (2CH₃), ^aweak signal; HRMS (ESI+) *m/z* calcd for C₂₆H₂₉N₅ (M + 2H)²⁺ 205.6206, found 205.6204; HPLC purity ≥ 96%, Method A: t_R = 21.99 min, λ = 240 nm.

6.1.43. 4-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)-*N,N*-diethylbutan-1-amine dihydrochloride (**28b**)

Compound **28b** was prepared according to general procedure A, starting from **24b** (46 mg, 0.081 mmol) and diethylamine (168 μL, 1.62 mmol, 20 eq.). The mixture was stirred overnight before addition of NaBH₄. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99:1 to 9:1). The dihydrochloride salt was washed with CH₂Cl₂ to give **28b** (19.5 mg, 0.039 mmol, 48%) as a white solid. *R*_f (free amine) = 0.34 (Et₃N-treated SiO₂, CH₂Cl₂/MeOH 95:5); Aspect: white solid; Mp > 160 °C (decomposition); IR (ATR) 3650–3125, 2920, 2830–2550, 1639, 1620, 1603, 1464, 1408, 1329, 742 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.65 (s, 1H), 9.38 (br s, 1H), 8.80 (s, 1H), 8.67 (d, *J* = 7.7, 1H), 8.40 (d, *J* = 7.8, 1H), 7.86 (d, *J* = 8.3, 1H), 7.79 (d, *J* = 8.1, 1H), 7.49 (t, *J* = 7.6, 1H), 7.43 (t, *J* = 7.6, 1H), 7.36 (t, *J* = 7.4, 1H), 7.30 (t, *J* = 7.4, 1H), 5.03

(t, $J = 6.5$, 2H), 3.01–2.90 (m, 6H), 1.96–1.87 (m, 2H), 1.79–1.69 (m, 2H), 1.07 (t, $J = 7.2$, 6H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 139.2 (C), 138.6 (C), 132.1^a (C), 130.1^b (CH), 127.7 (C), 124.0 (CH), 123.9 (CH), 122.5 (C), 122.1 (C), 121.6 (C), 121.0 (CH), 120.5 (CH), 120.1 (CH), 119.5 (CH), 112.4 (CH), 111.5 (C), 111.1 (C), 110.0 (CH), 104.0 (C), 50.5 (CH₂), 46.1 (2CH₂), 43.6 (CH₂), 27.8 (CH₂), 20.5 (CH₂), 8.3 (2CH₃), ^aestimated value from HMBC experiment, ^bestimated value from HSQC experiment; HRMS (ESI+) m/z calcd for C₂₇H₃₀N₅ (M + H)⁺ 424.2496, found 424.2494; HPLC purity \geq 95%, Method A: $t_{\text{R}} = 22.04$ min, $\lambda = 240$ nm.

6.1.44. 3-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)propan-1-amine dihydrochloride (**29a**)

To a suspension of **24a** (50 mg, 0.090 mmol) in absolute ethanol (4 mL) were added triethylamine (63 μL , 0.45 mmol, 5 eq.) and hydroxylamine hydrochloride (25 mg, 0.36 mmol, 4 eq.). The mixture was stirred for 2 h at room temperature and then concentrated under pressure. The residue was solubilized in THF (5 mL), then 2 M aqueous HCl (2 mL) and zinc powder (262 mg, 4.0 mmol, 45 eq.) were added. The mixture was refluxed for 24 h and then diluted with a saturated aqueous Na₂CO₃ solution (30 mL). The solution was extracted three times with CH₂Cl₂/MeOH (9:1). The combined organic extracts were dried over MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 95:5 to 85:15). After evaporation, the dihydrochloride salt was prepared in MeOH with 4 M aqueous HCl, and the mixture was concentrated under reduced pressure. Reverse flash chromatography (C18 4g, H₂O/MeOH 95:5 to 5:95) provided two fractions which were combined. After evaporation, the dihydrochloride salt was prepared in MeOH with 4 M aqueous HCl, the product was dried under reduced pressure and acetonitrile co-evaporation, and finally washed with dichloromethane to give **29a** (11 mg, 0.026 mmol, 29% in two steps) as a white solid. R_{f} (free amine) = 0.21 (CH₂Cl₂/MeOH 9:1 + 0.5% Et₃N); Mp > 160 °C (decomposition); IR (ATR) 3670–3539-3150, 3150–2490, 1639, 1620, 1604, 1466, 1409, 1330, 1087, 1013, 736 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 11.70 (s, 1H), 8.80 (s, 1H), 8.68 (d, $J = 7.6$, 1H), 8.41 (d, $J = 7.5$, 1H), 7.88 (d, $J = 8.2$, 1H), 7.80 (d, $J = 8.3$, 1H), 7.77 (br s, 3H), 7.49 (t, $J = 7.5$, 1H), 7.43 (t, $J = 7.6$, 1H), 7.37 (t, $J = 7.5$, 1H), 7.30 (t, $J = 7.3$, 1H), 5.10 (t, $J = 6.5$, 2H), 2.90–2.79 (m, 2H), 2.24–2.14 (m, 2H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 139.2 (C), 138.5 (C), 132.0 (C), 130.0 (CH), 127.7 (C), 124.0 (2CH), 122.4 (C), 122.0 (C), 121.6 (C), 121.1 (CH), 120.5 (CH), 120.2 (CH), 119.5 (CH), 112.5 (CH), 111.4 (C), 111.1 (C), 110.0 (CH), 104.0 (C), 41.7 (CH₂), 36.4 (CH₂), 28.6 (CH₂); HRMS (ESI+) m/z calcd for C₂₂H₂₀N₅ (M + H)⁺ 354.1713, found 354.1708; HPLC purity \geq 95%, Method A: $t_{\text{R}} = 20.87$ min, $\lambda = 240$ nm.

6.1.45. 4-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)butan-1-amine dihydrochloride (**29b**)

Compound **29b** was prepared according to general procedure A, starting from **24b** (60 mg, 0.106 mmol) and NH₃ (7 M in MeOH, 1 mL, 7.0 mmol, 66 eq.). The mixture was stirred overnight before addition of NaBH₄. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 95:5 + 0.5% Et₃N to 9:1 + 0.5% Et₃N). The dihydrochloride salt was washed with CH₂Cl₂ to give **29b** (25 mg, 0.057 mmol, 54%) as a white solid. *R*_f (free amine) = 0.24 (CH₂Cl₂/MeOH 9:1 + 0.5% Et₃N); Mp > 160 °C (decomposition); IR (ATR) 3650–2450, 1637, 1620, 1608, 1467, 1408, 1329, 1078, 736 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.60 (s, 1H), 8.79 (s, 1H), 8.67 (d, *J* = 7.6, 1H), 8.40 (d, *J* = 7.8, 1H), 7.83 (d, *J* = 8.3, 1H), 7.77 (d, *J* = 8.1, 1H), 7.56 (br s, 3H), 7.48 (t, *J* = 7.5, 1H), 7.43 (t, *J* = 7.6, 1H), 7.35 (t, *J* = 7.5, 1H), 7.30 (t, *J* = 7.4, 1H), 5.04 (t, *J* = 5.9, 2H), 2.73–2.62 (m, 2H), 1.98–1.87 (m, 2H), 1.57–1.45 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.1 (C), 138.6 (C), 132.1^a (C), 130.2^a (CH), 127.7 (C), 123.9 (CH), 123.8 (CH), 122.5 (C), 122.1 (C), 121.7 (C), 121.0 (CH), 120.5 (CH), 120.0 (CH), 119.5 (CH), 112.3 (CH), 111.5 (C), 111.1 (C), 110.1 (CH), 104.0 (C), 43.6 (CH₂), 38.5 (CH₂), 27.6 (CH₂), 24.3 (CH₂), ^aweak signal; HRMS (ESI+) *m/z* calcd for C₂₃H₂₂N₅ (M + H)⁺ 368.1870, found 368.1868; HPLC purity ≥ 95%, Method A: *t*_R = 21.03 min, λ = 240 nm.

6.1.46. 12-Butyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole (**30a**)

To a solution of **13a** (50 mg, 0.143 mmol) in MeOH (10 mL) was added Pd/C 10% (52 mg, 0.049 mmol, 0.34 eq.) and NH₄HCO₂ (344 mg, 5.46 mmol, 38 eq.). The mixture was refluxed for 6 h, then filtered through a pad of Celite and the solid was washed with dichloromethane. After evaporation of the solvents, the residue was solubilized in EtOAc and washed with a saturated aqueous solution of Na₂CO₃, dried over MgSO₄ and concentrated under reduced pressure. Evaporation provided **30a** (30 mg, 0.085 mmol, 60%) as a white solid. *R*_f = 0.36 (cyclohexane/EtOAc 6:4); Mp > 155 °C (decomposition); IR (ATR) 3480, 3163, 3055, 2954, 2926, 2871, 1610, 1461, 1326, 937, 737 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.70 (br s, 1H), 11.64 (s, 1H), 8.76 (s, 1H), 8.65 (br d, *J* = 7.2, 1H), 8.40 (d, *J* = 7.7, 1H), 7.81–7.74 (m, 2H), 7.445 (t, *J* = 7.5, 1H), 7.437 (t, *J* = 7.3, 1H), 7.36–7.27 (m, 2H), 4.94 (t, *J* = 6.6, 2H), 1.86–1.76 (m, 2H), 1.31–1.19 (m, 2H), 0.78 (t, *J* = 7.3, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.2 (C), 138.6 (C), 131.8^a (CH), 131.3^b (C), 125.7 (C), 123.84 (CH), 123.80 (CH), 123.4 (C), 122.7 (C), 121.6 (C), 120.8 (CH), 120.6 (CH), 119.9 (CH), 119.2 (CH), 111.9 (CH), 111.3 (C), 110.8 (C), 110.0 (CH), 104.8^b (C), 43.9 (CH₂), 32.9 (CH₂), 19.5 (CH₂), 13.9 (CH₃), ^aestimated from a ¹H-¹³C HSQC

experiment, ^bestimated from a ¹H-¹³C HMBC experiment; HRMS (ESI+) *m/z* calcd for C₂₃H₂₁N₄ (M + H)⁺ 353.1761, found 353.1755; HPLC purity ≥ 98%, Method A: t_R = 31.31 min, λ = 240 nm.

6.1.47. 12-Pentyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole (**30b**)

Compound **30b** was prepared using the same procedure described for **30a**, from **13b** (25 mg, containing 33% of **16**, 0.069 mmol of **13b** and isomers) in MeOH (5 mL), Pd/C 10% (36 mg, 0.034 mmol, 0.5 eq.) and NH₄HCO₂ (172 mg, 2.73 mmol, 40 eq.) to give **30b** (22 mg, 0.060 mmol, 88%) as a white solid. *R*_f = 0.41 (cyclohexane/EtOAc 6:4); Mp > 130 °C (decomposition); IR (ATR) 3472, 3164, 3055, 2926, 2847, 1461, 1325, 942, 923, 736 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.70 (br s, 1H), 11.63 (s, 1H), 8.75 (s, 1H), 8.66 (br d, *J* = 6.9, 1H), 8.40 (d, *J* = 7.5, 1H), 7.80–7.74 (m, 2H), 7.48–7.41 (m, 2H), 7.36–7.27 (m, 2H), 4.93 (t, *J* = 6.7, 2H), 1.83 (quint, *J* = 6.9, 2H), 1.31–1.16 (m, 4H), 0.72 (t, *J* = 6.9, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.1 (C), 138.6 (C), 131.9^a (CH), 131.0^b (C), 125.6 (C), 123.8 (CH), 123.7 (CH), 123.3^b (C), 122.7 (C), 121.6^c (C), 120.8 (CH), 120.6 (CH), 119.8 (CH), 119.2 (CH), 111.9 (CH), 111.2 (C), 110.8 (C), 110.0 (CH), 104.9^b (C), 43.9 (CH₂), 30.3 (CH₂), 28.2 (CH₂), 22.0 (CH₂), 13.8 (CH₃), ^aestimated from a ¹H-¹³C HSQC experiment, ^bestimated from a ¹H-¹³C HMBC experiment, ^cweak signal; HRMS (ESI+) *m/z* calcd for C₂₄H₂₃N₄ (M + H)⁺ 367.1917, found 367.1911; HPLC purity ≥ 97%, Method A: t_R = 32.73 min, λ = 240 nm.

6.1.48. 13-Butyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole (**31a**)

Compound **31a** was prepared using the same procedure described for **30a**, from **14a** (5.0 mg, 0.014 mmol) in MeOH (3 mL), Pd/C 10% (15.2 mg, 0.014 mmol, 1 eq.) and NH₄HCO₂ (71 mg, 1.12 mmol, 80 eq.) to give **31a** (4.6 mg, 0.013 mmol, 91%) as a white solid. *R*_f = 0.27 (CH₂Cl₂/MeOH 99:1); Mp > 140 °C (decomposition); IR (ATR) 3472, 3168, 3059, 2917, 2855, 1460, 1324, 1247, 1077, 939, 923, 732 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.67 (br s, 1H), 11.46 (s, 1H), 8.77 (s, 1H), 8.66 (br d, *J* = 7.0, 1H), 8.39 (d, *J* = 7.8, 1H), 7.79 (d, *J* = 8.3, 1H), 7.76 (d, *J* = 8.1, 1H), 7.46 (ddd, *J* = 8.2, 7.1, 1.2, 1H), 7.42 (ddd, *J* = 8.2, 7.0, 1.2, 1H), 7.34 (ddd, *J* = 7.9, 7.0, 0.9, 1H), 7.29 (ddd, *J* = 8.0, 6.9, 1.0, 1H), 4.96 (t, *J* = 6.7, 2H), 1.89–1.80 (m, 2H), 1.31–1.24 (m, 2H), 0.79 (t, *J* = 7.4, 3H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 139.0 (C), 138.7 (C), 131.9^a (CH), 131.2^b (C), 127.1 (C), 123.9 (CH), 123.6 (CH), 122.7 (C), 121.9^c (C), 121.6^c (C), 120.8 (CH), 120.5 (CH), 119.7 (CH), 119.3 (CH), 112.0 (CH), 111.7 (C), 111.2 (C), 109.9 (CH), 43.9 (CH₂), 32.8 (CH₂), 19.4 (CH₂), 13.8 (CH₃), ^aestimated from a ¹H-¹³C HSQC experiment, ^bestimated from a ¹H-¹³C HMBC experiment, ^cweak signal, one C could not be observed; HRMS (ESI+) *m/z* calcd for C₂₃H₂₁N₄ (M + H)⁺ 353.1761, found 353.1755; HPLC purity ≥ 98%, Method A: t_R = 31.02 min, λ = 240 nm.

6.1.49. 13-Pentyl-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole (**31b**)

Compound **31b** was prepared using the same procedure described for **30a**, from **14b** (25 mg, 0.069 mmol) in MeOH (5 mL), Pd/C 10% (36 mg, 0.034 mmol, 0.5 eq.) and NH₄HCO₂ (172 mg, 2.73 mmol, 40 eq.) to give **31b** (23.5 mg, 0.064 mmol, 93%) as a white solid. *R*_f = 0.38 (cyclohexane/EtOAc 6:4); Mp > 200 °C (decomposition); IR (ATR) 3472, 3168, 3051, 3922, 2847, 1460, 1324, 1247, 939, 923, 731 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.69 (s, 1H), 11.44 (s, 1H), 8.75 (s, 1H), 8.67 (d, *J* = 7.2, 1H), 8.40 (d, *J* = 7.5, 1H), 7.79 (d, *J* = 8.2, 1H), 7.75 (d, *J* = 8.1, 1H), 7.47 (t, *J* = 7.8, 1H), 7.42 (t, *J* = 7.7, 1H), 7.37–7.26 (m, 2H), 4.95 (t, *J* = 6.3, 2H), 1.86 (quint, *J* = 7.0, 2H), 1.33–1.16 (m, 4H), 0.73 (t, *J* = 6.8, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.0 (C), 138.7 (C), 131.9^a (CH), 131.2^b (C), 127.0 (C), 123.9 (CH), 123.6 (CH), 122.7 (C), 121.9^c (C), 121.6^c (C), 120.8 (CH), 120.5 (CH), 119.7 (CH), 119.4 (CH), 112.0 (CH), 111.7 (C), 111.3 (C), 109.9 (CH), 44.0 (CH₂), 30.3 (CH₂), 28.2 (CH₂), 22.0 (CH₂), 13.8 (CH₃), ^aestimated from a ¹H-¹³C HSQC experiment, ^bestimated from a ¹H-¹³C HMBC experiment, ^cweak signal, one C could not be observed; HRMS (ESI+) *m/z* calcd for C₂₄H₂₃N₄ (M + H)⁺ 367.1917, found 367.1910; HPLC purity ≥ 96%, Method A: *t*_R = 32.54 min, λ = 240 nm.

6.1.50. 3-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)propan-1-ol (**32a**)

To a solution of **13a** (100 mg, 0.285 mmol) in THF/H₂O 2:1 (15 mL) were added K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (306 mg, 1.43 mmol, 5 eq.). The mixture was protected from light and stirred overnight at room temperature. After addition of saturated aqueous solution of Na₂S₂O₃, the mixture was extracted 3 times with CH₂Cl₂, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99.6:0.4 to 88:12) to give the intermediate product (81 mg) which was directly used for the next step without further purification.

To a solution of intermediate (44 mg) in THF/MeOH 2:1 (7.5 mL) was added NaBH₄ (24 mg, 0.63 mmol, 5 eq.). The solution was stirred for 1 h at room temperature and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99.6:0.4 to 88:12) to give **32a** (39 mg, 0.110 mmol, 71% in two steps) as a pale yellow solid. *R*_f = 0.38 (CH₂Cl₂/MeOH 95:5); Mp > 250 °C; IR (ATR) 3470–2500, 1324, 727 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.71 (s, 1H), 11.75 (s, 1H), 8.75 (s, 1H), 8.67 (d, *J* = 7.7, 1H), 8.41 (d, *J* = 7.7, 1H), 7.77 (d, *J* = 8.3, 1H), 7.73 (d, *J* = 8.1, 1H), 7.49–7.41 (m, 2H), 7.33 (t, *J* = 7.3, 1H), 7.30 (t, *J* = 7.4, 1H), 5.09 (t, *J* = 4.5, 1H), 4.94 (t, *J* = 6.6, 2H), 3.52–3.46 (m, 2H), 2.08 (quint, *J* = 6.3, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.1 (C), 138.5 (C), 131.9 (CH), 131.2 (C), 125.7 (C), 123.9 (2CH), 123.3 (C), 122.6 (C), 121.6 (C), 120.8 (CH), 120.7 (CH), 119.9 (CH), 119.2 (CH), 111.7

(CH), 111.3 (C), 110.7 (C), 109.8 (CH), 104.8 (C), 57.7 (CH₂), 40.8 (CH₂), 33.6 (CH₂); HRMS (ESI+) m/z calcd for C₂₂H₁₉N₄O (M + H)⁺ 355.1553, found 355.1548; HPLC purity ≥ 95%, Method A: t_R = 26.50 min, λ = 240 nm.

6.1.51. 4-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)butan-1-ol (**32b**)

To a solution of **13b** (100 mg, containing 33% of **16**, 0.184 mmol of **13b**) in THF/H₂O 2:1 (15 mL) were added K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (294 mg, 1.37 mmol, 7.5 eq.). The mixture was protected from light and stirred overnight at room temperature. After addition of a saturated aqueous solution of Na₂S₂O₃, the mixture was extracted 3 times with CH₂Cl₂, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was solubilized in THF/MeOH 2:1 (15 mL) and NaBH₄ (52 mg, 1.37 mmol, 7.5 eq.) was added. The solution was stirred for 1 h 30 min at room temperature and concentrated under reduced pressure. The crude was purified by two column chromatographies (SiO₂, CH₂Cl₂/MeOH 99.6:0.4 to 92:8) to give **32b** (28 mg, 0.076 mmol, 41%) as a white solid. R_f = 0.33 (CH₂Cl₂/MeOH 96:4); Mp > 240 °C (decomposition); IR (ATR) 3401, 3140, 2927, 2845, 3330-2671, 1462, 1331, 980, 938, 740 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.70 (s, 1H), 11.71 (s, 1H), 8.74 (s, 1H), 8.67 (d, J = 7.6, 1H), 8.40 (d, J = 7.6, 1H), 7.78 (d, J = 8.3, 1H), 7.77 (d, J = 8.1, 1H), 7.48–7.41 (m, 2H), 7.36–7.27 (m, 2H), 4.94 (t, J = 6.9, 2H), 4.62 (t, J = 5.0, 1H), 3.41–3.36 (m, 2H), 1.91 (quint, J = 7.2, 2H), 1.45 (quint, J = 7.0, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 139.0 (C), 138.5 (C), 131.9^a (CH), 131.1^b (C), 125.6 (C), 123.8 (2CH), 123.4^c (C), 122.6 (C), 121.5^c (C), 120.8 (CH), 120.6 (CH), 119.8 (CH), 119.2 (CH), 111.8 (CH), 111.2 (C), 110.7 (C), 110.0 (CH), 104.7^b (C), 60.5 (CH₂), 43.8 (CH₂), 29.2 (CH₂), 27.4 (CH₂), ^aestimated from a ¹H-¹³C HSQC experiment, ^bestimated from a ¹H-¹³C HMBC experiment, ^cweak signal; HRMS (ESI+) m/z calcd for C₂₃H₂₁N₄O (M + H)⁺ 369.1710, found 369.1704; HPLC purity ≥ 95%, Method A: t_R = 26.47 min, λ = 240 nm.

6.1.52. 3-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)propan-1-ol (**33a**)

To a solution of **24a** (50 mg, 0.090 mmol) in THF/MeOH 2:1 (6 mL) was added NaBH₄ (7 mg, 0.185 mmol, 2 eq.). The solution was stirred for 1 h 30 min at room temperature and concentrated under reduced pressure. MeOH/H₂O 3:1 (10 mL) and then K₂CO₃ (97 mg, 0.70 mmol, 8 eq.) were added. The mixture was refluxed for 48 h and concentrated under reduced pressure. The crude was purified by column chromatography (SiO₂, cyclohexane/EtOAc 6:4 to 4:6) to give **33a** (20 mg, 0.056 mmol, 62%) as a light grey solid. R_f = 0.30 (cyclohexane/EtOAc 4:6); Mp > 250 °C; IR (ATR) 3415, 3093, 2924, 1323, 932, 830, 730 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.70 (s, 1H), 11.55 (s, 1H), 8.75 (s, 1H), 8.67 (d, J = 7.7, 1H), 8.41 (d, J = 7.7, 1H), 7.80 (d, J = 8.1, 1H), 7.71 (d, J = 8.1, 1H), 7.48 (t, J = 7.6, 1H), 7.43 (t, J = 7.5, 1H), 7.34 (t, J = 7.4, 1H), 7.30 (t, J =

7.4, 1H), 5.11 (t, $J = 4.5$, 1H), 4.97 (t, $J = 6.5$, 2H), 3.54–3.48 (m, 2H), 2.10 (quint, $J = 6.3$, 2H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 139.0 (C), 138.6 (C), 131.3^a (CH), 127.1 (C), 123.9 (CH), 123.7 (CH), 122.7 (C), 122.0 (C), 121.6 (C), 120.8 (CH), 120.6 (CH), 119.8 (CH), 119.4 (CH), 111.9 (CH), 111.7 (C), 111.3 (C), 109.7 (CH), 103.8^b (C), 57.7 (CH₂), 40.9 (CH₂), 33.5 (CH₂), ^aestimated from a ^1H - ^{13}C HSQC experiment, ^bestimated from a ^1H - ^{13}C HMBC experiment, one C could not be observed; HRMS (ESI+) m/z calcd for C₂₂H₁₉N₄O (M + H)⁺ 355.1553, found 355.1543; HPLC purity $\geq 95\%$, Method A: $t_{\text{R}} = 26.19$ min, $\lambda = 240$ nm.

6.1.53. 4-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)butan-1-ol (**33b**)

Compounds **33b** was prepared using the same procedure described for **32b** from **14b** (80 mg, 0.220 mmol) in THF/H₂O 2:1 (12.5 mL), K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (235 mg, 1.10 mmol, 5 eq.). Reduction was performed in THF/MeOH 2:1 (12.5 mL) with NaBH₄ (42 mg, 1.11 mmol, 5 eq.). Compound **33b** (31 mg, 0.084 mmol, 38%) was obtained as a white solid. $R_{\text{f}} = 0.38$ (CH₂Cl₂/MeOH 96:4); Aspect: white solid; Mp > 250 °C; IR (ATR) 3435, 3161, 2936, 1323, 1076, 730 cm⁻¹; ^1H NMR (400 MHz, DMSO- d_6) δ 13.69 (s, 1H), 11.52 (s, 1H), 8.75 (s, 1H), 8.67 (d, $J = 7.5$, 1H), 8.40 (d, $J = 7.5$, 1H), 7.81 (d, $J = 8.2$, 1H), 7.75 (d, $J = 8.1$, 1H), 7.47 (t, $J = 7.7$, 1H), 7.42 (t, $J = 7.7$, 1H), 7.34 (t, $J = 7.6$, 1H), 7.29 (t, $J = 7.5$, 1H), 4.96 (t, $J = 6.7$, 2H), 4.63 (t, $J = 4.9$, 1H), 3.42–3.36 (m, 2H), 1.94 (quint, $J = 7.3$, 2H), 1.48 (quint, $J = 7.0$, 2H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 139.0 (C), 138.6 (C), 131.9^a (CH), 130.5^b (C), 127.0 (C), 123.9 (CH), 123.6 (CH), 122.6 (C), 121.8^c (C), 121.6^c (C), 120.8 (CH), 120.5 (CH), 119.7 (CH), 119.3 (CH), 111.9 (CH), 111.6 (C), 111.2 (C), 109.9 (CH), 60.5 (CH₂), 43.9 (CH₂), 29.1 (CH₂), 27.3 (CH₂), ^aestimated from a ^1H - ^{13}C HSQC experiment, ^bestimated from a ^1H - ^{13}C HMBC experiment, ^cweak signal, one C could not be observed; HRMS (ESI+) m/z calcd for C₂₃H₂₁N₄O (M + H)⁺ 369.1710, found 369.1708; HPLC purity $\geq 97\%$, Method A: $t_{\text{R}} = 26.55$ min, $\lambda = 240$ nm.

6.1.54. (*Z/E*)-4-(5,13-Dihydro-12*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-12-yl)butanal oxime (**34b**)

To a solution of **13b** (100 mg, containing 33% of **16**, 0.184 mmol of **13b**) in THF/H₂O 2:1 (15 mL) were added K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (294 mg, 1.37 mmol, 7.5 eq.). The mixture was protected from light and stirred overnight at room temperature. After addition of a saturated aqueous solution of Na₂S₂O₃, the mixture was extracted 3 times with CH₂Cl₂, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was solubilized in MeOH/H₂O 14:1 (15 mL) then NaOAc (96 mg, 1.17 mmol, 6.4 eq.) and NH₂OH·HCl (81 mg, 1.17

mmol, 6.4 eq.) were added. The solution was stirred for 2 h at room temperature and concentrated under reduced pressure. The crude was purified by two column chromatographies (SiO₂, CH₂Cl₂/MeOH 99.6:0.4 to 88:12) to give **34b** (17 mg, 0.045 mmol, 24%) as a beige solid. $R_f = 0.41/0.35$ (CH₂Cl₂/MeOH 95:5); IR (ATR) 3670–2500, 1461, 1328, 921, 734 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ (*E/Z* mixture ~35:65) 13.71 (br s, 1H_E+1H_Z), 11.67 (s, 1H_E+1H_Z), 10.70 (s, 1H_Z), 10.40 (s, 1H_E), 8.78 (s, 1H_E+1H_Z), 8.65 (d, $J = 7.8$, 1H_E+1H_Z), 8.41 (d, $J = 7.8$, 1H_E+1H_Z), 7.79 (d, $J = 8.2$, 1H_Z), 7.78 (d, $J = 7.9$, 1H_E), 7.49–7.41 (m, 2H_E+2H_Z), 7.36–7.28 (m, 3H_E+2H_Z), 6.60 (t, $J = 5.1$, 1H_Z), 4.97 (t, $J = 6.8$, 2H_E+2H_Z), 2.32–2.25 (m, 2H_Z), 2.20–2.13 (m, 2H_E), 2.07–1.94 (m, 2H_E+2H_Z); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (*E/Z* mixture ~35:65) 149.5/149.0 (CH), 139.03/139.00 (C), 138.61/138.59 (C), 131.5^a (CH), 125.6/125.5 (C), 123.84 (CH), 123.81 (CH), 123.3^b (C), 122.73/122.68 (C), 121.6^b (C), 120.8 (CH), 120.6 (CH), 119.8 (CH), 119.3 (CH), 111.9 (CH), 111.2 (C), 110.84/110.82 (C), 109.9 (CH), 105.3^c (C), 43.6/43.4 (CH₂), 27.6/27.2 (CH₂), 26.4/22.2 (CH₂), ^aestimated from a ¹H-¹³C HSQC experiment, ^bweak signal, ^cestimated from a ¹H-¹³C HMBC experiment; HRMS (ESI+) m/z calcd for C₂₃H₂₀N₅O (M + H)⁺ 382.1662, found 382.1648; HPLC purity $\geq 95\%$, Method B: $t_R = 26.77$ min + 27.65 min, $\lambda = 240$ nm.

6.1.55. (*Z/E*)-4-(5,12-Dihydro-13*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazol-13-yl)butanal oxime (**35b**)

Compounds **35b** was prepared using the same procedure described for **34b** from **14b** (50 mg, 0.137 mmol) in THF/H₂O 2:1 (7.5 mL), K₂OsO₄·2H₂O (catalytic amount) and NaIO₄ (148 mg, 0.69 mmol, 5 eq.). Oxime formation was performed in MeOH/H₂O 14:1 (7.5 mL) with NaOAc (57 mg, 0.69 mmol, 5 eq.) and NH₂OH·HCl (48 mg, 0.69 mmol, 5 eq.). The crude was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 99:1 to 98:2) to give **35b** (13 mg, 0.034 mmol, 25%) as a white solid. $R_f = 0.41/0.46$ (CH₂Cl₂/MeOH 95:5); IR (ATR) 3600–3010, 2924, 2855, 1463, 1326, 1248, 1141, 931, 840, 737 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ (*E/Z* mixture ~6:4) 13.70 (s, 1H_Z+1H_E), 11.48 (s, 1H_Z+1H_E), 10.71 (s, 1H_Z), 10.41 (s, 1H_E), 8.77 (s, 1H_E+1H_Z), 8.66 (br d, $J = 6.4$, 1H_E+1H_Z), 8.40 (d, $J = 7.8$, 1H_E+1H_Z), 7.81 (d, $J = 8.2$, 1H_E+1H_Z), 7.76 (d, $J = 8.1$, 1H_E), 7.75 (d, $J = 7.9$, 1H_Z), 7.47 (t, $J = 7.9$, 1H_Z+1H_E), 7.43 (t, $J = 7.8$, 1H_Z+1H_E), 7.38–7.27 (m, 2H_Z+3H_E), 6.61 (t, $J = 5.1$, 1H_Z), 4.99 (t, $J = 6.7$, 2H_Z+2H_E), 2.35–2.27 (m, 2H_Z), 2.23–2.15 (m, 2H_E), 2.10–1.97 (m, 2H_Z+2H_E); ¹³C NMR (101 MHz, DMSO-*d*₆) δ (*E/Z* mixture ~6:4) 149.5/149.0 (CH), 139.1/139.0 (C), 138.61/138.58 (C), 132.1^a (C), 130.5^a (CH), 127.34/127.32 (C), 124.0 (CH), 123.8 (CH), 122.60/122.58 (C), 122.0/121.9 (C), 121.59/121.62 (C), 120.9 (CH), 120.6 (CH), 120.0 (CH), 119.5 (CH), 112.11/112.09 (CH), 111.6 (C), 111.2 (C), 109.9 (CH), 104.14/104.12 (C), 43.7/43.4 (CH₂), 27.6/27.2 (CH₂), 26.4/22.2 (CH₂), ^aweak signal; HRMS

(ESI+) m/z calcd for $C_{23}H_{20}N_5O$ ($M + H$)⁺ 382.1662, found 382.1658; HPLC purity, Method B: \geq 99%, $t_R = 26.67$ min + 27.47 min, $\lambda = 240$ nm.

6.2. Cell biology

6.2.1. Cell maintenance

The human AML cells MOLM-13 and OCI-AML3 were cultured in RPMI medium (R5886, Sigma-Aldrich, Merck) while the MV4-11 cells were cultured in IMDM (12440-053, Gibco, Thermo Fisher Scientific). The two non-cancerous cell lines, normal rat kidney epithelial (NRK) and rat cardiomyoblasts (H9c2) cells were cultured in DMEM medium (D6546, Sigma-Aldrich). All media were enriched with 10% fetal bovine serum (F7524; heat inactivated for MV4-11) and 2 mM L-glutamine (G7513; 8mM for MV4-11) as well as 100 IU/mL penicillin and 100 mg/mL streptomycin (P0781) (all from Sigma-Aldrich, Merck). Cells were cultured in a humidified atmosphere (37 °C, 5% CO₂) and routinely tested for mycoplasma infection using MycoAlert® (LT07-418, Lonza). No mycoplasma infection was detected for either cell line during this study.

6.2.2. Assessment of cell death

The new potential Pim kinase inhibitors as well as the pan-Pim kinase inhibitor AZD1208 (#6310, Tocris Bioscience) were prepared as stocks of 20 mM in DMSO (D5879, Sigma-Aldrich). For cytotoxic testing over 24 hours, MOLM-13, OCI-AML3 and MV4-11 cells were seeded at 30,000 cells/well, while NRK and H9c2 cells were seeded at 5,000 cells/well in 96-well microplates with 100 μ L medium/well. First, a survey of bioactivity of all the compounds were conducted with MOLM-13 cells with a dose-response experiment with 10-fold dilution steps, starting at 200 μ M. From these data and the results from Pim activity experiments, six compounds were selected for further analyses on all three AML cell lines and the non-cancerous cells. Here, the dose-response curve had 2-fold dilution steps to better pinpoint the EC₅₀ values. Cell viability was assessed by use of WST-1 reagent, in accordance with the manufacturer's instructions (Roche Diagnostics, Merck). Next, the cells were fixed in 2% buffered formaldehyde (pH 7.4) with the DNA-specific dye Hoechst 33342 (0.01 mg/mL, #14533, Sigma-Aldrich) and nuclear morphology evaluated under UV-microscopy to verify the viability results [5]. The highest dose of analogue corresponded to 1% of DMSO, which alone gave less than 5% cell death as judged by nuclear morphology. To calculate the EC₅₀ values, a four-parameter regression analyses of the WST-1 signal results was performed using SigmaPlot ver. 14.0 (Systat Software Inc., San Jose, CA, USA) as previously described [5].

6.3. In vitro kinase inhibition assays

These assays were performed at the International Centre for Kinase Profiling (ICKP) in Dundee, Scotland. The procedures for *in vitro* protein kinase assays have been described previously [16] and are detailed in ICKP web site (<https://www.kinase-screen.mrc.ac.uk/>).

Protein kinases were of human origin except ROCK2 (rat), and were either expressed as GST (glutathione transferase) fusion proteins in *Escherichia coli* (CAMK1, CAMKK2, CHK2, CK1 δ , Dyrk1a, EF2K, Eph-A2, HIPK2, IRAK4, MKK1, p38 α MAPK, PAK4, Pim-2, PKA α , SmMLCK, SRPK1) or as hexahistidine (His₆)-tagged proteins in Sf21 (*Spodoptera frugiperda* 21) insect cells (AMPK, Aurora B, BTK, CK2 α 1, GSK3 β , HER4, IGF1R, JAK3, JNK1, Lck, LKB1, RSK1, MARK3, MLK3, MSK1, MST2; NEK6, PDK1, Pim-1, Pim-3, PKB α , PKC α , PKD1, PLK1, PRK2, RIPK2, ROCK2, S6K1, SGK1, Src, SYK, TAK1, TBK1, Trka, TTK, VEGFR1). GST fusion proteins were purified by affinity chromatography on glutathione-Sepharose, and His₆-tagged proteins on nickel/nitrilotriacetate-agarose.

The assays (25.5 μ L volume, 5–20 mU of enzyme) were carried out robotically in a 96-well format at room temperature and were linear with respect to time and enzyme concentration under the conditions used. Unless stated otherwise, assays were performed for 30 min. Assays were stopped by addition of 5 μ L of 0.5 M (3%) orthophosphoric acid and then harvested onto P81 Unifilter plates with a wash buffer of 50 mM orthophosphoric acid. The concentration of magnesium acetate in the assays was 10 mM.

[γ -³³P]ATP (50-1000 cpm/pmol) was used at 5 μ M (CK2 α 1, EF2K, GSK3 β , HER4, HIPK2, IGF1R, MARK3, MKK1, PAK4, PKB α , PLK1, PRK2, Pim-2, TAK1), 20 μ M (AMPK, Aurora B, CAMKK2, CHK2, CK1 δ , IRAK4, JAK3, JNK1, LKB1, MLK3, MSK1, MST2, PDK1, Pim-1, Pim-3, PKA α , PKC α , RIPK2, ROCK2, S6K1, SGK1, SYK, TrkA, TTK, VEGFR1) or 50 μ M (BTK, CAMK1, DYRK1a, Eph-A2, Lck, RSK1, NEK6, p38 α MAPK, PKD1, SmMLCK, Src, SRPK1, TBK1), in order to be at or below the K_m for ATP for each enzyme.

Kinase substrates were: AMPK, HMRSAMSGHLVLR (SAMS substrate peptide, 400 μ M); Aurora B, LRRLSLGLRRLSLGLRRLSLGLRRLSLG (300 μ M); BTK, KVEKIGEGTYGVVYK (300 μ M); CAMK1, YLRRRLSDSNF (300 μ M); CAMKK2, DGEFLRTSCGSPNYAARRR (300 μ M); CHK2, KKKVSRSGLYRSPSPENLNRPR (CHKtide, 200 μ M CHKtide); CK1 δ , RRKDLHDDEEDEAMSITA (CKI peptide, 500 μ M); CK2 α 1, RRRDDSDDDD (CKII peptide, 165 μ M); DYRK1a, KKISGRLSPIMTEQ (Woodtide, 350 μ M); EF2K, RKKFGESKTKTKEFL (300 μ M); Eph-A2, Poly Glut Tyr (0.1 mg/mL); GSK3 β , YRRAAVPPSPSLSRHSSPHQS(PO₄)EDEEE (Phospho-GS2 peptide, 20 μ M); HER4, Poly Glut Tyr (1 mg/mL); HIPK2, myelin basic protein (MBP, 0.33 mg/mL); IGF1R, KKKSPGEYVNIIEFG

(300 μ M). IRAK4, myelin basic protein (MBP, 0.33 mg/mL); JAK3, KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC (PDKtide, 100 μ M); JNK1, ATF2 (activating transcription factor, 3 μ M); Lck, KVEKIGEGTYGVVYK (Cdc2 peptide, 250 μ M); LKB1, LSNLYHQGKFLQTFCGSPLYRRR (200 μ M); RSK1, KKLNRTLSVA (30 μ M); MARK3, KKKVSRSGLYRSPSPENLNRPR (CHKtide, 300 μ M); MKK1, activation of inactive MAPK (0.06 mg/mL); MLK3, myelin basic protein (MBP, 0.33 mg/mL); MSK1, GRPRTSSFAEGKK (modified Crosstide, 30 μ M); MST2, myelin basic protein (MBP, 0.33 mg/mL); NEK6, FLAKSFGSPNRAYKK, (NEK6 peptide, 300 μ M); p38 α MAPK, myelin basic protein (0.33 mg/mL); PAK4, RRRLSFAEPG (300 μ M); PDK1, KTFCGTPEYLAPEVRREPRILSEEEQ-EMFRDFDYIADWC (PDKtide, 100 μ M); Pim-1, RSRHSSYPAGT (300 μ M); Pim-3, RSRHSSYPAGT (300 μ M); Pim-2, RSRHSSYPAGT (300 μ M); PKA α , LRRASLG (Kemptide, 30 μ M); PKB α , GRPRTSSFAEGKK (modified Crosstide, 30 μ M); PKC α , Histone H1 (0.1 mg/mL); PKD1, KKLNRTLSVA (30 μ M); PLK1, ISDELMDATFADQEAKKK (300 μ M); PRK2, KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK (Long S6 peptide, 30 μ M); RIPK2, myelin basic protein (MBP, 0.33 mg/mL); ROCK2, KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK, (Long S6 substrate peptide, 30 μ M); S6K1, KKRNRRLTV (100 μ M); SGK1, GRPRTSSFAEGKK (modified Crosstide, 30 μ M); SmMLCK, KKRPRATS NVFA (300 μ M); Src, KVEKIGEGTYGVVYK (300 μ M); SRPK1, RSRRSRSRSRSRSR (300 μ M); SYK, Poly Glut Tyr (1 mg/mL); TAK1, RLGRDKYKTLRQIRQ (300 μ M); TBK1, KKKKERLLDDRHDSGLDSMKDEE (300 μ M); TrkA, Poly Glut Tyr (1 mg/mL); TTK, RSRRSRSRSRSRSR (300 μ M); VEGFR1, KKKSPGEYVNIEFG (300 μ M).

AMPK (diluted in 50 mM Hepes pH 7.5, 1 mM DTT, 0.02% Brij35) was assayed in 50 mM Hepes pH 7.5, 1 mM DTT, 0.02% Brij35, 0.196 mM AMP; Aurora B (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol; BTK (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; CaMK1a (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris, 0.1 mM EGTA, 0.5 mM CaCl₂, 0.3 μ M calmodulin, 0.1% 2-mercaptoethanol; CaMKK2 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.5 mM CaCl₂, 0.3 μ M calmodulin, 0.1% 2-mercaptoethanol; CHK2 (diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.1% 2-mercaptoethanol, 0.01% Brij-35, 5% glycerol, 1 mg/mL BSA) was assayed in 8 mM MOPS pH 7.0, 0.2 mM EDTA; CK1 δ (diluted in 20 mM Hepes pH 7.5, 0.15 M NaCl, 0.1 mM EGTA, 0.1% Triton X-100, 5 mM DTT, 50%

glycerol) was assayed in 20 mM Hepes pH 7.5, 0.15 M NaCl, 0.1 mM EDTA, 5 mM DTT, 0.1% Triton-X 100; CK2 α 1 (diluted in 20 mM Hepes pH 7.5, 0.15 M NaCl, 0.1 mM EGTA, 0.1% Triton X-100, 5 mM DTT, 50% glycerol) was assayed in 20 mM Hepes pH 7.5, 0.15 M NaCl, 0.1 mM EDTA, 5 mM DTT, 0.1% Triton-X 100; DYRK 1A (of diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; EF2K (diluted in 50 mM Hepes pH 6.6, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Hepes pH 6.6, 0.2mM CaCl₂, 0.3 μ M Calmodulin, 0.05% 2-mercaptoethanol; EPH-A2 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; GSK3 β (diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01% Brij35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 8 mM MOPS pH 7.0, 0.2 mM EDTA; HER4 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; HIPK2 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1%, 2-mercaptoethanol; IGF1R (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; IRAK4 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; JAK3 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol; JNK1 α 1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol; LCK (diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01% Brij35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1 mM Na₃VO₄, and incubated for 15 min; LKB1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; MAPKAP-K1a/RSK1 (diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01% Brij35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Na- β -glycerophosphate pH 7.5, 0.5 mM EDTA, and incubated for 40 min; MARK3 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol; MKK1: This is a two-step assay where inactive MAPK (0.06 mg/mL) is activated by MKK1 (diluted in 25 mM Tris, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 0.01% Brij35, 1 mg/mL BSA) in 25.5 μ L containing 25 mM Tris, 0.1 mM EGTA, 0.01% Brij35, 10 mM magnesium acetate and 0.005 mM ATP. After incubating at room temperature for 30 min, 5 μ L from the first reaction is pipetted into 20 μ L of the second reaction mix containing (final concentration) 25 mM Tris pH 7.5, 0.1 mM EGTA, 0.1 mM Na₃VO₄, 0.66 mg/mL myelin basic protein (MBP), 10 mM magnesium acetate and 0.05 mM

[³²P-γ-ATP] (500 -1000 cpm/pmole) and incubated for 30 min at room temperature; MLK3 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; MSK1 (diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01% Brij35, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 8 mM MOPS pH7.0, 0.2 mM EDTA; MST2 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 100 μM Na₃VO₄) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol; NEK6 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.01% Brij, 0.1% 2-mercaptoethanol; p38α MAPK (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1 mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 25 mM Tris pH 7.5, 0.1 mM EGTA; PAK4 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol.

PDK1 (diluted in 50 mM Tris pH 7.5, 0.05% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.05% 2-mercaptoethanol; PIM1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol; PIM2 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA, 0.1% 2-mercaptoethanol) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.5 mM CaCl₂, 0.3 μM calmodulin, 0.1% 2-mercaptoethanol; PIM3 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% β-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol; PKAα (diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01% Brij35, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 8 mM MOPS pH 7.5, 0.2 mM EDTA; ΔPH-PKBα-S473D (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.05% 2-mercaptoethanol; PKCα (diluted in 20 mM Hepes pH 7.4, 0.03% Triton X-100) was assayed in the presence of PtdSerine and DAG (0.1 mg/mL and 10 μg/mL) and 0.1 mM CaCl₂. The assay was carried out in 20 mM Hepes pH 7.4, 0.03% Triton X-100; PKD1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol; PLK1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA, 100 μM Na₃VO₄) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.05% 2-mercaptoethanol, 10 μM Na₃VO₄; PRK2 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol; RIPK2 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; ROCK2 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was

assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; S6K1/p70S6K (T412E) (diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01% Brij35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 8 mM MOPS pH 7.0, 0.2 mM EDTA; SGK1 (diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01% Brij35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 8 mM MOPS pH 7.0, 0.2 mM EDTA; SmMLCK (diluted in 50 mM Hepes pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Hepes pH 7.5, 0.1 mM EGTA, 5 mM CaCl₂, 10 μM Calmodulin; Src (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; SRPK1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol; SYK (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; TAK1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-Mercaptoethanol, 0.5 mM MnCl₂; TBK1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; TrkA (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 10 mM MnCl₂, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; TTK (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA; VEGFR1/FLT1 (diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/mL BSA) was assayed in 50 mM Tris pH 7.5, 0.1 mM EGTA.

The inhibitory profile of tested compounds was expressed as the percentage of residual kinase activity for the inhibitor concentration of 1 μM or 0.5 μM. The IC₅₀ values of inhibitors were determined after testing 10 different concentrations of each compound. The results are shown as the mean ± standard deviation for duplicate determinations.

6.4. Molecular Modelling

Pim-3 three-dimensional structure (Uniprot accession number Q86V86) was obtained from Alphafold structure database [31] as a PDB file. The model was prepared using UCSF Chimera 1.14 software [30] by addition of hydrogen atoms, removal of water molecules and considering 1YHS Pim-1 crystal structure from the Protein Data Bank (PDB). AM1 charges were calculated with Chimera. Rigid and flexible (side chains of the ATP-binding pocket) pdbqt files were prepared with AutoDockTools 1.5.7 (ADT) [32]. Apolar hydrogen atoms were removed and AM1 charges were added. The geometry of compound **19a** (6*H*-tautomer) was optimized using Gaussian 16 C.01 software [33] (B3LYP/6-31G) and AM1 charges were generated. Docking studies in the

Pim-3 ATP-binding pocket were performed with AutoDock 4.2 [32]. Results were analysed with Chimera.

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