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Synthesis and biological evaluation of 1*H*-pyrrolo[3,2-*g*]isoquinolines

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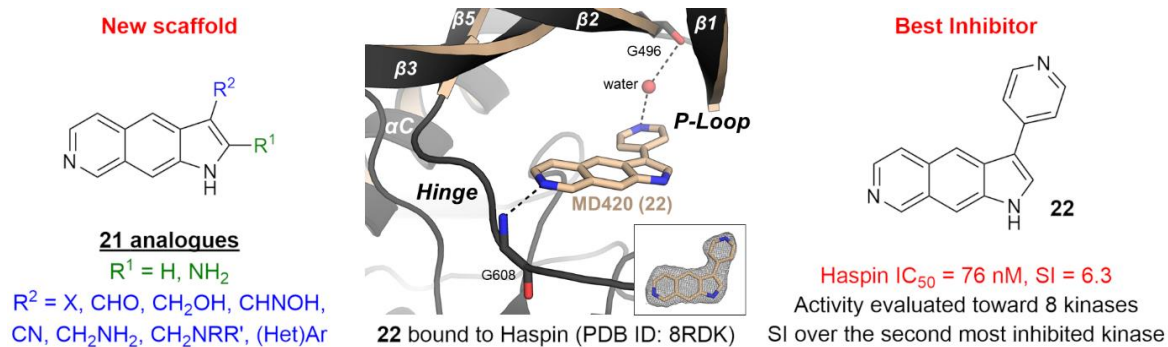
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Abstract

A structure-activity relationship study performed on 1*H*-pyrrolo[3,2-*g*]isoquinoline scaffold identified new haspin inhibitors with nanomolar potencies and selectivity indices (SI) over 6 (inhibitory potency evaluated against 8 protein kinases). Compound **22** was the most active of the series (haspin $IC_{50} = 76$ nM). Cellular evaluation of **22** confirmed its activity for endogenous haspin in U-2 OS cells and its anti-proliferative activity against various cell lines. In addition, the binding mode of analog **22** in complex with haspin was determined by X-ray crystallography.

Keywords: pyrroles; indoles; isoquinolines; kinase inhibition; Haspin; cellular activity

Graphical abstract



1. Introduction

As part of our research program focusing on the synthesis and development of new heteroaromatic scaffolds demonstrating potent protein kinase inhibitory potencies, we recently focused on the histone associated protein kinase haspin (GSG2, Germ Cell-Specific Gene 2 Protein). Through its modulation of Aurora kinases and its role phosphorylating histone H3 threonine 3 (H3T3), haspin is essential for mitosis. Moreover, haspin does not belong to a canonical protein kinase family (Group: Other kinases) and exhibits an unusual kinase domain structure facilitating its selective inhibition [1-3]. Therefore, haspin is an attractive biological target for the identification of new anticancer drugs as demonstrated in HCT-116 cells xenografted mice models [4,5]. In addition, the upregulation of haspin has also been reported in pancreatic cancer tissues and human pancreatic cell lines [6] as well as in bladder cancer tissues [7].

Despite its potential as a target for drug discovery, very few high quality inhibitors have been described (*e.g.* acridines, β -carbolines, ribofuranosyl derivatives, ARC-type bisubstrate inhibitors, CHR-6494) [8]. The compound currently used as a reference inhibitor is polyazaheteroaromatic derivative CHR-6494 (Figure 1). We recently reported new pyridoquinazoline series and identified compound **I** as a potent Haspin inhibitor with a good overall selectivity profile when evaluated toward a large panel of 486 kinases [9] (Figure 1). Inspired by CHR-6494 indazole ring and to evaluate the impact of ring A size on the biological activity, new pyrazoloisoquinolines with a pyrazole 5-membered ring instead of a pyrimidine were also synthesized [10]. Enzyme kinetic assays demonstrated that this structural modification was not detrimental to haspin inhibition (*e.g.* Compound **II**) (Figure 1). However, the selectivity profile was less favorable than the one observed for the quinazoline series.

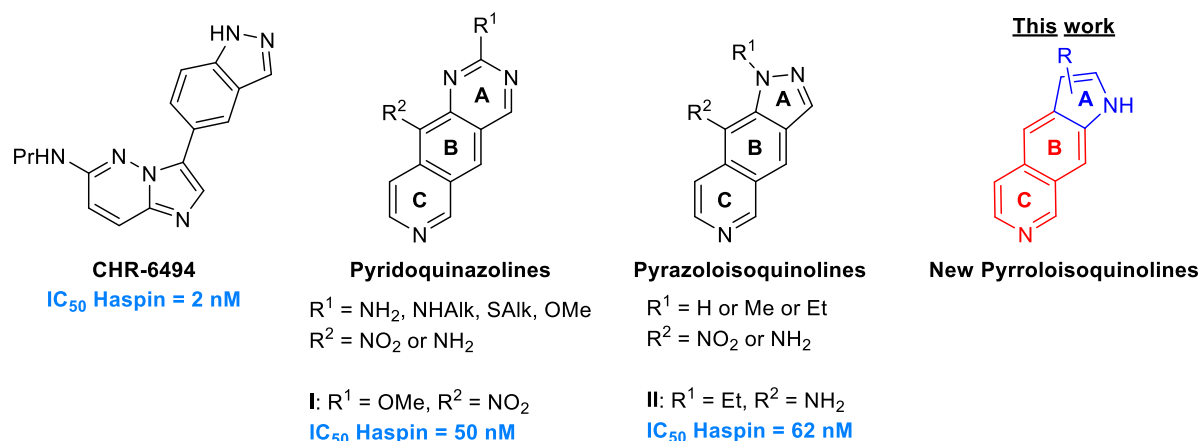


Figure 1. Structure and haspin IC_{50} values of **CHR-6494** and most potent inhibitor of the pyridoquinazoline (**I**) and pyrazoloisoquinoline (**II**) series developed by our group. New pyrroloisoquinoline scaffold designed from previous pyridoquinazoline and pyrazoloisoquinoline series.

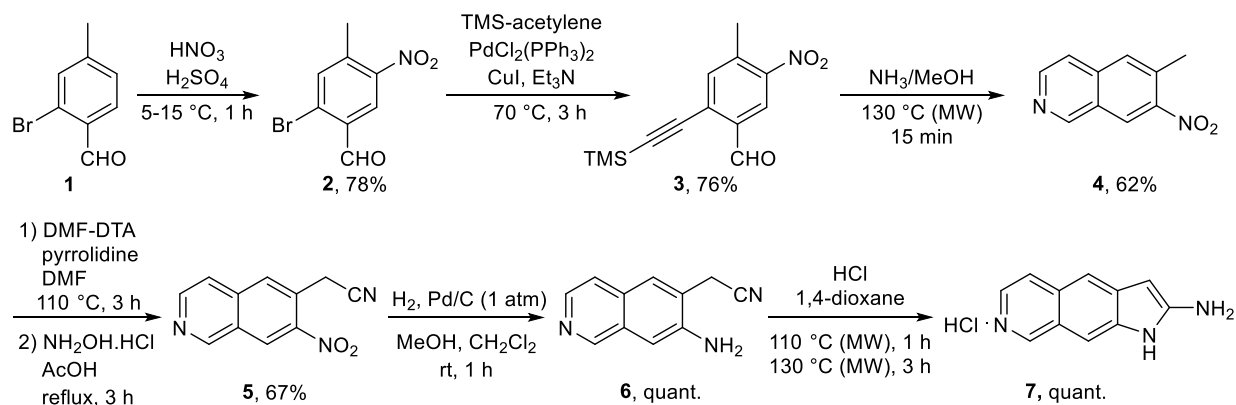
Based on previous results and to enlarge the structure-activity relationship studies performed around this tricyclic scaffold, a new pyrroloisoquinoline series was designed. It has been demonstrated previously that the nitrogen atom of the isoquinoline moiety (B and C rings) is involved in hydrogen bonding with the hinge backbone of the targeted protein kinase, thus, this

part was conserved and combined to a diversely substituted pyrrole 5-membered ring (A) (Figure 1).

2. Results and Discussion

2.1. Synthesis and protein kinases inhibitory potencies

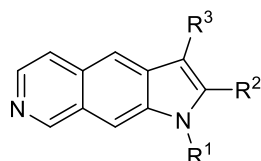
Numerous synthetic pathways were assayed to obtain targeted compound **7**, bearing a 2-amino group in order to mimic the corresponding 2-amino function which was present in the 2-aminopyrimidine moiety of the pyridoquinazoline series. The successful approach started by nitration of relatively cheap commercially available 2-bromo-4-methylbenzaldehyde **1** [11] followed by Sonogashira coupling in the presence of TMS-acetylene and cyclization in the presence of ammonia in methanol under microwave irradiation to afford isoquinoline **4** in 37% overall yield [12] (Scheme 1). Nitrile **5** was prepared from **4** after enamine formation in the presence of DMF-di-*tert*-butylacetal (DMF-DTA) followed by hydrolysis leading to an intermediate aldehyde, which was converted to the corresponding nitrile by reaction with hydroxylamine hydrochloride to give the oxime intermediate, and further elimination under acidic conditions [13]. With intermediate **5** in hands, synthesis of **7** was achieved quantitatively in two steps by first reduction of the nitro group and finally cyclization in acidic medium under microwave irradiation [14].



Scheme 1. Synthetic route to pyrroloisoquinoline **7**.

As depicted in Table 1, to measure the impact of ring C structural modification on biological activities, 2-aminopyrroloisoquinoline **7** was early evaluated at 10 μ M and 1 μ M compound concentration using ADP-Glo assay [15] toward a panel of eight human protein kinases used for previous series as they have been identified as frequent off-targets of these scaffolds (haspin, CLK1, DYRK1A, CDK9/Cyclin T, GSK-3 β , CK1 ϵ , CDK5/p25, PIM1).

Table 1. Kinase inhibitory activities of references **I**, **II** and compounds **7-19**, **21-24** and **26**.



7 R ¹ , R ³ = H, R ² = NH ₂	14 R ¹ , R ² = H, R ³ = CHNOH	18 R ¹ = Me, R ² , R ³ = H
8 R ¹ , R ² , R ³ = H	15 R ¹ , R ² = H, R ³ = CN	19 R ¹ = Me, R ² = H, R ³ = I
9 R ¹ , R ² = H, R ³ = Br	16 R ¹ , R ² = H, R ³ = CH ₂ NH ₂ .TFA	21 R ¹ , R ² = H, R ³ = phenyl
10 R ¹ , R ² = H, R ³ = I	17a R ¹ , R ² = H, R ³ = CH ₂ NHMe	22 R ¹ , R ² = H, R ³ = pyridin-4-yl
11 R ¹ , R ² = H, R ³ = Cl	17b R ¹ , R ² = H, R ³ = CH ₂ NHEt	23 R ¹ , R ² = H, R ³ = pyrimidin-5-yl
12 R ¹ , R ² = H, R ³ = CHO	17c R ¹ , R ² = H, R ³ = CH ₂ NMe ₂	24 R ¹ , R ² = H, R ³ = acetyl
13 R ¹ , R ² = H, R ³ = CH ₂ OH	17d R ¹ , R ² = H, R ³ = CH ₂ NEt ₂	26 R ¹ , R ² = H, R ³ = 2-aminopyrimidin-4-yl

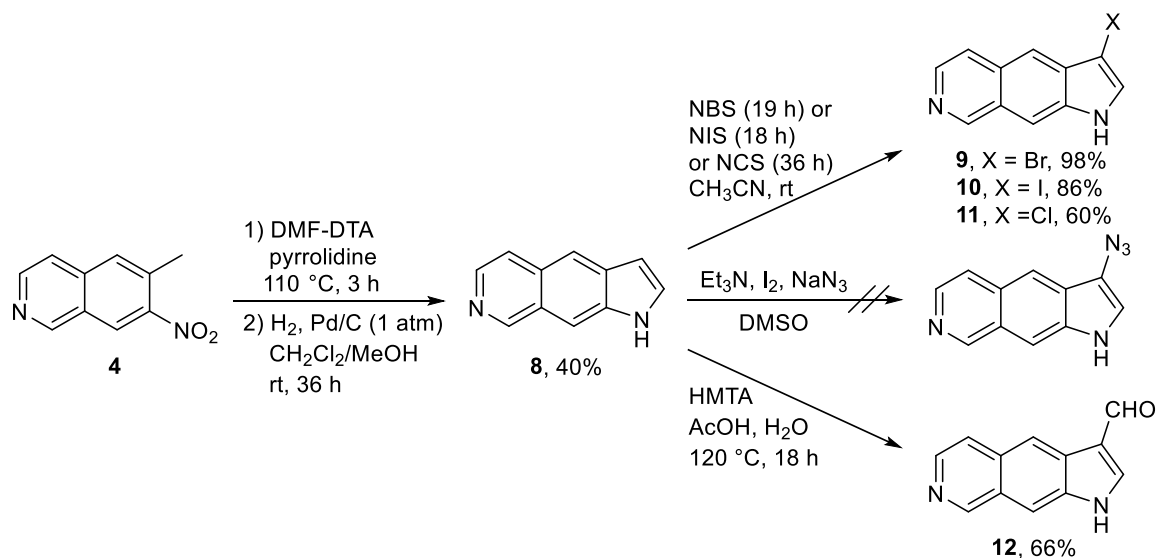
Cpds	Kinase inhibition (% residual activity at 10 or 1 μM). IC ₅₀ (nM) values under brackets																
	Haspin		CLK1		DYRK1A		CDK9		GSK3		CK1		CDK5		PIM1		SI
	10	1	10	1	10	1	10	1	10	1	10	1	10	1	10	1	
I	14	23	8	34	20	49	43	59	29	76	53	95	80	100	55	100	8.9 (CLK1)
II	3	17	8	34	6	23	50	80	42	82	48	84	28	65	60	91	4.0 (DYRK1A)
7	22	61	20	73	39	100	27	74	64	69	52	79	99	89	88	100	
8	35	80	67	87	37	99	34	76	100	100	90	97	98	100	45	90	
9	6	16	32	72	11	36	27	39	62	89	77	100	48	100	24	48	5.4 (PIM1)
10	10	18	27	69	16	50	35	62	74	77	27	69	100	ND	23	50	5.0 (DYRK1A)
11	28	53	39	78	21	61	25	62	82	92	82	100	68	100	16	63	
12	10	17	26	53	7	21	7	7	23	55	34	80	57	84	33	64	0.96 (CDK9)
13	18	42	55	96	62	77	55	81	81	84	98	100	94	ND	100	100	
14 Z/E	5	13	ND	15	1	9	2	13	27	80	46	89	32	51	11	43	1.1 (CLK1)

15	7 15 (75)	0 19 (360)	5 38 (182)	6 34 (178)	26 59	47 92	34 69	95 100	2.4 (CDK9)
16	31 74	86 99	62 77	58 93	80 94	96 99	94 100	84 97	
17a	32 69	74 95	92 ND	37 58	83 87	98 100	88 93	90 89	
17b	27 62	80 94	63 80	37 74	80 76	100 100	89 ND	74 76	
17c	29 58	62 91	67 74	18 57	66 78	99 100	97 100	67 66	
17d	43 70	84 89	76 89	51 86	77 79	100 98	100 ND	47 65	
18	15 58	58 92	29 67	60 89	83 100	85 92	38 53	35 67	
19	14 30 (243)	22 61	14 46	26 57	60 81	72 100	22 95	26 48	
21	9 37 (482)	44 74	19 53	63 83	77 66	88 100	73 97	18 53	
22	14 24 (76)	17 46 (693)	11 46 (513)	38 78	54 92	25 61	10 79	9 49 (479)	6.3 (PIM1)
23	4 17 (249)	14 54	20 51	43 79	59 68	43 81	72 100	33 63	
24	0 8 (157)	10 21 (265)	0 25 (860)	20 34	27 69	62 98	13 31	27 80	1.7 (CLK1)
26	0 0 (122)	5 16 (205)	0 25 (560)	16 56	35 76	10 46	21 79	4 20 (225)	1.7 (CLK1)

Assays were performed in triplicate using the ADP-Glo assay in the presence of 10 μ M ATP. Typically, the standard deviation of single data points was below 10%. Percentage of kinases residual activity in the presence of a 10 μ M and 1 μ M compound concentration. IC₅₀ values in nM (in brackets) were determined for haspin when residual activity was < 45% at 1 μ M. For other kinases, IC₅₀ values measured for targets that were most potently inhibited (inhibition % \geq 50% at 1 μ M). SI haspin/other most potently inhibited kinase, selectivity index = CLK1 or DYRK1A or CDK9 or PIM1 IC₅₀/haspin IC₅₀. ND : not determined.

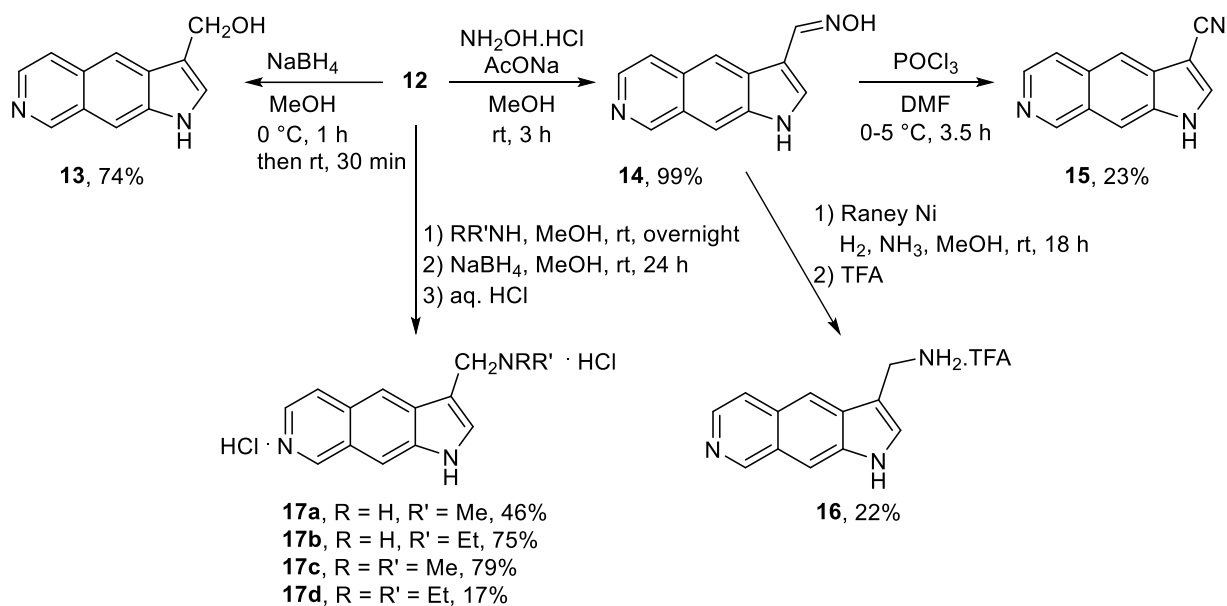
Unfortunately, the screening results obtained demonstrated that compound **7**, compared to references **I** and **II**, was less active on haspin with an IC₅₀ value > 1 μ M (Table 1). This indicated that a 2-aminopyrrole moiety as ring C was not suitable for the development of potent haspin inhibitor. Despite this negative result, as we had developed a synthetic pathway to this new scaffold, we decided to explore further this pyrroloisoquinoline series. Thus, new analogues bearing various substituents at the 3-position, that are expected to favor molecular interactions with the haspin ATP-binding site, were synthesized.

The key pyrroloisoquinoline **8** was obtained from **4** by Leimgruber-Batcho reaction [16]. Then, various substituents which were further modified were introduced at the 3-position. Firstly, 3-halogeno analogues **9-11**, were easily prepared in the presence of *N*-bromo-, *N*-iodo- or *N*-chlorosuccinimide [17] (Scheme 2). Our attempts to synthesize 3-azido derivative by reacting compound **8** with NaN_3/I_2 in the presence of trimethylamine in DMSO [18], only led to the isolation of the corresponding iodo analogue **10**. Duff formylation of **8** in the presence hexamethylenetetramine (HMTA) in acidic medium was more successful leading to compound **12** in 66% yield [19] (Scheme 2).



Scheme 2. Synthesis of compounds **8-12**.

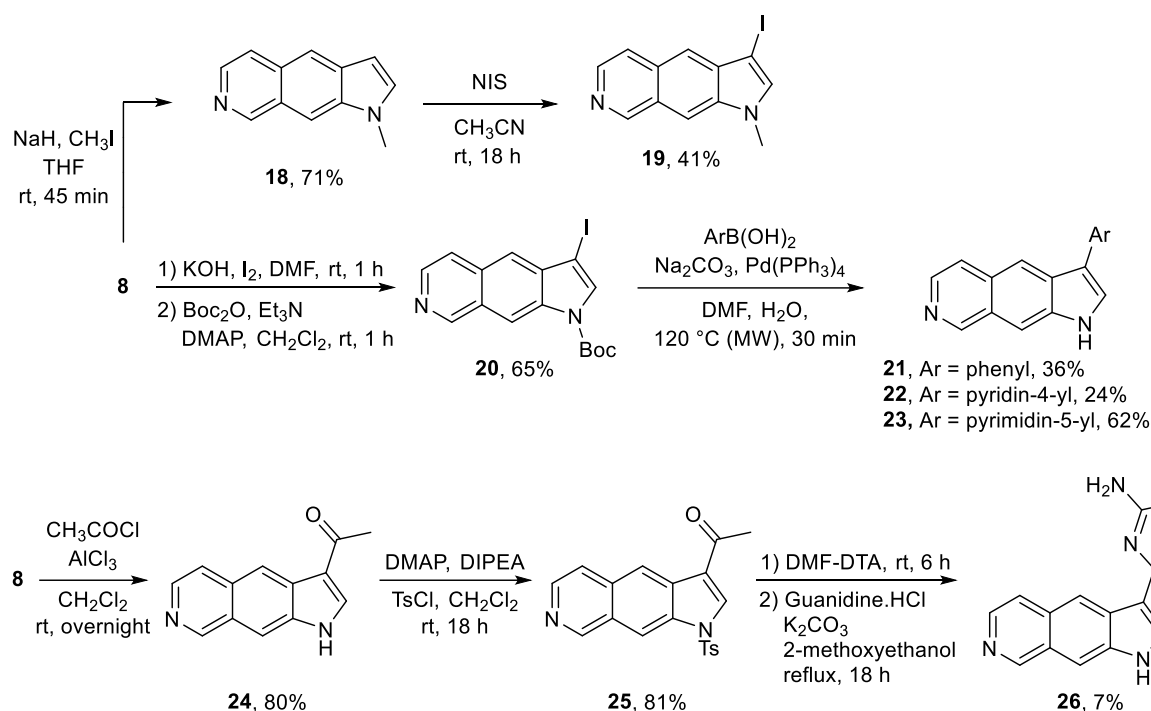
Formylpyrroloisoquinoline **12** was an interesting intermediate which could be easily converted to additional diversely 3-substituted analogues (Scheme 3). Indeed, reduction using sodium borohydride led to compound **13** in 74% yield [20]. On the other hand, reaction of hydroxylamine hydrochloride with **12** led to oxime **14** obtained quantitatively as a mixture of 80/20 *Z/E* isomers ratio [21]. Next, oxime **14** led either to the nitrile **15** after treatment with phosphorus oxychloride [22] or aminomethyl analogue **16** by reduction in the presence of Raney nickel [23]. At last, reductive amination of **12** led to corresponding 3-aminomethyl derivatives **17a-d** in 17% to 79% yield after dihydrochloride salt formation (Scheme 3) [24].



Scheme 3. Synthesis of compounds **13-17**

In addition, in order to determine the relevance of a potential H-bond interaction between the indole N-H and the enzyme active site, two *N*-methylated analogues of compound **8** were also prepared by methylation with iodomethane [25] and subsequent iodination with NIS. Derivatives **18** and **19** were obtained in 71% and 41% yield respectively (Scheme 4).

Finally, analogues bearing (hetero)aryl substituents at the 3-position were also synthesized. Therefore, compound **8** was first iodinated in the presence of iodine and KOH before Boc protection to give analogue **20** in 65% yield [26]. In a second step, Suzuki cross-coupling reaction in the presence of various boronic acids led to the corresponding coupling products **21-23** in 24% to 62% yields. (Scheme 4). Lastly, compound **26** bearing a pyrimidine moiety at the 3-position was prepared using a similar approach to the one previously reported by our group for the preparation of diversely substituted meridianin derivatives [27]. Acylation of **8** [28], followed by protection of the nitrogen atom with a tosyl group [29] led to compound **25** in 65% overall yield. Subsequent reaction of **25** with DMF/dimethylformamide-*di-tert*-butylacetal afforded an enaminone intermediate which was cyclized to the corresponding pyrimidine scaffold under basic conditions in the presence of guanidine hydrochloride in 2-methoxyethanol.



Scheme 4. Synthesis of new pyrroloisoquinolines **18**, **19**, **21-23** and **26**.

The ability of new compounds (**8-19**, **21-24** and **26**) to inhibit the protein kinases from the same panel used for **7** was evaluated (Table 1). Similarly, in order to 2-amino-derivative **7**, the non-substituted pyrroloisoquinoline **8** was almost inactive towards the tested kinases. Contrarily, the 3-halogenated analogues **9** and **10** bearing respectively a bromine or iodine atom demonstrated sub-micromolar inhibitory potencies for haspin. Moreover, in both cases, the kinase DYRK1A (compound **8**) and PIM1 (compound **9**) as dominant off-targets of this series were only moderately inhibited compared to the potency determined for haspin. Surprisingly, the chloro analogue **11** was less active (47% of inhibition at 1 μ M). However, other 3-substituted derivatives (**12**, **14**, **15**) also exhibited haspin inhibitory activity but were less selective. Thus, 3-CHO analogue (**12**) was almost an equipotent inhibitor of CDK9 and haspin. These results indicated that, compared to **12**, inhibition of haspin can be recovered by substitution of the 3-position, with the 3-CN (**15**) group being preferred over 3-CHO (**12**), 3-CHNOH (**14**), 3-CH₂OH (**13**) and 3-CH₂NRR' (**16**, **17a-d**) substituents considering potency and selectivity.

The *N*-methylated products **18** and **19** were prepared to evaluate the importance of the pyrrole NH group. In analogy to the NH series, the 3-iodo derivative **19** was more potent than the 3-unsubstituted analogue **18** which was not very active ($IC_{50} \approx 1 \mu$ M, 42% inhibition at 1 μ M), but this compound seemed somewhat more potent than its corresponding NH analogue **8** (20% inhibition at 1 μ M). Conversely, *N*-methylated 3-iodo derivative **19** was less active on haspin compared to its NH analogue **10**. Another 3-substituted derivative, compound **24**, bearing a 3-acetyl group, did inhibit haspin but with modest selectivity within our screening panel.

Finally, the results obtained for the 3-(hetero)aryl substituted derivatives demonstrated that haspin inhibition was conserved using bulky hydrophobic substituents. However, haspin inhibition was improved using nitrogen containing heteroaryl substituents (**22**, **23**, **26** compared to **21**). The best potency and selectivity within our selectivity panel was achieved by compound **22** bearing a pyridin-4-yl moiety at the 3-position.

2.2. Biological evaluation on cancer cell lines.

To better characterize the phenotypic effects of the most active derivatives in terms of potency and selectivity, **9**, **10** and **22** as well as previously prepared analogues **I** and **II** (Figure 1) and the reference CHR-6494 were evaluated in cell viability assays (Table 2).

The compounds were tested on the osteosarcoma cell line U-2 OS, the colorectal cancer cell line HCT116, the breast cancer cell line HBL-100, SH-SY5Y (neuroblastoma) and RPE-1 (retinal fibroblast immortalized with hTERT). Cell viability was assessed in dose-response after 48 h incubation and EC₅₀ values were determined (Table 2).

Table 2. Cell viability assays (MTS). Effects of selected compounds on cell viability of cancerous and non-cancerous cell lines after 48 h incubation.

Compounds	EC ₅₀ (μM)				
	RPE1	HBL100	HCT116	SH-SY5Y	U-2 OS
I	4.5	9.6	5.8	4.3	5.9
II	0.3	7.3	1.2	1.2	2.7
9	> 50	> 50	> 50	> 50	> 50
10	> 50	> 50	> 50	> 50	> 50
22	7.8	24.1	7.3	20.0	11.2
CHR-6494	1.4	25.7	2.2	>50	0.9

EC₅₀ (μM) were determined from dose-response curves realized in triplicate.

Our results revealed that compounds **9** and **10** had no effect on cell viability for any of the tested cell lines at a concentration of 50 μM. This observation may result from various factors, such as a reduced solubility of the compounds in aqueous cell culture media, a limited cellular uptake or a fast degradation in the cellular environment. Compound **II** appeared to be the most efficient inhibiting cell viability with EC₅₀ ranging from 0.3 μM for RPE1 cells to 9.6 μM using HBL100 breast cancer cells. Only compounds **I** and **II** appeared to affect the viability of highly resistant SH-SY5Y neuroblastoma cells with EC₅₀ of 4.3 μM and 1.2 μM respectively.

Compounds **I**, **II** and **22** were generally the most efficient at inhibiting the viability of transformed RPE-1 cells but active on all other cancerous cell lines tested with EC₅₀ ranging from 1.2 μM for compound **II** using HCT116 and SH-SY5Y to 24.1 μM for compound **22** on HBL100. Overall, these results showed that, compound **I**, **II** and **22** display anti-proliferative activity on several cancer cell lines.

We next tested the ability of compound **22** to inhibit endogenous haspin in cells alongside compounds **I**, **II** and CHR-6494. We treated U-2 OS cells at 5 μM and 2.5 μM of each compound or 0.5 μM of CHR-6494 or 0.1% DMSO for 18 h and proceeded to the quantification of H3T3P haspin specific signal by immunofluorescence (Figure 2). By comparing the H3T3P/DAPI (DNA) signal ratios, we were able to demonstrate that all tested compounds had the ability to inhibit endogenous haspin kinase activity to various extent when compared to the

DMSO control in a dose-dependent manner. Compound **22** showed a strong inhibitory effect when used at 5 μM , which was similar compared to the reference CHR-6494 at 0.5 μM , and inhibited haspin activity signal by 50 % when used at 2.5 μM compared to the DMSO signal. These results showed that compounds **I**, **II** and **22** were able to penetrate the cell plasma membrane as well as locate and inhibit their target haspin in U-2 OS cells at low micromolar concentrations.

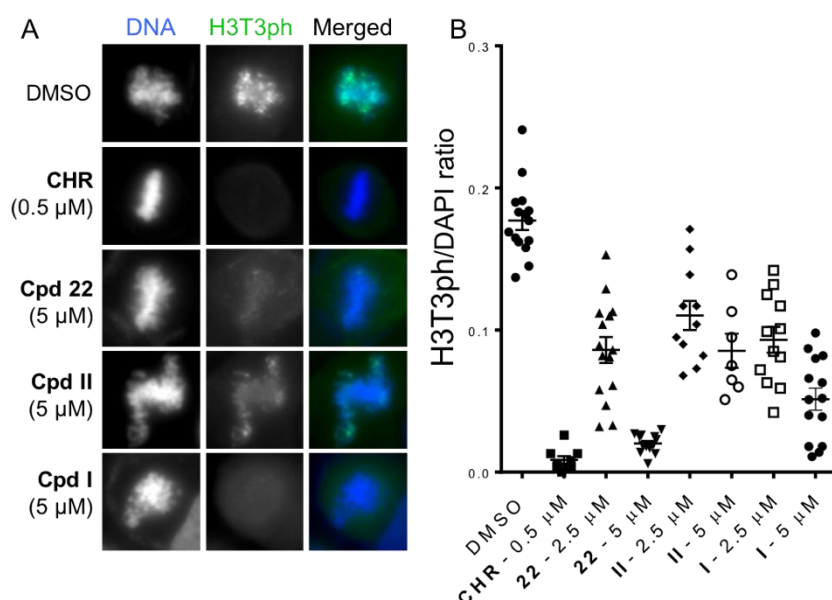


Figure 2. Effect of CHR-6494 and selected compounds (**I**, **II**, **22**) on endogenous haspin kinase activity in U-2 OS cells. U-2 OS cells were treated for 18 h with 0.5 μM of CHR-6494 or 2.5 μM and 5 μM of each compound or 0.1 % of DMSO. (A) Endogenous haspin kinase activity was monitored by immunofluorescence staining of phosphorylated Histone H3 on threonine 3 (H3T3P) and staining of DNA by DAPI; representative images are shown. (B) Both fluorescent signals were quantified in prometaphase/metaphase cells and H3T3P/DAPI ratio is shown on a scatter plot, $n \geq 12$ (apart for compound **II** for which mitotic figures were very rare).

In order to assess the effect of **I**, **II** and **22** on the cell cycle, we treated U-2 OS cells with 2.5 and 5 μM of each compound or 0.5 μM of CHR6494 or 0.1 % of DMSO and measured DNA content by flow cytometry after 24 h (Figure 3). At the concentrations tested for compounds **22** and **II** as well as CHR-6494, we observed minimal influence of these inhibitors on the cell cycle. However, a more pronounced accumulation of cells in the G2/M phase was observed for compound **II** (25% compared to 16% in the control) which was consistent with a M-phase arrest. Strikingly, compound **I** showed a dose-dependent effect, blocking cells in S-phase (61% at 2.5 μM and 78% at 5 μM compared to 39% in DMSO control condition). This marked increase of cells in S-Phase strongly suggested an off-target activity of compound **I** possibly by modulating key DNA replication checkpoints, which however remains to be tested.

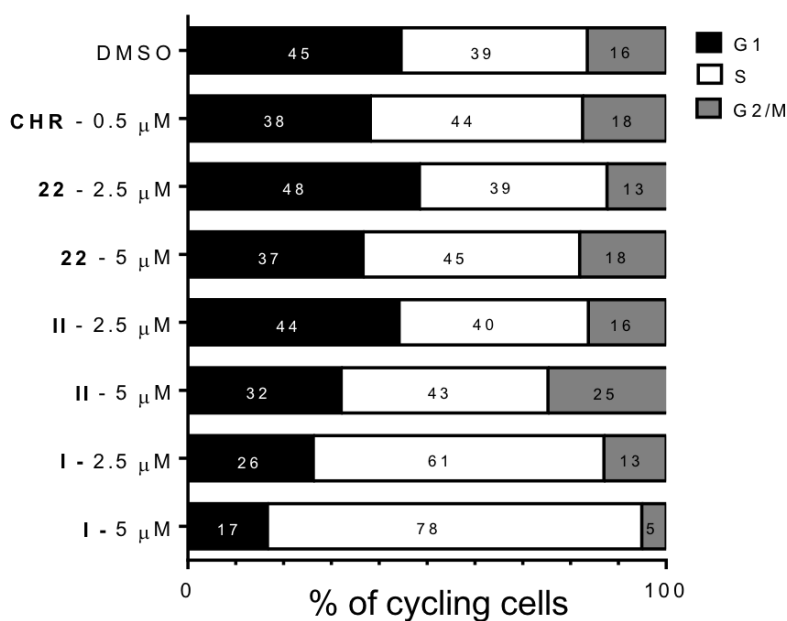


Figure 3. Effects of selected compounds on the cell cycle. U-2 OS cells were treated with 0.5 μ M of CHR-6494 or 2.5 and 5 μ M of each compound for 24 h. DNA content was measured by flow cytometry and quantification of the percentage of cells in each phase of the cell cycle using FCS Express software is represented on a bar graph.

2.3. Structural analysis of the binding mode of compound 22 with Haspin

To elucidate the binding mode to haspin of this series, we conducted a crystallographic analyses of haspin in complex with compound **22** (**MD420**). Crystals obtained diffracted at a resolution of 2.0 Å (Figure 4A). The ligand bound in a canonical type I binding mode, exclusively occupying the ATP binding pocket. This pocket comprises predominantly hydrophobic residues F605, A501, I686, L656, I490, V498 and I557 (Figure 4B), that formed non-polar interactions with the ligand. In addition, the nitrogen atom of the isoquinoline formed a hydrogen bond with

G608 located in the hinge region, and the pyridine moiety was in a position to form a water-mediated hydrogen bond with P-Loop residue G496.

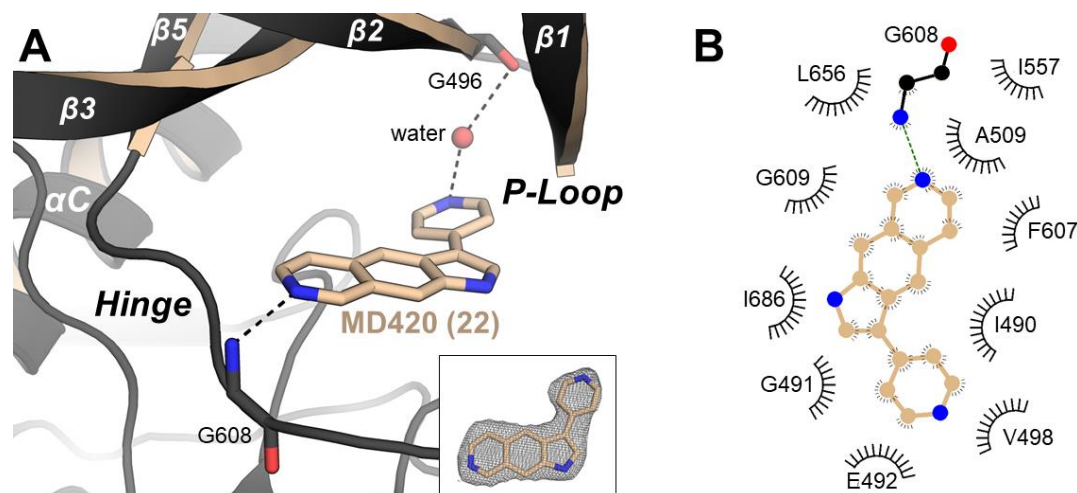


Figure 4: (A) Crystal structure of compound **22** in complex with haspin (pdb code: 8RDK). The protein backbone is shown in cartoon representation (black color) and compound **22** is shown as a stick model (wheat color). Hydrogen bonds are depicted as black dashed lines and water molecules as red spheres. The inset shows a $2F_o - F_c$ electron density map for the inhibitor at a contour level of 1.0σ . (B) Two dimensional representation of the inhibitor interactions with predominantly hydrophobic residues, created with LigPlot⁺ [30].

3. Conclusion

In this study we established a synthetic approach allowing the preparation of diversely substituted pyrrolo[3,2-g]isoquinolines that, apart from analogues with a quinone central core, are very poorly documented in the literature. The results obtained demonstrated that this series represent a new family of haspin inhibitors with different selectivity profiles suggesting that this scaffold could be optimized to inhibit other kinases evaluated by our selectivity panel. This structure-activity relationship revealed that *N*-methylation at the 1-position was not favorable for haspin activity. Conversely, introduction of hydrophobic groups at the 3-position led to potent haspin inhibitors. Main off-targets identified were CLK1, DYRK1A, CDK9 and PIM1 possibly due to structural similarity of their back pockets [31]. Most active compounds exhibited nanomolar potencies. The most potent inhibitors of this series had an potency of 76 nM to 150 nM with robust on-target activity in U-2 OS cells. In addition, selected analogues (**I**, **II** and **22**,) were evaluated in cancer cell proliferation assays and showed anti-proliferative activity. Finally, the binding mode of analogue **22** in haspin was determined using crystallographic methods providing a valuable structural model for further optimization studies of this series.

4. Experimental section

4.1 Chemistry

4.1.1. General.

Starting materials were obtained from commercial suppliers and used without further purification. IR spectra were recorded on a Perkin-Elmer Spectrum 65 or Smart Orbit, Nicolet 5700 thermo electron FT-IR spectrometer (ν in cm^{-1}). NMR spectra, performed on a Bruker AVANCE 400 III HD (^1H : 400 MHz, ^{13}C : 101 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), multiplet (m) and broad signal (br s). Coupling constants are expressed in Hertz. Experiments under microwave irradiation were performed in a sealed tube using a CEM Discover 2.0 Benchmate apparatus. High resolution mass spectra were determined on a high-resolution Waters Micro Q-ToF or Thermo Scientific Q Exactive Q-Orbitrap apparatus (UCA START, Université Clermont Auvergne, Clermont-Ferrand, France). Chromatographic purifications were performed by column chromatography using 40–63 μm silica gel, by preparative TLC using silica gel-coated glass plates 60 F254 from Macherey Nagel, by Interchim XS 520 Plus puriFlash system with 200-800 nm detector and pre-packed IR 50SI cartridges or by Buchi Pure Chromatography system for reverse phase chromatography. 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 were uncorrected.

The purity of all tested compounds was established by HPLC analysis using a VWR Hitachi chromatograph. A Macherey Nagel Nucleodur gravity column (4.6 mm \times 250 mm, 5 μM) was used for all compounds. The flow rate was 0.5 mL/min, and the analysis was performed at 25 $^\circ\text{C}$ for each compound. Solvents were (A) Acetonitrile, (B) Water/0.1% TFA. Three methods were designed: method A was a gradient of 5:95 A/B for 5 min to 95:5 A/B in 20 min and then 95:5 A/B for 10 min, whereas method B was a gradient of 2:98 A/B for 8 min to 95:5 A/B in 25 min and then 95:5 A/B for 20 min, whereas method C was an isocratic mode at 40:60 A/B for 40 min.

4.1.2. 2-bromo-4-methyl-5-nitrobenzaldehyde (2)

To a solution of 2-bromo-4-methyl benzaldehyde **1** (5.00 g, 25.1 mmol) in concentrated sulfuric acid (30 mL) was added dropwise a 70% nitric acid solution (5 mL, 50.2 mmol) at 5 $^\circ\text{C}$. After being stirred for 1 h at a temperature below 15 $^\circ\text{C}$, the reaction mixture was poured into ice/water. The white precipitate formed was filtered off and washed with water several times. The powder was dissolved in EtOAc, dried over MgSO_4 and concentrated under reduced pressure. The solid was triturated in cyclohexane yielding compound **2** (4.80 g, 19.7 mmol, 78%) as a white powder. $R_f = 0.44$ (Cyclohexane/EtOAc 9:1). Mp. 110-111 $^\circ\text{C}$. IR (ATR): 3096, 3052, 2879, 1686, 1513, 1336 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 2.60 (s, 3H), 8.06 (s, 1H), 8.34 (s, 1H), 10.16 (s, 1H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 19.6 (CH_3), 125.8, 138.1 (CH_{arom}), 129.5, 132.1, 140.6, 148.3 (C_{arom}), 189.9 (CHO). HRMS (ESI) calcd for $\text{C}_8\text{H}_5\text{NO}_3^{79}\text{Br}$ (M - H^+) 241.9447, found 241.9451.

4.1.3. 4-methyl-2-(2-(trimethylsilyl)ethynyl)-5-nitrobenzaldehyde (3)

To a solution of compound **2** (380 mg, 1.55 mmol) in TEA (10 mL), under argon atmosphere, were successively added CuI (12 mg, 0.0623 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (22 mg, 0.0311 mmol) and

trimethylsilylacetylene (538 μL , 3.89 mmol). The mixture was stirred at 70 $^{\circ}\text{C}$ for 3 h. The solvent was removed in vacuo. Residue was purified through column chromatography using Cyclohexane/EtOAc (9:1) yielding compound **3** (308 mg, 1.18 mmol, 76%) as a pale brown powder. $R_f = 0.46$ (Cyclohexane/EtOAc 95:5). Mp. 97-98 $^{\circ}\text{C}$. IR (ATR): 2961, 2860, 2161, 1698, 1602, 1520, 1345 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 0.30 (s, 9H), 2.59 (s, 3H), 7.85 (s, 1H), 8.33 (s, 1H), 10.30 (s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ -0.5 (3 CH_3), 19.5 (CH_3), 98.9, 106.1 (C_{alkyne}), 123.6, 137.8 (CH_{arom}), 128.2, 134.6, 139.2, 148.5 (C_{arom}), 189.2 (CHO). HRMS (ESI $^+$) calcd for $\text{C}_{13}\text{H}_{16}\text{NO}_3^{28}\text{Si}$ ($\text{M} + \text{H}$) $^+$ 262.0894, found 262.0893.

4.1.4. 6-methyl-7-nitroisoquinoline (4)

A 10 mL CEM Discover microwave tube was charged with compound **3** (300 mg, 1.15 mmol) and 3 mL of a solution of ammonia in methanol (7 M). The reaction flask was sealed and irradiated for 15 min (Discover mode, Control Type = Standard, P = 75 W, T = 130 $^{\circ}\text{C}$). The solvent was removed under reduced pressure. Residue was purified through column chromatography (Cyclohexane/EtOAc 3:7 to 0:10) yielding the isoquinoline **4** (134 mg, 0.712 mmol, 62%) as a grey powder. $R_f = 0.46$ (Cyclohexane/EtOAc 3:7). Mp. 125-128 $^{\circ}\text{C}$. IR (ATR): 2971, 1519, 1336, 1330 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 2.68 (s, 3H), 7.87 (d, $J = 5.6$ Hz, 1H), 8.07 (s, 1H), 8.66 (d, $J = 5.6$ Hz, 1H), 8.93 (s, 1H), 9.51 (1H, s). ^{13}C NMR (101 MHz, DMSO- d_6) δ 19.9 (CH_3), 119.5, 125.1, 130.0, 145.9, 153.6 (CH_{arom}), 125.8, 133.5, 136.6, 148.2 (C_{arom}). HRMS (ESI $^+$) calcd for $\text{C}_{10}\text{H}_9\text{N}_2\text{O}_2$ ($\text{M} + \text{H}$) $^+$ 189.0659, found 189.0657.

4.1.5. 2-(7-nitroisoquinolin-6-yl)acetonitrile (5)

To a solution of compound **4** (50 mg, 0.266 mmol) in DMF (0.35 mL) under argon were successively added pyrrolidine (33 μL , 0.399 mmol) and *N,N*-Dimethylformamide di-*tert*-butyl acetal (127 μL , 0.531 mmol). After stirring for 3 h at 110 $^{\circ}\text{C}$, the red mixture was poured into water (50 mL) and extracted with EtOAc (7 x 20 mL). The combined organic layers were washed with water (8 x 20 mL) and brine, dried over MgSO_4 and concentrated under reduced pressure. The crude redish glassy solid (72 mg) was used in the next step without any further purification. To a solution of the crude enamine in acetic acid (1.5 mL) was added hydroxylamine hydrochloride (24 mg, 0.345 mmol). After stirring for 3 h at reflux, the reaction mixture was diluted with ice (10 mL) before adding an aqueous solution of NaOH (1M) until pH = 8. The mixture was then extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over MgSO_4 and concentrated under reduced pressure yielding compound **5** (38 mg, 0.178 mmol, 67% over 2 steps) as a pale brown powder. $R_f = 0.45$ (Cyclohexane/EtOAc 3:7). Mp. 174-175 $^{\circ}\text{C}$. IR (ATR): 2973, 2200, 1519, 1335, 1330, 1245 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 4.54 (s, 2H), 8.06 (d, $J = 5.6$ Hz, 1H), 8.31 (s, 1H), 8.76 (d, $J = 5.6$ Hz, 1H), 9.14 (s, 1H), 9.62 (s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 22.2 (CH_2), 117.9 (CN), 120.1, 126.9, 130.0, 146.7, 154.0 (CH_{arom}), 126.3, 127.3, 136.8, 146.2 (C_{arom}). HRMS (ESI $^-$) calcd for $\text{C}_{11}\text{H}_6\text{N}_3\text{O}_2$ ($\text{M} - \text{H}$) $^+$ 212.0455, found 212.0455.

4.1.6. 2-(7-aminoisoquinolin-6-yl)acetonitrile (6)

To a solution of compound **5** (50 mg, 0.235 mmol) in a mixture of DCM/MeOH 3:2 (1 mL) was added Pd/C (9 mg). The mixture was stirred under 1 atm. of H_2 for 1 h at rt. and filtered through Celite $^{\circledR}$. The Celite $^{\circledR}$ pad was washed several times with a mixture of DCM/MeOH 1:1. Combined filtrates were concentrated under reduced pressure yielding compound **6** (43 mg, 0.235 mmol, quant.) as a pale brown powder without purification. $R_f = 0.20$ (Cyclohexane/

EtOAc 3:7). Mp. 117-118 °C. IR (ATR): 3333, 3201, 2921, 2248, 1655, 1504, 1402, 1275 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 4.05 (s, 2H), 5.69 (s, 2H), 7.12 (s, 1H), 7.60 (d, $J = 5.6$ Hz, 1H), 7.77 (s, 1H), 8.15 (d, $J = 5.6$ Hz, 1H), 8.97 (s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 19.9 (CH_2), 118.2 (CN), 106.4, 119.8, 126.3, 138.9, 149.5 (CH_{arom}), 123.6, 127.9, 129.7, 145.7 (C_{arom}). HRMS (ESI $^+$) calcd for $\text{C}_{11}\text{H}_{10}\text{N}_3$ ($\text{M} + \text{H}^+$) 184.0869, found 184.0865.

4.1.7. 1H-pyrrolo[3,2-g]isoquinolin-2-amine hydrochloride (7)

A 10 mL CEM Discover microwave tube was charged with compound **6** (50 mg, 0.273 mmol) and a 4 N HCl solution in 1,4-dioxane (3 mL). The reaction flask was sealed and irradiated for 1 h (Control Type = Dynamic, P = 200 W, T = 110 °C) and again for 3 h (Control Type = Dynamic, P = 200 W, T = 130 °C). The solvent was removed under reduced pressure yielding compound **7** (60 mg, 0.273 mmol, quant.) as a pale rose powder. $R_f = 0$ (EtOAc). Mp. > 250 °C. IR (ATR): 3105, 3037, 2937, 1704, 1441, 1428, 1120 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 5.62 (s, 1H), 7.14 (br s, 2H), 7.55 (s, 1H), 7.89 (d, $J = 5.6$ Hz, 1H), 7.93 (s, 1H), 8.01 (d, $J = 5.6$ Hz, 1H), 9.29 (s, 1H), 11.24 (s, 1H), 14.12 (s, 1H). ^{13}C NMR (101 MHz, TFA- d_1) δ 113.0, 126.5, 128.4, 133.0, 147.8 (CH_{arom}), 130.7, 140.1, 140.5, 146.6, 174.1 (C_{arom}). One CH_{arom} peak could not be observed. HRMS (ESI $^+$) calcd for $\text{C}_{11}\text{H}_{10}\text{N}_3$ ($\text{M} + \text{H}^+$) 184.0869, found 184.0866. HPLC method A: purity > 98%, $\lambda = 240$ nm, $t_R = 12.4$ min.

4.1.8. 1H-pyrrolo[3,2-g]isoquinoline (8)

To a solution of compound **4** (400 mg, 2.13 mmol) in dry DMF (2.4 mL) under argon were successively added pyrrolidine (350 μL , 4.25 mmol) and *N,N*-Dimethylformamide di-*tert*-butyl acetal (1.02 mL, 4.25 mmol). After stirring for 3 h at 110 °C, the red mixture was poured into water (100 mL) and extracted with EtOAc (7 x 60 mL). The combined organic layers were washed with water (8 x 60 mL) and brine, dried over MgSO_4 and concentrated under reduced pressure. The crude redish glassy solid (420 mg) was used in the next step without any further purification. To a solution of the crude enamine in EtOH (6.8 mL) was added Pd/C (40 mg). The mixture was stirred under 1 atm. of H_2 for 36 h at rt., and filtered through Celite $^{\text{®}}$. The Celite $^{\text{®}}$ pad was washed several times with EtOH then with MeOH. The residue was purified through column chromatography using DCM/MeOH (97:3) as eluant, yielding the pure compound **8** (144 mg, 0.856 mmol, 40% over 2 steps) as a white powder. $R_f = 0.28$ (DCM/MeOH 95:5). Mp. 154-156 °C. IR (ATR): 3253-2354, 1600, 1430, 1281 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 6.67 (s, 1H), 7.77 (d, $J = 6.0$ Hz, 1H), 7.82 (t, $J = 2.8$ Hz, 1H), 8.11 (s, 1H), 8.13 (s, 1H), 8.20 (d, $J = 6.0$ Hz, 1H), 9.33 (s, 1H), 11.48 (br s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 100.5, 107.1, 115.3, 120.1, 132.5, 138.1, 152.6 (CH_{arom}), 124.9, 128.7, 132.7, 136.8 (C_{arom}). HRMS (ESI $^+$) calcd for $\text{C}_{11}\text{H}_9\text{N}_2$ ($\text{M} + \text{H}^+$) 169.0760, found 169.0758. HPLC method A: purity > 98%, $\lambda = 240$ nm, $t_R = 18.7$ min.

4.1.9. General procedure for synthesis of compounds 9, 10, 11

To a solution of compound **8** in ACN was added the corresponding *N*-halogenosuccinimide (NBS, NIS or NCS) at rt. The mixture was stirred at rt for the appropriate time. After completion of the reaction, the mixture was neutralized with a saturated solution of NaHCO_3 then extracted with EtOAc. The combined organic layers were washed with brine (20 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. Residue was purified by chromatography.

3-bromo-1H-pyrrolo[3,2-g]isoquinoline (9)

Compound **8** (36 mg, 0.214 mmol), NBS (38 mg, 0.214 mmol), ACN (3 mL), rt. 19 h. Purification by column chromatography (DCM/MeOH 95:5) yielded compound **9** (52 mg, 0.210 mmol, 98%) as a pale brown powder. $R_f = 0.53$ (DCM/MeOH 94:6). Mp. > 225 °C (decomp.). IR (ATR): 3113-2373, 1603, 1429, 1278, 643 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 7.89 (d, $J = 6.0$ Hz, 1H), 8.03 (s, 1H), 8.05 (d, $J = 2.4$ Hz, 1H), 8.18 (s, 1H), 8.26 (d, $J = 6.0$ Hz, 1H), 9.40 (s, 1H), 11.88 (br s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 88.0 (C-Br), 108.4, 113.8, 121.0, 131.8, 138.8, 152.9 (CH_{arom}), 125.6, 128.9, 130.8, 135.9 (C_{arom}). HRMS (ESI $^+$) calcd for $\text{C}_{11}\text{H}_8^{79}\text{BrN}_2$ ($\text{M} + \text{H}^+$) 246.9865, found 246.9863. HPLC method A: purity > 99%, $\lambda = 240$ nm, $t_{\text{R}} = 20.4$ min.

3-iodo-1H-pyrrolo[3,2-g]isoquinoline (10)

Compound **8** (50 mg, 0.297 mmol), NIS (67 mg, 0.297 mmol), ACN (4 mL), rt. 18 h. Purification by Puriflash chromatography (DCM/MeOH 98:2 to 90:10) yielded compound **10** (75 mg, 0.255 mmol, 86%) as a pale yellow powder. $R_f = 0.53$ (DCM/MeOH 94:6). Mp. > 163 °C (decomp.). IR (ATR): 3118-2227, 1604, 1428, 1275 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 7.891 (d, $J = 6.0$ Hz, 1H), 7.901 (s, 1H), 8.04 (d, $J = 2.4$ Hz, 1H), 8.16 (s, 1H), 8.26 (d, $J = 6.0$ Hz, 1H), 9.39 (s, 1H), 11.93 (br s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 55.3 (C-I), 108.1, 115.6, 120.2, 136.8, 138.7, 152.7 (CH_{arom}), 125.3, 129.1, 134.1, 136.4 (C_{arom}). HRMS (ESI $^+$) calcd for $\text{C}_{11}\text{H}_8\text{IN}_2$ ($\text{M} + \text{H}^+$) 294.9728, found 294.9727. HPLC method A: purity > 99%, $\lambda = 240$ nm, $t_{\text{R}} = 20.4$ min.

3-chloro-1H-pyrrolo[3,2-g]isoquinoline (11)

Compound **8** (50 mg, 0.297 mmol), NCS (40 mg, 0.297 mmol), ACN (1 mL), rt. 36 h. Purification by column chromatography (DCM/MeOH 95:5) yielded compound **11** (36 mg, 0.178 mmol, 60%) as a brown powder. $R_f = 0.53$ (DCM/MeOH 94:6). Mp. > 233 °C (decomp.); IR (ATR): 3114-2376, 1602, 1443, 1221, 756 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 7.88 (d, $J = 6.0$ Hz, 1H), 8.02 (d, $J = 2.4$ Hz, 1H), 8.10 (s, 1H), 8.18 (s, 1H), 8.26 (d, $J = 6.0$ Hz, 1H), 9.40 (s, 1H), 11.77 (br s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 102.5 (C-Cl), 108.5, 112.9, 120.2, 129.5, 138.8, 152.9 (CH_{arom}), 125.2, 128.9, 129.3, 135.5 (C_{arom}). HRMS (ESI $^+$) calcd for $\text{C}_{11}\text{H}_8^{35}\text{ClN}_2$ ($\text{M} + \text{H}^+$) 203.0371, found 203.0367. HPLC method A: purity > 97%, $\lambda = 240$ nm, $t_{\text{R}} = 20.2$ min.

4.1.10. 1H-pyrrolo[3,2-g]isoquinoline-3-carbaldehyde (12)

To a solution of compound **8** (352 mg, 2.09 mmol) in 3.15 mL of a mixture of AcOH/H $_2$ O (1:2) was added hexamethylenetetramine (323 mg, 2.30 mmol). The mixture was stirred for 18 h at 120 °C. After cooling, the reaction mixture was basified until pH = 8 with an aqueous saturated solution of NaHCO $_3$ then extracted with EtOAc (8 x 20 mL). The combined organic layers were dried over MgSO $_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM/ MeOH 98:2 to 90:10) yielding compound **12** (270 mg, 1.38 mmol, 66%) as a pale yellow powder. $R_f = 0.45$ (DCM/MeOH 94:6). Mp. > 220 °C (decomp.). IR (ATR): 2848-2123, 1650, 1426, 1370, 1271 cm^{-1} . ^1H NMR (400 MHz, DMSO- d_6) δ 7.93 (d, $J = 6.0$ Hz, 1H), 8.26 (s, 1H), 8.33 (d, $J = 6.0$ Hz, 1H), 8.71 (s, 1H), 8.75 (s, 1H), 9.43 (s, 1H), 10.03 (s, 1H), 12.55 (br s, 1H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 109.0, 117.11, 120.5, 139.7, 145.2, 152.9 (CH_{arom}), 117.05, 125.7, 128.2, 130.9, 137.4 (C_{arom}), 184.7 (CHO). HRMS (ESI $^-$) calcd for $\text{C}_{12}\text{H}_7\text{N}_2\text{O}$ ($\text{M} - \text{H}^+$) 195.0553, found 195.0552. HPLC method A: purity > 97%, $\lambda = 240$ nm, $t_{\text{R}} = 17.8$ min.

4.1.11. (1*H*-pyrrolo[3,2-*g*]isoquinolin-3-yl)methanol (**13**)

To a suspension of **12** (20 mg, 0.102 mmol) in MeOH (0.5 mL) at 0 °C under Argon was added NaBH₄ (12 mg, 0.306 mmol). The mixture was stirred at 0 °C for 1 h. After completion of the reaction, a saturated aqueous solution of NH₄Cl was added at 0 °C and allowed to stir again for 30 minutes at rt. A saturated aqueous solution of NaHCO₃ was added and the aqueous layer was extracted with EtOAc (4 x 20 mL). Combined organic layers were dried over MgSO₄ and then evaporated under reduced pressure. Residue was purified by column chromatography (DCM/MeOH 95:5) yielding the compound **13** (15 mg, 0.076 mmol, 74%) as a pale yellow powder. *R*_f = 0.32 (DCM/MeOH 93:7). Mp. >180 °C (decomp.). IR (ATR): 3394, 3298-2481, 1604, 1571, 1435, 1366, 991 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.77 (d, *J* = 5.2 Hz, 2H), 4.91 (t, *J* = 5.4 Hz, 1H), 7.70 (d, *J* = 2.0 Hz, 1H), 7.80 (d, *J* = 5.6 Hz, 1H), 8.06 (s, 1H), 8.17 (s, 1H), 8.20 (d, *J* = 5.6 Hz, 1H), 9.33 (s, 1H), 11.28 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 55.3 (CH₂), 107.0, 114.3, 121.5, 130.3, 138.1, 152.5 (CH_{arom}), 115.3, 124.6, 128.4, 131.8, 137.4 (C_{arom}). HRMS (ESI⁺) calcd for C₁₂H₁₁N₂O (M + H⁺) 199.0871, found 199.0862. HPLC method A: purity > 98%, λ = 240 nm, t_R = 17.7 min.

4.1.12. (*Z/E*)-1*H*-pyrrolo[3,2-*g*]isoquinoline-3-carbaldehyde oxime (**14**)

To a solution of compound **12** (105 mg, 0.535 mmol) in 3.3 mL of a mixture MeOH /H₂O (10:1) were added hydroxylamine hydrochloride (112 mg, 1.61 mmol) and sodium acetate (132 mg, 1.61 mmol). The mixture was stirred at rt. for 3 h. The solvent was then removed under reduced pressure. Residue was purified by chromatography (DCM/MeOH 95:5 to 90:10) yielding a mixture of *Z*- and *E*- isomers of compound **14** (112 mg, 0.530 mmol, 99%) as an orange powder. *R*_f = 0.39 (DCM/MeOH 94:6). IR (ATR): 3500-2950, 1599, 1267, 1229, 1180 cm⁻¹. (*E*)-isomer: ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.81 (d, *J* = 6.0 Hz, 1H), 8.09 (d, *J* = 2.4 Hz, 1H), 8.15 (s, 1H), 8.26 (d, *J* = 6.0 Hz, 1H), 8.38 (s, 1H), 8.55 (s, 1H), 9.38 (s, 1H), 10.70 (br s, 1H), 11.82 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 108.1, 117.6, 120.7, 135.4, 138.9, 144.5 (CH_{arom}), 109.0, 125.5, 129.1, 129.7, 137.7 (C_{arom}), 153.1 (C_{NOH}). (*Z*)-isomer: ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.81 (d, *J* = 6.0 Hz, 1H), 7.97 (s, 1H), 8.19 (s, 1H), 8.27 (d, *J* = 6.0 Hz, 1H), 8.48 (s, 1H), 8.67 (d, *J* = 2.4 Hz, 1H), 9.39 (s, 1H), 11.40 (br s, 1H), 12.07 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 108.2, 114.1, 120.5, 137.4, 138.0, 138.8 (CH_{arom}), 105.6, 125.2, 129.4, 131.2, 135.8 (C_{arom}), 152.9 (C_{NOH}). HRMS (ESI⁺) calcd for C₁₂H₁₀N₃O (M + H⁺) 212.0818, found 212.0818. HPLC method C: purity > 95%, λ = 240 nm, t_R = 4.2 min.

4.1.13. 1*H*-pyrrolo[3,2-*g*]isoquinoline-3-carbonitrile (**15**)

POCl₃ (27 μL, 0.284 mmol) was added dropwise in DMF (88 μL, 1.136 mmol) keeping the temperature between 10 °C and 20 °C and the mixture was stirred for 30 minutes at a temperature below 20 °C. Then a solution of compound **14** (60 mg, 0.284 mmol) in 0.5 mL of DMF was added dropwise keeping the temperature between 0 °C and 5 °C. The mixture was stirred for 3 h 30. After completion of reaction, the mixture was poured into crushed ice and then extracted with EtOAc (3 x 20 mL). Combined organic layers were dried over MgSO₄ and evaporated under reduced pressure. Residue was purified by two successive chromatography columns (first one with DCM/MeOH 95:5 and the second one with diethyl ether/MeOH 95:5 mixture) yielding the compound **15** (13 mg, 0.067 mmol, 23%) as a pale yellow powder. *R*_f = 0.33 (DCM/MeOH 95:5). Mp. > 220 °C (decomp.). IR (ATR): 3196, 2162, 1598, 1506, 1325, 1270 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.93 (d, *J* = 6.0 Hz, 1H), 8.29 (s, 1H), 8.33 (s, 1H), 8.34 (d, *J* = 6.0 Hz, 1H), 8.71 (s, 1H), 9.45 (s, 1H), 12.61 (br s, 1H). ¹³C NMR (101 MHz,

DMSO-*d*₆) δ 109.8, 114.6, 120.1, 139.7, 141.4, 153.1 (CH_{arom}), 83.6, 116.0, 130.0, 130.8, 135.5 (C_{arom}), 125.5 (CN). HRMS (ESI⁺) calcd for C₁₂H₈N₃ (M + H⁺) 194.0713, found 194.0711. HPLC method A: purity > 95%, λ = 240 nm, t_R = 18.7 min.

4.1.14. (1*H*-pyrrolo[3,2-*g*]isoquinolin-3-yl)methanamine Trifluoroacetic Acid Salt (16)

To a solution of compound **14** (40 mg, 0.189 mmol, 1.0 eq.) in 0.5 mL of a 7 N NH₃ solution in MeOH was added Raney Nickel (10 mg) under argon. The mixture was stirred at rt. for 18 h. After completion of the reaction, the solvent was removed under reduce pressure. The residue was purified by preparative HPLC (ACN/H₂O+0.1% TFA 2:98 to 95:5) yielding compound **16** (13 mg, 0.042 mmol, 22%) as a yellow powder. R_f = 0 (DCM/MeOH 94:6). Mp. > 185 °C (decomp.). IR (ATR): 3306-3000, 2936, 1659, 1442, 1178, 1114 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.40 (m, 2H), 8.18 (br s, 3H), 8.25 (d, *J* = 2.4 Hz, 1H), 8.34-8.39 (m, 2H), 8.65 (s, 1H), 8.66 (s, 1H), 9.92 (s, 1H), 12.33 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 33.4 (CH₂), 112.2, 115.6, 124.2, 126.9, 137.5, 148.1 (CH_{arom}), 107.9, 122.2, 129.7, 135.3, 137.9 (C_{arom}). HRMS (ESI⁺) calcd for C₁₂H₁₂N₃ (M + H⁺) 198.1026, found 198.1020. HPLC method B: purity > 96%, λ = 255 nm, t_R = 6.7 min.

4.1.15. General procedure for the synthesis of compounds 17a-d:

To a suspension of **12** in EtOH or MeOH under argon atmosphere was added a solution of corresponding amine. The mixture was stirred overnight at rt. Then NaBH₄ was added at 0 °C and the mixture was stirred for additional 24 h at rt. After completion of the reaction 1 mL of MeOH was added and the solvent was removed under reduced pressure. Residue was purified by reverse phase flash chromatography (H₂O/MeOH 5:95 to 95:5, 4 g column) and trituration with DCM. The fractions containing the desired product were acidified with a 1 M aqueous solution of HCl (3 mL) before concentration to dryness.

N-methyl-1-(1*H*-pyrrolo[3,2-*g*]isoquinolin-3-yl)methanamine dihydrochloride (17a)

Compound **12** (50 mg, 0.255 mmol), methylamine (33% w/w in EtOH, 0.765 mmol, 95 μ L), EtOH (1 mL), NaBH₄ (29 mg, 0.765 mmol). Purification by reverse phase flash chromatography (H₂O/MeOH 5:95 to 95:5) then acidification afforded the compound **17a** (33 mg, 0.116 mmol, 46%) as a yellow powder. R_f = 0 (DCM/MeOH 94 :6). Mp. > 216 °C (decomp.). IR (ATR): 3499-2485, 1641,1564, 1440, 1378, 1250, 1116 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.58 (t, *J* = 5.6 Hz, 3H), 4.47 (t, *J* = 5.2 Hz, 2H), 8.31-8.42 (m, 3H), 8.72 (s, 1H), 8.77 (s, 1H), 9.26 (br s, 2H), 9.98 (s, 1H), 12.58 (br s, 1H), 15.77 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 31.6 (CH₃), 41.8 (CH₂), 112.3, 115.8, 124.3, 126.1, 138.8, 147.4 (CH_{arom}), 105.6, 122.0, 129.7, 135.7, 137.8 (C_{arom}). HRMS (ESI⁺) calcd for C₁₃H₁₄N₃ (M + H⁺) 212.1182, found 212.1177. HPLC method B: purity > 99%, λ = 240 nm, t_R = 18.0 min.

N-((1*H*-pyrrolo[3,2-*g*]isoquinolin-3-yl)methyl)ethanamine dihydrochloride (17b)

Compound **12** (50 mg, 0.255 mmol), ethylamine (2M in MeOH, 1.27 mL, 2.55 mmol), MeOH (1 mL), NaBH₄ (29 mg, 0.765 mmol). Purification by reverse phase flash chromatography (H₂O/MeOH 5:95 to 95:5) then acidification afforded the compound **17b** (57 mg, 0.191 mmol, 75%) as a yellow powder. R_f = 0 (DCM/MeOH 94:6). Mp. > 226 °C (decomp.). IR (ATR): 3507-2485, 1639, 1564, 1440, 1378, 1302, 1121 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.26 (t, *J* = 7.2 Hz, 3H), 2.91-3.10 (m, 2H), 4.47 (t, *J* = 5.2 Hz, 2H), 8.35-8.42 (m, 3H), 8.74 (s, 1H), 8.79 (s, 1H), 9.31 (br s, 2H), 10.00 (s, 1H), 12.61 (br s, 1H), 15.82 (br s, 1H). ¹³C NMR (101

MHz, DMSO-*d*₆) δ 11.0 (CH₃), 1 CH₂ under solvent signal, 41.3 (CH₂), 112.3, 115.8, 124.3, 126.1, 138.8, 147.5 (CH_{arom}), 105.8, 122.0, 129.7, 135.8, 137.8 (C_{arom}). HRMS (ESI⁺) calcd for C₁₄H₁₆N₃ (M + H⁺) 226.1339, found 226.1334. HPLC method B: purity > 99%, λ = 240 nm, *t*_R = 18.0 min.

***N,N*-dimethyl-1-(1*H*-pyrrolo[3,2-*g*]isoquinolin-3-yl)methanamine dihydrochloride (17c)**

Compound **12** (50 mg, 0.255 mmol), dimethylamine (2M in MeOH, 2.55 mmol, 1.28 mL), MeOH (1 mL), NaBH₄ (29 mg, 0.765 mmol). Purification by reverse phase flash chromatography (H₂O/MeOH 5:95 to 95:5) then acidification afforded the compound **17c** (60 mg, 0.201 mmol, 79%) as a yellow powder. *R*_f = 0 (DCM/MeOH 94:6). Mp. > 220 °C (decomp.). IR (ATR): 3463-2397, 1640,1569, 1441, 1320, 1272, 1124 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.76 (d, *J* = 4.0 Hz, 6H), 4.63 (d, *J* = 3.2 Hz, 2H), 8.33-8.39 (m, 2H), 8.43 (d, *J* = 2.8 Hz, 1H), 8.75 (s, 1H), 8.80 (s, 1H), 9.99 (s, 1H), 10.74 (br s, 1H), 12.70 (br s, 1H), 15.77 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 41.0 (2 CH₃), 50.0 (CH₂), 112.5, 115.7, 124.3, 126.3, 140.0, 147.5 (CH_{arom}), 103.7, 122.1, 130.0, 136.4, 137.8 (C_{arom}). HRMS (ESI⁺) calcd for C₁₄H₁₇N₃ (M + 2 H⁺) 113.5706, found 113.5703. HPLC method B: purity > 99%, λ = 240 nm, *t*_R = 18.0 min.

***N*-((1*H*-pyrrolo[3,2-*g*]isoquinolin-3-yl)methyl)-*N*-ethylethanamine dihydrochloride (17d)**

Compound **12** (50 mg, 0.255 mmol), diethylamine (264 μ L, 2.55 mmol), MeOH (2 mL), NaBH₄ (29 mg, 0.765 mmol). Purification by reverse phase flash chromatography (H₂O/MeOH 5:95 to 95:5) then acidification and trituration with DCM afforded the compound **17d** (14 mg, 0.043 mmol, 17%) as a yellow powder. *R*_f = 0 (DCM/MeOH 94:6). Mp. > 176 °C (decomp.). IR (ATR): 3483-2501, 1624,1572, 1441, 1381, 1029 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.32 (t, *J* = 7.2 Hz, 6H), 3.11-3.14 (m, 4H), 4.65 (d, *J* = 4.4 Hz, 2H), 8.38 (s, 2H), 8.49 (s, 1H), 8.73 (s, 1H), 8.76 (s, 1H), 9.97 (s, 1H), 10.35 (br s, 1H), 12.68 (br s, 1H), 15.65 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 8.6 (2 CH₃), 45.4 (2 CH₂), 45.8 (CH₂), 112.5, 115.6, 124.4, 126.3, 140.0, 147.5 (CH_{arom}), 103.5, 122.1, 130.0, 136.3, 137.8 (C_{arom}). HRMS (ESI⁺) calcd for C₁₆H₂₁N₃ (M + 2 H⁺) 127.5862, found 127.5863. HPLC method B: purity > 98%, λ = 240 nm, *t*_R = 17.3 min.

4.1.16. 1-methyl-1*H*-pyrrolo[3,2-*g*]isoquinoline (18)

To a solution of compound **8** (30 mg, 0.178 mmol) in THF (6 mL) at 0 °C under argon was added NaH 60% dispersion in mineral oil (9 mg, 0.232 mmol). The solution was stirred for 10 minutes at 0 °C before adding the iodomethane (14 μ L, 0.223 mmol). The mixture was then stirred at rt. for 45 minutes in the dark. A saturated aqueous solution of NH₄Cl was added to the mixture. The THF was removed under reduce pressure and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtered and evaporated under reduced pressure. Residue was purified by column chromatography (DCM/MeOH 96:4) yielding compound **18** (23 mg, 0.126 mmol, 71%) as a white powder. *R*_f = 0.57 (DCM/MeOH 94:6). Mp. 150-153 °C. IR (ATR): 3093, 2924, 1592, 1520, 1455, 1367, 1269, 1083 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.94 (s, 3H), 6.68 (d, *J* = 3.2 Hz, 1H), 7.80 (d, *J* = 3.2 Hz, 1H), 7.81 (br s, 1H), 8.15 (s, 1H), 8.17 (s, 1H), 8.24 (br s, 1H), 9.36 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 32.8 (CH₃), 99.8, 105.6, 115.9, 120.3, 136.5, 138.4, 152.6 (CH_{arom}), 125.0, 128.6, 132.9, 137.4 (C_{arom}). HRMS (ESI⁺) calcd for

C₁₂H₁₁N₂ (M + H⁺) 183.0917, found 183.0917. HPLC method A: purity > 95%, λ = 240 nm, t_R = 19.5 min.

4.1.17. 3-iodo-1-methyl-1*H*-pyrrolo[3,2-*g*]isoquinoline (19)

N-iodosuccinimide (25 mg, 0.110 mmol) was added in one portion to a solution of **18** (20 mg, 0.110 mmol) in ACN (2 mL) at rt. The mixture was stirred for 18 h at rt. Then an aqueous saturated solution of NaHCO₃ was added and the mixture was extracted with DCM (2 x 20 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtered and evaporated under reduced pressure. Residue was purified by column chromatography (DCM/MeOH 98:2). The residue was then taken up in DCM (10 mL) and washed with 10 mL of an aqueous solution of HCl (1 N) to remove succinimide, basified with an aqueous saturated solution of NaHCO₃ (10 mL) and extracted with DCM (2 x 10 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtered and evaporated under reduced pressure and triturated with pentane yielding compound **19** (14 mg, 0.045 mmol, 41%) as a white powder. R_f = 0.48 (DCM/MeOH 95:5). Mp. > 115 °C (decomp.). IR (ATR): 3101, 2924, 1600, 1511, 1450, 1410, 1322, 1157, 1089 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.96 (s, 3H), 7.91 (s, 1H), 7.92 (d, *J* = 6.0 Hz, 1H), 8.03 (s, 1H), 8.25 (s, 1H), 8.28 (d, *J* = 6.0 Hz, 1H), 9.41 (s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 33.1 (CH₃), 53.9 (C-I), 106.6, 116.2, 120.2, 138.9, 140.2, 152.7 (CH_{arom}), 125.1, 129.0, 134.3, 137.2 (C_{arom}). HRMS (ESI⁺) calcd for C₁₂H₁₀IN₂ (M + H⁺) 308.9883, found 308.9882. HPLC method A: purity > 95%, λ = 240 nm, t_R = 21.0 min.

4.1.18. 1-(*tert*-Butoxycarbonyl)-3-iodo-1*H*-pyrrolo[3,2-*g*]isoquinoline (20)

To a solution of compound **8** (200 mg, 1.189 mmol) in DMF (3 mL) were added freshly crushed KOH pellets (166 mg, 2.973 mmol) and I₂ (302 mg, 1.189 mmol). The mixture was stirred for 1 h at rt. then quenched with an aqueous saturated solution of Na₂S₂O₃ and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water (5 x 20 mL), brine (20 mL), dried over MgSO₄, filtered and evaporated under reduced pressure. Residue was suspended in DCM (6 mL) before adding TEA (248 μL, 1.784 mmol), DMAP (14 mg, 0.119 mmol) and Boc₂O (285 mg, 1.31 mmol). The mixture was stirred for 1 h at rt. Then the solution was diluted with water and extracted with EtOAc. The combined organic layers were washed with a saturated solution of NaCl (20 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM/MeOH 98:2 to 92:8) yielding compound **20** (304 mg, 0.771 mmol, 65%) as a white powder. R_f = 0.63 (DCM/MeOH 96:4). Mp. > 120 °C (decomp.). IR (ATR): 3169, 2984, 1729, 1366, 1341, 1221, 1150 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.67 (s, 9H), 8.01 (s, 1H), 8.02 (d, *J* = 5.2 Hz, 1H), 8.24 (s, 1H), 8.45 (d, *J* = 5.2 Hz, 1H), 8.81 (s, 1H), 9.51 (s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 27.7 (3CH₃), 66.6 (C-I), 84.7 (C), 112.2, 118.1, 120.9, 135.5, 141.0, 153.3 (CH_{arom}), 127.4, 131.2, 133.9, 135.6 (C_{arom}), 148.0 (C=O). HRMS (ESI⁺) calcd for C₁₆H₁₆IN₂O₂ (M + H⁺) 395.0251, found 395.0239.

4.1.19. General procedure for the synthesis of compounds **21**, **22**, **23**:

A 10 mL CEM Discover microwave tube was charged with compound **20** (80 mg, 0.203 mmol), the corresponding boronic acid (1.6 eq.), Na₂CO₃ (65 mg, 0.609 mmol), Pd(PPh₃)₄ (5 mg, 0.00406 mmol) and 2.5 mL of a mixture of DMF/H₂O (4:1) under argon. The reaction flask was sealed and irradiated for 30 min (Control Type = Dynamic, P = 175 W, T = 120 °C). The

mixture was filtered on Celite[®] then washed with EtOAc and water. The aqueous layer was extracted twice with EtOAc and the combined organic layers were washed with water (5 x 20 mL) and brine before being dried over MgSO₄, filtered and evaporated under reduced pressure. Residue was purified by column chromatography (DCM/MeOH 98:2 to 90:10).

3-phenyl-1H-pyrrolo[3,2-g]isoquinoline (21), obtained as a yellow powder (18 mg, 0.074 mmol, 36%). $R_f = 0.53$ (DCM/MeOH 94:6). Mp. > 230 °C (decomp.). IR (ATR): 3173-2405, 1604, 1439, 1246 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.30 (t, $J = 7.6$ Hz, 1H), 7.50 (t, $J = 7.6$ Hz, 2H), 7.83 (d, $J = 7.6$ Hz, 2H), 7.88 (d, $J = 7.6$ Hz, 1H), 8.17 (s, 1H), 8.18 (d, $J = 3.6$ Hz, 1H), 8.24 (d, $J = 5.6$ Hz, 1H), 8.47 (s, 1H), 9.38 (s, 1H), 11.80 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 107.7, 114.7, 120.5, 125.7, 126.7 (2CH), 128.9 (2CH), 130.5, 138.5, 152.6 (CH_{arom}), 114.8, 124.9, 129.2, 129.9, 135.1, 137.7 (C_{arom}). HRMS (ESI⁺) calcd for C₁₇H₁₃N₂ (M + H⁺) 245.1073, found 245.1069. HPLC method A: purity > 95%, λ = 240 nm, t_R = 21.8 min.

3-(pyridin-4-yl)-1H-pyrrolo[3,2-g]isoquinoline (22), obtained as a yellow powder (12 mg, 0.049 mmol, 24%). $R_f = 0.43$ (DCM/MeOH 94:6). Mp. > 142 °C (decomp.). IR (ATR): 3201-2336, 1595, 1431, 1272 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.90 (d, $J = 6.0$ Hz, 2H), 7.94 (d, $J = 6.0$ Hz, 1H), 8.25 (s, 1H), 8.29 (d, $J = 6.0$ Hz, 1H), 8.56 (d, $J = 2.8$ Hz, 1H), 8.61 (d, $J = 6.0$ Hz, 2H), 8.67 (s, 1H), 9.43 (s, 1H), 12.17 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 108.5, 115.3, 120.5 (2CH), 120.7, 133.3, 138.4, 149.8 (2CH), 152.5 (CH_{arom}), 111.8, 124.8, 129.4, 129.7, 137.8, 142.7 (C_{arom}). HRMS (ESI⁺) calcd for C₁₆H₁₂N₃ (M + H⁺) 246.1026, found 245.1020. HPLC method A: purity > 95%, λ = 240 nm, t_R = 17.5 min.

3-(pyrimidin-5-yl)-1H-pyrrolo[3,2-g]isoquinoline (23), obtained as a yellow powder (31 mg, 0.126 mmol, 62%). $R_f = 0.43$ (DCM/MeOH 94:6). Mp. > 250 °C (decomp.). IR (ATR): 3101-2248, 1604, 1411, 1274 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.92 (d, $J = 5.6$ Hz, 1H), 8.23 (s, 1H), 8.27 (d, $J = 5.6$ Hz, 1H), 8.50 (d, $J = 2.8$ Hz, 1H), 8.64 (s, 1H), 9.11 (s, 1H), 9.33 (s, 2H), 9.41 (s, 1H), 12.14 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 108.2, 115.0, 120.5, 132.1, 138.7, 152.7, 153.8 (2CH), 155.4 (CH_{arom}), 107.8, 125.0, 129.2, 129.4, 129.6, 137.5 (C_{arom}). HRMS (ESI⁺) calcd for C₁₅H₁₁N₄ (M + H⁺) 247.0984, found 247.0978. HPLC method A: purity > 96%, λ = 240 nm, t_R = 18.4 min.

4.1.20. 1-(1H-pyrrolo[3,2-g]isoquinolin-3-yl)ethanone (24)

To a suspension of compound **8** (50 mg, 0.297 mmol) in DCM (1.40 mL) was added AlCl₃ (198 mg, 1.486 mmol) at rt. under argon. The mixture was stirred for 30 minutes at rt. before adding acetyl chloride (106 μL, 1.486 mmol). The mixture was stirred overnight at rt. before being cautiously quenched with MeOH at 0 °C. The solution was then concentrated under reduced pressure. Residue was purified by two successive chromatography columns (DCM/MeOH 98:2 to 85:15) yielding compound **24** (50 mg, 0.238 mmol, 80%) as a pale yellow powder. $R_f = 0.44$ (DCM/MeOH 94 :6). Mp. > 250 °C. IR (ATR): 3499-2775, 1612, 1532, 1427, 1375, 1270, 1161 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.53 (s, 3H), 7.91 (d, $J = 6.0$ Hz, 1H), 8.20 (s, 1H), 8.30 (d, $J = 6.0$ Hz, 1H), 8.78 (s, 2H), 9.40 (s, 1H), 12.35 (br s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 27.3 (CH₃), 108.6, 117.5, 120.7, 139.1, 141.3, 152.7 (CH_{arom}), 115.7, 125.3, 129.6, 130.7, 137.2 (C_{arom}), 192.3 (C=O). HRMS (ESI⁺) calcd for C₁₃H₁₁N₂O (M + H⁺) 211.0866, found 211.0863. HPLC method A: purity > 97%, λ = 240 nm, t_R = 18.6 min.

4.1.21. 1-(1-tosyl-1H-pyrrolo[3,2-g]isoquinolin-3-yl)ethanone (25)

To a suspension of compound **24** (78 mg, 0.371 mmol) in DCM (2 mL) under argon at rt. was added (*i*Pr)₂NEt (97 μ L, 0.557 mmol), DMAP (2 mg, 0.0186 mmol) and tosyl chloride (78 mg, 0.408 mmol). The mixture was stirred for 18 h at rt. A saturated aqueous solution of NaHCO₃ was added and the aqueous layer was extracted with DCM (3 x 20 mL). Combined organic layers were dried over MgSO₄ and then evaporated under reduced pressure. Residue was purified by Puriflash chromatography (DCM/MeOH 99:1 to 90:10) yielding compound **25** (109 mg, 0.299 mmol, 81%) as a pale yellow powder. *R*_f = 0.63 (DCM/MeOH 96:4). Mp. > 213 °C (decomp.). IR (ATR): 3141, 2827, 1672, 1544, 1455, 1379, 1162 cm⁻¹. NMR (400 MHz, DMSO-*d*₆) δ 2.31 (s, 3H), 2.66 (s, 3H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.98 (d, *J* = 6.0 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 2H), 8.44 (d, *J* = 6.0 Hz, 1H), 8.76 (s, 1H), 8.79 (s, 1H), 9.13 (s, 1H), 9.58 (s, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 21.0, 27.9 (CH₃), 110.7, 119.5, 120.4, 127.4 (2CH), 130.5 (2CH), 139.5, 141.4, 153.3 (CH_{arom}), 120.1, 126.0, 130.2, 132.1, 133.2, 133.6, 146.5 (C_{arom}), 193.7 (C=O). HRMS (ESI⁺) calcd for C₂₀H₁₇N₂O₃S (M + H⁺) 365.0954, found 365.0947.

4.1.22. 4-(1*H*-pyrrolo[3,2-*g*]isoquinolin-3-yl)pyrimidin-2-amine (**26**)

To a solution of compound **25** (88 mg, 0.241 mmol) in dry DMF (1.5 mL) under argon was added dimethylformamide di-*tert*butyl acetal (87 μ L, 0.362 mmol). The mixture was stirred for 6 h at rt. A saturated aqueous solution of NaHCO₃ was added and the aqueous layer was extracted with EtOAc (3 x 20 mL). Combined organic layers were washed with water (5 x 20 mL) and brine, dried over MgSO₄ and evaporated under reduced pressure. The crude enaminone was used in the next step without any further purification. To a solution of the enaminone in 2-methoxyethanol (1 mL) under Argon were added guanidine hydrochloride (35 mg, 0.362 mmol) and K₂CO₃ (67 mg, 0.483 mmol). The mixture was refluxed for 18 h. Then an aqueous saturated solution of Na₂CO₃ was added and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water (5 x 20 mL) and brine, dried over MgSO₄, filtered and evaporated under reduced pressure. Residue was first purified by column chromatography (DCM/MeOH 99:1). Finally, two preparative TLC plates (first elution pure DCM then DCM/MeOH 90:10 + 0.1% TEA) yielded pure compound **26** as a red powder (4.7 mg, 0.018 mmol, 7%). *R*_f = 0.33 (DCM/MeOH 93:7). Mp. > 215 °C (decomp.). IR (ATR): 3468, 3226-2776, 1538, 1426, 1278, 1195 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.54 (br s, 2H), 7.13 (d, *J* = 5.2 Hz, 1H), 7.84 (d, *J* = 5.2 Hz, 1H), 8.16 (d, *J* = 5.2 Hz, 1H), 8.17 (s, 1H), 8.29 (d, *J* = 5.2 Hz, 1H), 8.68 (s, 1H), 9.24 (s, 1H), 9.38 (s, 1H), 12.21 (br s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 99.6, 105.2, 107.8, 118.6, 135.1, 138.9, 152.8, 157.2 (CH_{arom}), 112.7, 120.7, 125.2, 129.8, 137.7, 162.1, 163.6 (C_{arom}). HRMS (ESI⁺) calcd for C₁₅H₁₂N₅ (M + H⁺) 262.1087, found 262.1081. HPLC method A: purity > 95%, λ = 255 nm, *t*_R = 17.8 min.

4.2 Protein kinase assays

Kinase enzymatic activities were assayed in 384 well plates using the ADP-Glo™ assay kit (Promega, Madison, WI, USA) according the manufacturer's guidance [27]. Briefly, the reactions were carried out in a final volume of 6 μ L for 30 min at 30 °C in an appropriate kinase buffer (10 mM MgCl₂, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 μ g/mL heparin), with either protein or peptide as substrate in the presence of 10 μ M ATP (40 mM Tris pH 7.5, 20 mM MgCl₂ and 0.1 mg/mL of BSA). Afterward, 6 μ L of ADP-Glo™ Kinase Reagent was

added to stop the kinase reaction. After an incubation time of 50 min at room temperature (RT), 12 μL of Kinase Detection Reagent was added for 1 h at RT. The transmitted signal was measured using the Envision (Perkin Elmer, Waltham, MA, USA) microplate luminometer and expressed in relative light unit (RLU). In order to determine the half maximal inhibitory concentration (IC_{50}), the assays were performed in duplicate in the absence or presence of increasing doses of the tested compounds. GraphPad Prism6 software (GraphPad Software, San Diego, CA, USA) was used to fit dose–response curves and to determine the IC_{50} values. Kinase activities are expressed in percentage of maximal activity, i.e., measured in the absence of inhibitor. Peptide substrates were obtained from Proteogenix (Schiltigheim, France).

The following protein kinases were analyzed in this study: ABL1 (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed with 0.17 $\mu\text{g}/\mu\text{L}$ of the following peptide: EAIYAAPFAKKK, as substrate; CDK5/p25 (human, recombinant, expressed in bacteria) was assayed with 0.8 $\mu\text{g}/\mu\text{L}$ of histone H1 as substrate; CDK9/CyclinT (human, recombinant, expressed by baculovirus in Sf9 insect cells) were assayed with 0.27 $\mu\text{g}/\mu\text{L}$ of the following peptide: YSPTSPSYSPTSPSYSPTSPSKKKK, as substrate; CK1 ϵ (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed with 0.022 $\mu\text{g}/\mu\text{L}$ of the following peptide: RRKHAAIGSpAYSITA (“Sp” stands for phosphorylated serine) as CK1-specific substrate; GSK3 β (human, recombinant, expressed by baculovirus in Sf9 insect cells) were assayed with 0.010 $\mu\text{g}/\mu\text{L}$ of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpEDEEE); Haspin-kd (human, kinase domain, amino acids 470–798, recombinant, expressed in bacteria) was assayed with 0.007 $\mu\text{g}/\mu\text{L}$ of histone H3 (1–21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate; JAK3 (human, recombinant, expressed by baculovirus in Sf9 insect) was assayed with 0.17 $\mu\text{g}/\mu\text{L}$ of the following peptide: GGEEEEYFELVKKKK, as substrate; Pim-1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed with 0.8 $\mu\text{g}/\mu\text{L}$ of histone H1 as substrate; MmCLK1 (from *Mus musculus*, recombinant, expressed in bacteria) was assayed with 0.027 $\mu\text{g}/\mu\text{L}$ of the following peptide: GRSRSRSRSR as substrate; RnDYRK1A-kd (*Rattus norvegicus*, amino acids 1–499 including the kinase domain, recombinant, expressed in bacteria, DNA vector kindly provided by Dr. W. Becker, Aachen, Germany) was assayed with 0.033 $\mu\text{g}/\mu\text{L}$ of the following peptide: KKISGRLSPIMTEQ as substrate.

4.3 Cellular evaluation

4.3.1. Cell culture

HCT116, SH-SY5Y, HBL100, U-2 OS and RPE-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (unless otherwise specified). Cells were cultured at 37 °C in a 5% CO_2 humidified atmosphere.

4.3.2. Cell viability on monolayer cultures (2D)

Briefly, cells were grown in 96-well plates in the presence of increasing concentrations of each compound (from 50 to 0.01 μM) for 48 h. Cell viability was then assessed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay from Promega according to manufacturer's instructions. Each experiment was done in triplicate. The data were processed

using GraphPad PRISM Software (GraphPad Software, San Diego, CA, USA) to determine the IC₅₀ values according to the signal given by the control (0.5% DMSO) set at 100% viability.

4.4.3. H3T3ph immunofluorescence

U-2 OS cells were grown on coverslips, treated for 16 h then fixed with 4% paraformaldehyde in PBS, permeabilized by 0.15% Triton-X100 for 2 minutes, blocked for 15 minutes in 4% BSA in PBS and processed using standard immunofluorescence protocols. The primary antibody was an anti-phospho-Thr3 Histone H3 from Millipore (1/1000 dilution). Coverslips were mounted with Vectashield containing DAPI (Vector Laboratories). Images were acquired with a Coolsnap HQ₂ CCD camera (Photometrics) on a Zeiss Axio microscope (Carl Zeiss) using a 63x NA 1.40 objective. Image acquisition and processing were performed using Zen Blue (Carl Zeiss). Quantification of signal intensity was performed using ImageJ software (NIH).

4.4. Protein expression, crystallization and data collection

4.4.1 Expression and purification

Haspin kinase domain (residues G465-K798) with a TEV-cleavable N-terminal His₆-tag was transformed in BL21(DE3) cells. Bacteria were grown in Terrific Broth medium containing 50 mg/mL kanamycin. Protein expression was induced at an OD₆₀₀ of 2 by using 0.5 mM (IPTG) at 18 °C for 12 hours. Cells expressing GSG2 were lysed by sonication in lysis buffer containing 50 mM HEPES pH 7.5, 500 mM NaCl, 25 mM imidazole, 5% glycerol, and 0.5 mM TCEP. After centrifugation, the supernatant was loaded onto a Nickel-Sepharose column equilibrated with 30 mL lysis buffer. The column was washed with 60 mL lysis buffer. Proteins were eluted by an imidazole step gradient (50, 100, 200, 300 mM). Fractions containing protein were pooled together and loaded onto Superdex 75 16/60 Hi-Load gel filtration column equilibrated with final buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, and 0.5 mM TCEP). The purity of the protein was assessed by SDS PAGE; pure protein fractions were concentrated to approximately 11 mg/mL.

4.4.2 Protein crystallization

Haspin (11 mg/mL) crystallized using the sitting-drop vapour diffusion method by mixing protein and well solutions in 2:1, 1:1, and 1:2 ratios (Drop size 200 nL) at 4 °C. The reservoir solution contained 45-60% MPD as precipitant and 0.1 M SPG pH 7.5 as buffer. Compound **22** (**MD420**) from a 50 mM DMSO stock was dissolved in reservoir solution (final concentration 500 μM) and soaked into the crystal for a few days.

4.4.3 Data collection, structure solution and refinement

Crystals were fished without any additional cryoprotectant and flash-frozen in liquid nitrogen. Diffraction data were collected at beamline I03 at Diamond Light Source (UK) at a wavelength of 1.0 Å at 100 K. Data were processed using XDS [32] and scaled with aimless [33]. The PDB structure with the accession code 2VUW [2] was used as an initial search MR model using the program MOLREP [34]. The final model was built manually using Coot [35] and refined with REFMAC5 [36]. Data collection and refinement statistics are summarized in Table 3 below.

Dictionary files for compound **22 (MD420)** was generated using the Grade Web Server (<http://grade.globalphasing.org>).

Table 3. Data collection and refinement statistics

Data collection	Haspin-Compound 22 (MD420)
Beamline	I03/DLS
Wavelength (Å)	0.976254
Space group	P 2 ₁ 2 ₁ 2 ₁
Cell dimensions	
<i>a, b, c</i> (Å)	69.51, 78.20, 86.87
α, β, γ (°)	90, 90, 90
Resolution (Å)*	86.87-2.00 (2.05-2.00)
unique observations*	32717 (2387)
<i>R_{pim}</i> *	0.040 (0.537)
Completeness (%)*	100.0 (100.0)
Multiplicity*	13.5 (14.0)
mean <i>I</i> / σ <i>I</i> *	12.1 (1.6)
CC1/2*	0.999 (0.790)
Refinement	
<i>R</i> _{work} / <i>R</i> _{free}	19.85 / 23.68
No. of atoms	2774
overall B-factors (Å ²)	39.53
Rms deviations	
Bond lengths (Å)	0.0071
Bond angles (°)	0.8596
Ramachandran outlier (%)	0.0
Protein Data Bank entry	8RDK

*Values for the highest resolution shell are shown in parentheses.

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