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Short communication

6-Substituted 2-(N-trifluoroacetylamino)imidazopyridines induce cell cycle arrest and apoptosis in SK-LU-1 human cancer cell line

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ABSTRACT

A series of 6-substituted 2-(N-trifluoroacetylamino)imidazopyridines have been synthesized and their bioactivities were evaluated. Compounds **6a**, **6c**, and **11a** were the most active compounds with modest cytotoxic activity against six human cancer cell lines U251 (glioma), PC-3 (prostate), K-562 (leukemia), HCT-15 (colon), MCF7 (breast) and SK-LU-1 (lung). The cell cycle analysis showed that compounds **6a**, **6c**, and **11a** induce a G2/M phase cell cycle arrest on SK-LU-1 cell line where inhibition of CDK-1 and CDK-2 may be implicated.

1. Introduction

Advances in molecular and cellular biology through the last decades have revealed some of the many process implicated in cancer approach. However, at the present time the prognosis against of most human malignancies has not been improved significantly. The continuous search for novel agents which target pathological processes of human carcinogenesis has lead to the synthesis of small molecules which may modulate cell cycle and apoptotic pathways [1]. Special interest has been focused on molecules that can arrest cell cycle mechanism by the inhibition effect for cyclin-dependent kinases (CDK) family, because perturbation of the cell cycle has been related to human neoplastic diseases [2]. From various subtypes of CDKs, the most important explored targets in cancer therapy have been CDK-1, -2, and -4 [3].

Nitrogen-bridgehead fused heterocycles containing an imidazole ring have been shown as a common structural moiety in pharmacologically important molecules, displaying a wide range of activities and spreading on diverse number of targets. Probably the most widely used heterocyclic system from this group is imidazo[1,2-a]pyridine [4]. Imidazo[1,2-a]pyridines shown anticytomegalo-zoster and anti-varicella-zoster virus [5], antibacterial [6], hypnoselective, and anxioselective activities [7]. They work as α-amyloid formation inhibitors [8] and constitute a novel class of orally active nonpeptide bradykinin B2 receptor antagonists [9]. Recently [10] it was described that 2-aminoimidazo[1,2-a]-pyridine scaffold represents a novel structural class of protein serine/threonine kinase inhibitors and these kinds of compounds affect potently the cyclin-dependent kinases by competing with ATP for binding to a catalytic subunit of the protein.

In addition, Jaramillo et al. [11] disclose a detailed SAR study of 2-aminoimidazo[1,2-a]pyridines around the role of substituent at 3-position, and with respect the optimal spacer between phenyl and imidazopyridine rings, in this study the presence of an sp2 carbon was shown to be necessary in order to keep activity. Carbonyl, vinyl, or Z-configurated cyanovinyl substituents, were also efficient; nevertheless activity was lost partially when either E-configurated olefins or bulkier substituents such as tetrazole were introduced.

Because most of the actual SAR reports over this subject have been made on substituents 2 and 3 of imidazopyridine ring as well as the spacer between the aromatic bicyclic moiety, it appears to be interesting to determine the effect of removing the substituent at 3-position, and the effect of other groups such as heterocyclic compounds at 6-position of the imidazo[1,2-*a*]pyridine ring. Herein we described the synthesis of a series of 6-substituted 2-(N-trifluoroacetylamino)imidazopyridines compounds **6a–e** and **11a–c**, which displayed cytotoxicity against six human cancer cell lines U251 (glioma), PC-3 (prostate), K-562 (leukemia), HCT-15 (colon), MCF7 (breast) and SK-LU-1 (lung). The effects on the cell cycle,

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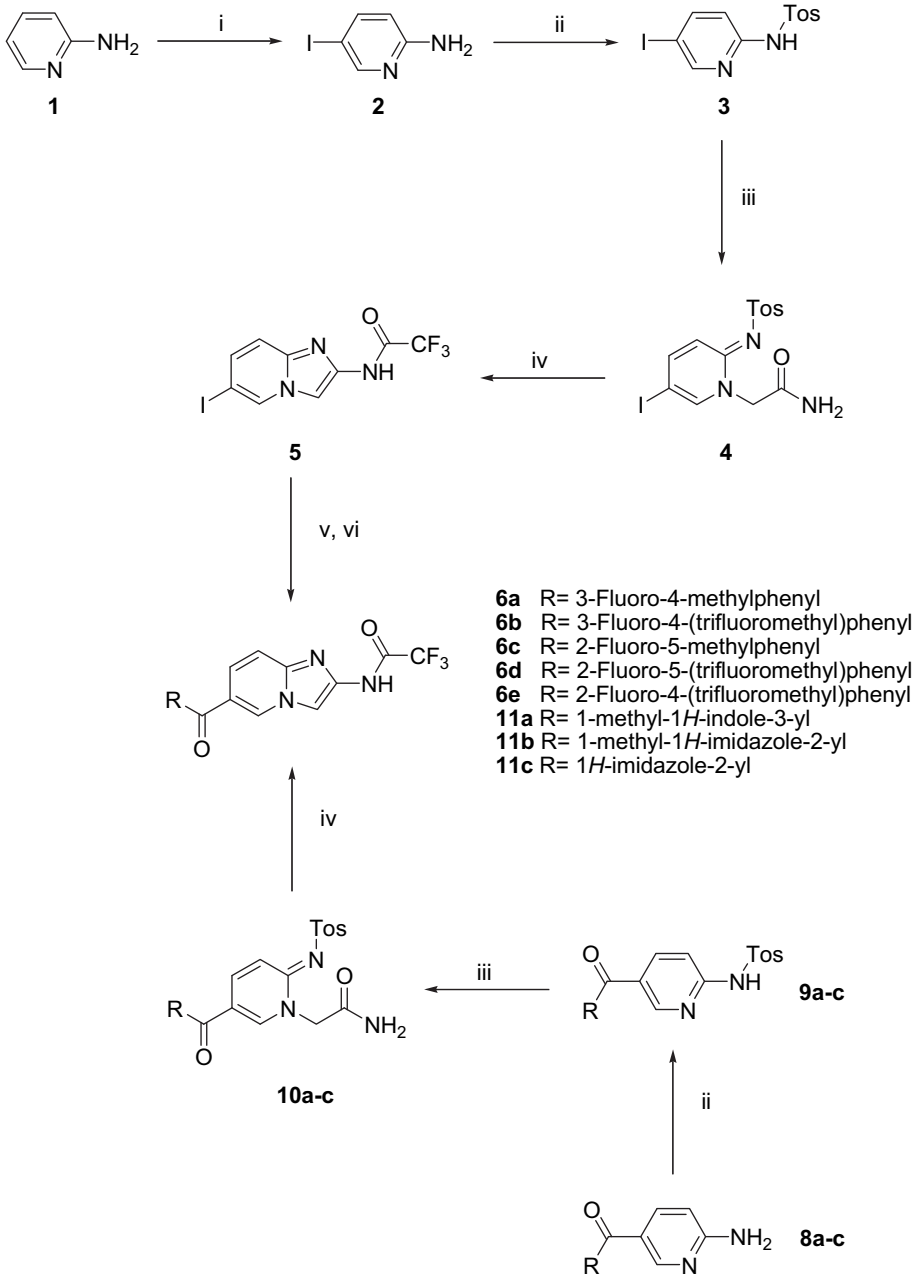
apoptosis induction, and inhibitory activity on CDK-1 and CDK-2 of selected compounds were also studied.

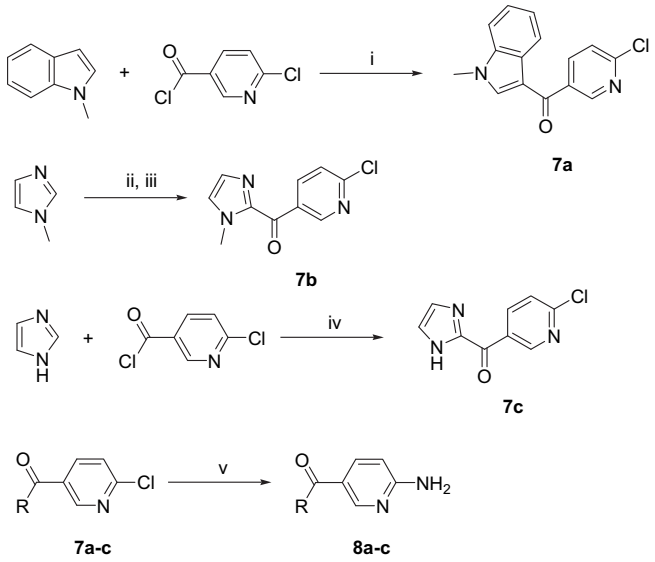
2. Results and discussion

2.1. Chemistry

The synthesis of compounds **6a–e** (Scheme 1) was accomplished according to the previous reported proceedings [12]. The protection of 2-amino-5-iodo-pyridine **2** with p-tolylsulfonyl chloride in pyridine and subsequent treatment with iodoacetamide in the presence of diisopropylethylamine in DMF provided compound **4**. Conversion of **4** to the desired 2-(N-trifluoroacetylamino)imidazopyridine **5** was achieved by treatment with trifluoroacetic anhydride in refluxing methylene chloride. Metalation of compound **5** with *i*-propyl magnesium chloride, and then reaction with 4 equivalents of the corresponding fluorinated aldehyde with further oxidation of the crude reaction with manganese dioxide afforded diarylketones **6a–e** in yields over 30%. Synthesis of compounds **8a–c** is summarized in Scheme 2. Compound **7a** was obtained by classical Friedel-Crafts acylation of *N*-methylindole with 6-chloronicotinyl chloride. Reaction of *N*-methylimidazole with *n*-Butyllithium and quenching with 6-chloro-N-methoxy-N-methylnicotinamide (Weinreb amide) leads to the desired compound **7b**. Diarylketone **7c** was obtained by acylation of imidazole with 6-chloronicotinyl chloride under conditions reported by Bastiaansen and Godefroi [13]. Finally, treatment of **7a–c** with ammonia yields corresponding amines



Scheme 1. Reagents and conditions i) $I_2/HIO_4/H_2SO_4/CH_3COOH$, 80 °C; ii) TsCl, py, 85 °C; iii) ICH_2CONH_2, DIPEA, DMF, r.t.; iv) TFAA, CH_2Cl_2 reflux; v) i-PrMgCl, THF, −40 °C, aldehyde; vi) MnO_2, CH_2Cl_2.



Scheme 2. Reagents and conditions i) $AlCl_3$, CH_2Cl_2; ii) *n*-BuLi, THF, −70 °C; iii) 6-chloro-N-methoxy-N-methylnicotinamide, −70 °C to r.t.; iv) $(C_2H_5)_3N$/Pyridine r.t. to reflux; v) NH_3 anh./EtOH 150 °C.

8a–c. Synthesis of compounds **11a–c** (Scheme 1) was performed just as it was described for synthesis of **5**, using as starting material the amines **8a–c** instead of 2-amino-5-iodo-pyridine **3**.

2.2. Biological activities

2.2.1. Cytotoxicity

All compounds were evaluated for cytotoxic activity *in vitro* against six human cell lines (glia carcinoma U251, prostate PC-3, leukemia K-562, colon HCT-15, human breast MCF7 and lung carcinoma SK-LU-1). To compare cytotoxic effects between a known CDK inhibitor and the compounds herein described we used Olomucine as a positive control and DMSO as solvent control. A starting screening with a fixed concentration of 50 μM showed citotoxicity for everyone of these six human cell lines (Table 1) with growth inhibition activity greatest than the observed for intermediary **5** and Olomucine. Compounds **6a**, **6b**, **6c**, **6e**, **11a**, and **11c** displayed to be the most active. In addition, the IC_{50} values for these compounds were determined and the results are summarized in Table 2.

As shown in Table 2, MCF7 and SK-LU-1 displayed to be the most sensitive cell lines. Comparing data of compounds **6a–e** (Tables 1 and 2) it is possible to observe that substitution with fluorine at 2-position (**6c**, **6d** and **6e**) of the phenyl ring shown better cytotoxic activity than those having a substitution at 3-position (**6a** and **6b**). Negligible effect was observed when substitution with trifluoromethyl instead methyl attached to phenyl ring occurs (compounds **6a** and **6c** vs. **6b** and **6d**). When heterocyclic substituents at 6-position were introduced (compounds **11a–c**) comparable or improved cytotoxic activity was detected (Tables 1 and 2).

Table 1
Cytotoxicity of target compounds against six human cancer cell lines. Primary screening at 50 μM.[a]

Compound	Cell line (growth inhibition %)					
	U251	PC-3	K-562	HCT-15	MCF7	SK-LU-1
5	20.8	50.9	33.1	56.3	36.7	25.6
6a	37.9	30.7	52.2	65.5	57.3	81.5
6b	38.4	32.6	50.1	59.2	56.5	48.3
6c	61.5	59.2	92.6	91.2	66.3	71.7
6d	59.3	61.5	88.0	91.7	65.1	70.5
6e	80.1	92.6	90.2	100	97.2	100
11a	92.7	63.7	95.5	78.0	80.4	69.2
11b	11.9	6.1	45.3	NA	30.8	29.2
11c	80.2	58.1	88.3	47.8	60.7	97.5
Olomucine	32.0	22.7	19.8	28.9	37.6	36.9

[a] Each experiment was independently performed two times at a time of 48 h. NA, not active.

In order to examine the effect on cell cycle, apoptosis induction, and enzyme inhibition we selected the most active compounds (in the cytotoxicity assay) representing each substitution pattern (compounds **6a**, **6c**, and **11a**) and we used the most sensitive cell lines, MCF7 and SK-LU-1 (Table 2). Furthermore, the origin of these cell lines is representative of the most frequent cancer types worldwide [14].

2.2.2. Cell cycle analysis

Asynchronously growing MCF7 and SK-LU-1 cells were exposed to the chosen compounds at concentrations of $2\times IC_{50}$ for 24 h, stained with propidium iodide and analyzed by flow cytometry in order to determine the total population distribution in the different phases (G0/G1, S, and G2/M). Control cells treated only with DMSO using the highest amount into the experiments proceeded through a normal cell cycle (Fig. 1a, and Table 3). However, under the same conditions, treatment of MCF7 cells with compound **6a** (95 μM) did not show any difference compared with DMSO control (Fig. 1b, and Table 3). When MCF7 cells were treated with compound **6c** (22 μM) it was observed a statistically significant increase of cell population in phase G0/G1 from 57.2% for control to 74.1% after treatment. Nevertheless for S and G2/M phase a decreasing of cell population was displayed (Fig. 1c, and Table 3). Treatment of MCF7 cells with compound **11a** (89 μM) led to the enrichment of G0/G1 cell population going from 57.2% for control to 70.6% after treatment, while a decreasing of cells in S phase was observed, remaining cells in G2/M phase unchanged (Fig. 1d, and Table 3). The use of Olomucine as control did not have any effect on cell cycle of MCF7 cells (Table 3) at the selected concentration for these experiments (112 μM). Control (DMSO), SK-LU-1 cell cycle histograms and percentage of population distribution are shown in Fig. 1e and Table 3. When SK-LU-1 cells were treated with compound **6a** (106 μM) a statistically significant accumulation of cell population in G2/M phase was observed with 16.7% for control to 60.6% for treated cells. With respect to cells in S phase, they were unchanged and population in G0/G1 decreased significantly from 61.7% for control to 22.6% for treated cells (Fig. 1f, and Table 3). Compound **6c** (70 μM) causes a most evident arrest at G2/M phase with a 75.7% of overall cell population in this phase only a 10.4% of cells in G0/G1 phase and cells in S phase were observed without change (Fig. 1g, and Table 3). Treatment with compound **11a** (50 μM) induced weaker cell cycle alteration with arrest in G2/M phase (47.5%) decreasing the population in G0/G1 phase (32.9%) and displaying S phase without change (Fig. 1h, and Table 3). Olomucine treatment (64 μM) showed a decrease of cells in G0/G1 phase and a weak arrest of cells in G2/M phase while cells in S phase remained unchanged. Differences observed with the stage of arrest between MCF7 and SK-LU-1 cells could be due to the genetic differences of each both cell line.

2.2.3. Apoptosis

In this work we also examined the effect of compounds **6a**, **6c** and, **11a** on cell death (apoptosis) for SK-LU-1 cells. The appearance of phosphatidylserine (PS) residues (normally hidden within the plasma membrane) on the surface of the cell is used as a parameter to detect and measure apoptosis. The presence of PS on the cell surface creates one of the specific signals for recognition and removal of apoptotic cells by macrophages. These PS changes can be detected with the anticoagulant (annexin V), which has shown a high affinity for binding to PS. As the apoptotic process progresses, cell membrane integrity is lost. Using DNA specific viability

Table 2
Cytotocicity IC_{50} values for compounds **6a**, **6c**, **6e**, **11a** and Olomucine against six cancer cell lines.[a]

Compound	Cell line IC_{50} (μM)					
	U251	PC-3	K-562	HCT-15	MCF7	SK-LU-1
6a	80.6 ± 2.1	ND	83.6 ± 0.1	56.2 ± 2.7	47.5 ± 0.9	53.0 ± 7.7
6b	84.2 ± 4.5	69.1 ± 1.5	83.2 ± 3.1	57.1 ± 3.2	50.8 ± 1.3	57.1 ± 3.1
6c	51.9 ± 2.3	36.3 ± 3.0	10.9 ± 2.0	10.5 ± 0.8	10.7 ± 1.6	11.2 ± 1.0
6e	52.3 ± 4.6	28.0 ± 1.9	56.6 ± 3.3	25.0 ± 1.5	24.9 ± 2.1	35.1 ± 4.3
11a	27.8 ± 1.5	ND	54.6 ± 5.4	65.1 ± 4.3	44.3 ± 0.1	24.8 ± 0.4
11c	37.7 ± 5.5	52.3 ± 2.5	51.5 ± 3.3	56.8 ± 8.5	42.4 ± 4.1	25.1 ± 1.1
Olomucine	81.5 ± 2.1	81.0 ± 7.4	89.3 ± 1.9	62.5 ± 3.0	55.9 ± 1.1	68.1 ± 0.41

[a] Results express the mean IC_{50} ± SD obtained from three independent experiments performed at 48 h. ND, not determinate at tested concentrations.

dyes, such as Propidium Iodide (PI) it is possible to distinguish between early apoptotic, late apoptotic, and dead cells. In order to prove these findings, selected cells were treated with compounds **6a**, **6c** and **11a** for 48 h at 106 μM, 70 μM and 50 μM respectively ($2\times IC_{50}$). FACS analyses (Fig. 2) showed populations of annexin V-stained cells (early apoptotic) and annexin V-PI stained cells (late apoptotic) with the drug concentrations used. Control cells treated only with DMSO alone showed a 5% of apoptosis. However when cells were treated with compound **6a** an average of 39.5% of cells underwent apoptosis, apoptosis was evident inclusive at 24 h of exposition as shown by the appearance of an evident sub-G1 peak in cell cycle analysis histograms (Fig. 1f), while compound **6c** and compound **11a** causes a 20% and 24.1% of apoptotic death respectively (Fig. 3). A weaker effect was observed at 24 h of treatment with compounds **6c** and **11a** as shown in cell cycle analysis histograms (Fig. 1g and h).

2.2.4. Inhibiting CDK-1 and CDK-2 activities

Since imidazo[1,2-a]pyridines have shown both cytotoxic activity and inhibition against CDK-2 and CDK-1 *in vitro* [10,11] we examined the activities for cell cycle relative complex cyclin B/CDK-1, and cyclin E/CDK-2 in presence of compounds **6a**, **6c**, and **11a**. In order to compare the inhibition grade of the activity of cyclin B/CDK-1, and cyclin E/CDK-2 complexes by tested compounds with that of Olomoucine (a CDK inhibitor), we performed a one-point quantitative determination at IC_{50} of Olomoucine (7 μM) [15]. We found a strong inhibition effect for cyclin B/CDK-1 activity when compound **6c** and compound **11a** were presented, 83% and 61% respectively (Table 4). Both compounds were most active than Olomoucine. A weaker inhibition of cyclin B/CDK-1 was observed with compound **6a** (Table 4). Evaluation of cyclin E/CDK-2 inhibition activities enabled us to detect inhibition percents of 44, 49, 45 and 51% for compounds **6a**, **6c**, **11a**, and Olomoucine respectively (Table 4). These results allow us to assume that compounds **6a**, **6c**, and **11a** may affect the cell cycle via modifying cyclin B/CDK-1, and cyclin E/CDK-2 activities; even though other targets can be involved so further experiments are necessary to make sure the exact mechanism.

3. Conclusion

The antiproliferative activity of 2-(N-trifluoroacetylamino)-imidazopyridines 6-substituted against a variety of cancer cell lines was evaluated. Most of synthesized compounds displayed modest cytotoxic activity in the micromolar range, especially against MCF7 and SK-LU-1. Compounds **6a**, **6c**, and **11a** showed significant arrest in G2/M phase, followed by apoptotic cell death in SK-LU-1 cells. Compounds **6a**, **6c**, and **11a** could affect the cell cycle via inhibition of cyclin B/CDK-1, and cyclin A/CDK-2 activities but since synthesized compounds are each basically equitoxic against MCF7 and SK-LU-1

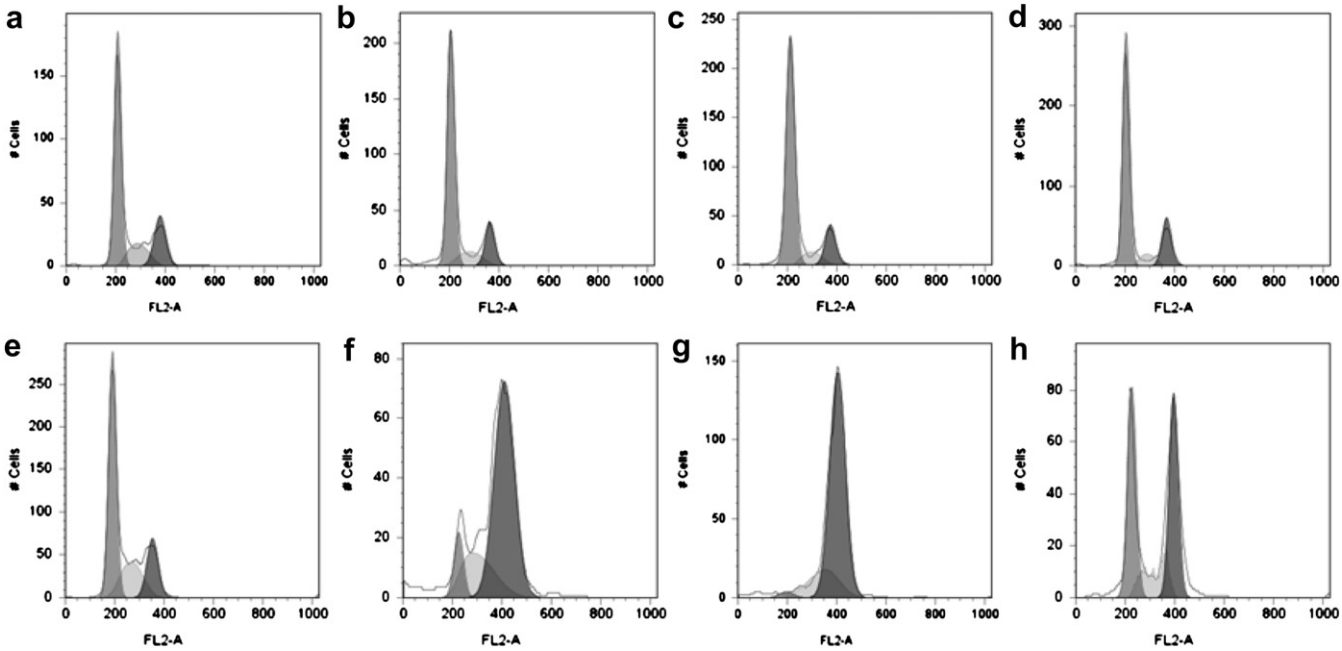


Fig. 1. Cell cycle distribution by flow cytometry in MCF7 cells treated with a) DMSO control, b) **6a** (95 μM), c) **6c** (22 μM), d) **11a** (89 μM); and SK-LU-1 cells treated with e) DMSO control, f) **6a** (106 μM), g) **6c** (70 μM), h) **11a** (50 μM). Compounds were tested at $2\times IC_{50}$. Histograms are the most representative of three independent experiments.

Table 3
Cell cycle distribution percentage by flow cytometry in MCF7 and SK-LU-1 cells treated for 24 h at $2\times IC_{50}$.[a]

Compound	MCF7			SK-LU-1		
	%G0/G1	%S	%G2/M	%G0/G1	%S	%G2/M
Control	57.2 ± 3.4	17.0 ± 1.2	22.1 ± 0.1	61.7 ± 2.9	18.2 ± 1.5	16.7 ± 0.9
6a	63.5 ± 4.9	14.5 ± 3.3	18.1 ± 3.5	22.6 ± 5.7*	16.2 ± 5.3	60.6 ± 1.1*
6c	74.0 ± 2.4*	7.1 ± 2.1*	13.4 ± 2.4	10.4 ± 11.9*	21.2 ± 1.8	75.7 ± 11.5*
11a	70.6 ± 3.2	6.3 ± 1.2*	20.2 ± 0.3	32.9 ± 6.1*	14.7 ± 6.5	47.5 ± 7.7*
Olomucine	60.6 ± 3.1	15.0 ± 2.4	23.2 ± 0.4	44.2 ± 2.9*	19.7 ± 8.5	31.8 ± 3.6*

[a] Results express the means ± SD obtained from three independent experiments. *$P < 0.05$ as compared with solvent (DMSO) on 24 h treatment. Tested concentrations for MCF7 cells were **6a** (95 μM), **6c** (22 μM), **11a** (89 μM), and for SK-LU-1 cells **6a** (106 μM), **6c** (70 μM), **11a** (50 μM).

cells (Table 2) but have minimal cell cycle effects in MCF7 cells and significant cell cycle effects in SK-LU-1 cells we could consider that other targets may be involved and further experiments are necessary to assess the exact mechanism.

4. Experimental protocols

All reactions involving air-sensitive reagents were performed under inert atmosphere (N_2 or argon) using syringe–septum cap techniques. All glassware was oven-dried prior to use. All melting points were determined with a MEL-TEMP® capillary melting point apparatus and were uncorrected. Infrared spectra were determined in a spectrophotometer Perkin–Elmer 282-B and Nicolet FT-IR magna 55×. The mass spectra were recorded on a JEOL JMS-SX 10217 instrument (EI). The ^{1}H NMR spectra were recorded on a Varian Gemini 200 MHz, Varian Unity 300 MHz. High resolution spectra were recorded using a Varian Inova 500 MHz (^{1}H NMR and 125 MHz ^{13}C NMR) spectrometers. Chemical shifts are expressed as δ values relative to TMS as internal standard, *J* values are given in Hz and spectra were recorded in $CDCl_3$, DMSO-d_6 or a mixture of both. Flash chromatography was performed on silica gel 60 (230–400 mm) by Merck.

All the aldehydes and reagents herein used were acquired from Sigma–Aldrich (St. Louis, MO) and used without further purification unless otherwise stated. THF was distilled from Na–benzophenone under N_2.

4.1. Synthesis

4.1.1. 2-Amino-5-iodo-pyridine (*2*)

A mixture of 2-aminopyridine **1** (2.4 g, 25 mmol), periodic acid dihydrate (0.86 g, 3.75 mmol), and iodine (2.7 g, 10.7 mmol) was heated in a mixed solution of acetic acid (60 mL), water (3 mL), and sulphuric acid (0.5 mL) at 80 °C for 4 h. The reaction mixture was then poured into 10% aqueous $Na_2S_2O_3$ solution to quench any unreacted iodine and extracted with ether. The extract was washed with 10% aqueous NaOH, dried (Na_2SO_4), and concentrated in vacuum. The residue was purified by flash chromatography on silica gel. Recrystallization from ethanol gave pale yellow prisms of **2** (4.75 g, 86% yield).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.51 (s, 2H), 6.35 (d, 1H, *J* = 8 Hz), 7.62 (d, 1H, *J* = 8 Hz), 8.21 (s, 1H). $C_5H_5IN_2$, MW calcd. 220.96.

4.1.2. 5-Chloro-N-tosylpyridin-2(1H)-imine (*3*)

2-Amino-5-iodo-pyridine **2** (2.2 g, 10 mmol) was dissolved in anhydrous pyridine (10 mL). *p*-Toluensulphonyl chloride (2.1 g, 11 mmol) was added and the solution was heated at 90 °C under Nitrogen overnight. Pyridine was removed *in vacuo* to yield a brown solid. Water (200 mL) was added and the mixture was stirred for 1 h. The solid was collected and dried in vacuum to give 3.52 g (94% yield) of **3** as a beige solid; m.p. 177–178 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.34 (s, 3H), 6.92 (d, 1H, *J* = 8.7 Hz), 7.56 (AA′BB′ system, 4H, *J* = 8.1 Hz), 7.98 (dd, 1H, *J* = 8.6 and 2.1 Hz), 8.34 (d, 1H, *J* = 2.2 Hz), 11.23 (bs, 1H, NH). $C_{12}H_{11}IN_2O_2S$, MW calcd. 374.2. Mass (FAB^+) *m*/*z* = 374.9 $(M+H)^+$.

4.1.3. (E)-2-(5-iodo-2-(tosylimino)pyridin-1(2H)-yl)acetamide (*4*)

To a suspension of **3** (3.7 g, 10 mmol) in anhydrous DMF (20 mL) was added *i*-Pr_2Net (1.42 g, 11 mmol) under argon. To the solution was added 2-iodoacetamide (2.1 g, 11 mmol) and the mixture was stirred at r.t. overnight. The solution was poured onto water (50 mL), filtered, washed with water (200 mL) and dried in vacuum to give **4** as a white solid (3.8 g, 88% yield) m.p. 245 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.32 (s, 3H), 4.75 (s, 2H), 7.15 (d, 1H, *J* = 9.4 Hz), 7.38 (bs, 1H, NH), 7.43 (AA′BB′ system, 4H, *J* = 8.3 Hz), 7.77 (bs, 1H, NH), 7.90 (dd, 1H, *J* = 9.5 and 2.2 Hz), 8.35 (d, 1H, *J* = 2.2 Hz). $C_{14}H_{14}IN_3O_3S$, MW calcd. 431.25.

4.1.4. 6-Iodo-2-(trifluoroacetamido)imidazo[1,2-a]pyridine (*5*)

To a suspension of **4** (2.16 g, 5 mmol) in anhydrous dichloromethane (40 mL) was added trifluoroacetic anhydride until dissolution and the solution was refluxed for 6 h. Solvents were removed

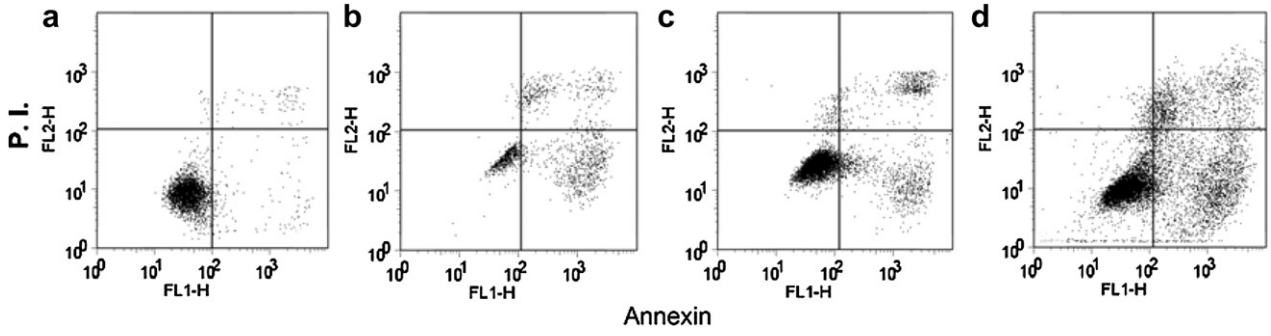


Fig. 2. Apoptosis induced in SK-LU-1 cell line. Apoptosis was assessed by phosphatidylserine exposure on the cell membrane after 48 h treatment with a) DMSO control, b) compound **6a** (106 μM), c) compound **6c** (70 μM), d) compound **11a** (50 μM). Concentrations are $2\times IC_{50}$. Histograms are the most representative of three independent experiments.

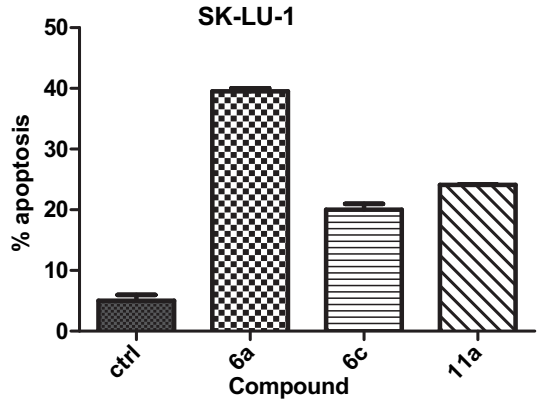


Fig. 3. Apoptosis percentage induced in SK-LU-1 cells. Apoptosis was assessed by phosphatidylserine exposure on the cell membrane after 48 h treatment at $2\times IC_{50}$. The percentages were 5.1% for DMSO control, 39.5% for compound **6a** (106 μM), 20.0% for compound **6c** (70 μM), and 24.1% for compound **11a** (50 μM). Percentages are the means of three independent experiments.

in vacuum and the solid was suspended in EtOAc (100 mL) and stirred for 30 min. The solid was collected and again stirred in water for 30 min. The solid was collected and dried in vacuum to give 1.2 g (65% yield) of **5** m.p. 245–246 °C.

^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ = 7.28 (d, 1H, J = 9.5 Hz), 7.39 (dd, 1H, J = 1.5 and 9.5 Hz), 8.17 (s, 1H), 8.69 (dd, 1H, J = 0.5 and 1.5 Hz), 12.10 (bs, 1H, NH).

^{13}C NMR (125.7 MHz, $CDCl_3$ + DMSO-d_6): δ = 74.6 (C), 102.1 (C), 115.3 (q, J_{CF} = 287.2 Hz, $COCF_3$), 116.7 (CH), 130.7 (CH), 132.3 (CH), 139.32 (CH), 139.7 (C), 154.0 (q, J_{CF} = 38.6, $COCF_3$). $C_9H_5F_3IN_3O$, MW calcd. 355.06. Mass (FAB$^+$): m/z = 356 $(M+H)^+$.

*4.1.5. General procedure for the synthesis of compounds **6a–e***

To a stirred solution of **5** (354.9 mg, 1 mmol) in THF (10 mL) at −40 °C under argon was added 2 M *i*-PrMgCl in THF (1 mL, 2 mmol) over a period of 2 min. Stirring was continued for 1 h at −40 °C before triethylamine (0.140 mL, 2 mmol) was added. After 15 min corresponding aldehyde (4 mmol) was added. The reaction mixture was stirred overnight at r.t. before quenching with sat. NH_4Cl (10 mL). Water (5 mL) was added to dissolve the formed precipitate and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried (Na_2SO_4) and column chromatography on silica gel afforded **6a–e**.

*4.1.6. 2,2,2-Trifluoro-N-(6-(3-fluoro-4-methylbenzoyl)imidazo[1,2-a]pyridin-2-yl)acetamide (**6a**)*

Compound **6a** was prepared starting from 3-fluoro-4-methylbenzaldehyde as electrophile and isolated as a yellow solid (109 mg, 30% yield); m.p. 210 °C.

^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6): δ = 2.31 (d, 3H, J = 1.5 Hz), 7.33 (dd, 1H, J_{HH} = 7.5 and J_{HF} = 7.5 Hz), 7.41 (dd, 1H, J = 2 and 8 Hz), 7.46 (dd, 1H, J = 2 and 7.5 Hz), 7.47 (d, 1H, J = 7 Hz), 7.60 (dd, 1H, J = 1.5 and 9.0 Hz), 8.26 (bs, 1H), 8.92 (bs, 1H), 12.09 (bs, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$ + DMSO-d_6): δ = 14.2 (d, CH, J = 2.8 Hz), 103.6 (CH), 115.2 (CH), 115.3 (q, J_{CF} = 288.98 Hz), 115.4 (d, CH, J_{CF} = 24 Hz), 122.3 (C), 124.3 (CH), 129.5 (d, C, J_{CF} = 17.50 Hz), 129.9 (d, CH, J_{CF} = 1.7 Hz), 131.2 (d, CH, J_{CF} = 4.7 Hz), 131.5 (CH), 136.2 (d, CH, J_{CF} = 6.4 Hz), 140.7 (C), 141.6 (C), 154.1 (q, J_{CF} = 38.7 Hz), 160.2 (d, CF, J_{CF} = 246.6 Hz), 190.8 (C). $C_{17}H_{11}F_4N_3O_2$, MW calcd. 365.28. Mass (m/z, %): 365 (M^+, 100), 296 (35), 256 (20), 137 (22).

Table 4
Enzyme inhibition data for compounds **6a**, **6c**, and **11a**.[a]

CDK/cyclin	Compound (% inhibition)			
	6a	**6c**	**11a**	Olomucine
CDK-1/B	29	83	61	50
CDK-2/E	44	49	45	51

[a] Values are the means for two experiments performed at 7 μM of each tested compound.

*4.1.7. 2,2,2-Trifluoro-N-(6-(3-fluoro-4-(trifluoromethyl)benzoyl)-imidazo[1,2-a]pyridin-2-yl)acetamide (**6b**)*

Compound **6b** was prepared starting from 2-fluoro-5-trifluoromethylbenzaldehyde as electrophile and isolated as a yellow solid (126 mg, 30% yield); m.p. 120 °C.

^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6): δ = 7.35 (dd, 1H, J_{HH} = 7.5 and J_{HF} = 7.5 Hz), 7.44 (dd, 1H, J = 2 and 8 Hz), 7.47 (dd, 1H, J = 2 and 7.5 Hz), 7.48 (d, 1H, J = 7 Hz), 7.57 (dd, 1H, J = 1.5 and 9.0 Hz), 8.36 (bs, 1H), 8.89 (bs, 1H), 12.21 (bs, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$ + DMSO-d_6): δ = 102.4 (CH), 115.0 (CH), 115.4 (q, J_{CF} = 287.2 Hz), 115.6 (d, CH, J_{CF} = 24 Hz), 122.1 (C), 122.3 (q, J_{CF} = 288.9 Hz) 124.3 (CH), 128.9 (d, C, J_{CF} = 17.50 Hz), 129.6 (d, CH, J_{CF} = 1.7 Hz), 131.2 (d, CH, J_{CF} = 4.7 Hz), 131.5 (CH), 136.2 (d, CH, J_{CF} = 6.4 Hz), 140.7 (C), 141.6 (C), 154.3 (q, J_{CF} = 38.7 Hz), 160.6 (d, CF, J_{CF} = 246.6 Hz), 189.8 (C). $C_{17}H_8F_7N_3O_2$, MW calcd. 419.25. Mass (m/z, %): 419 (M^+, 100), 400 (15), 350 (95), 191 (63). IR (KBr, cm^{-1}): 3012, 2874, 1750, 1472, 1309.

*4.1.8. 2,2,2-Trifluoro-N-(6-(2-fluoro-5-methylbenzoyl)imidazo[1,2-a]pyridin-2-yl)acetamide (**6c**)*

Compound **6c** was prepared starting from 2-fluoro-5-methylbenzaldehyde as electrophile and isolated as a yellow solid (128 mg, 35% yield); m.p. 208–210 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.36 (s, 3H, CH_3), 7.29 (dd, 1H, J = 1.5 and 8.5 Hz), 7.43 (dd, 1H, J = 2.0 and 4.5 Hz), 7.49 (qddd, 1H, J = 0.5, 2.0, 7 and 8.5 Hz), 7.63 (t, 1H, J = 1 and 2 Hz), 7.69 (dd, 1H, J = 2 and 7.5 Hz), 8.41 (s, 1H), 9.16 (dd, 1H, J = 1 and 1.5 Hz), 12.58 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 20.0 (CH_3), 104.31 (CH), 115.56 (CH), 116.10 (d, CH, J = 22.12 Hz), 123.10 (C), 123.8 (CH), 125.6 (C), 130.4 (CH), 133.48 (CH), 133.9 (d, CH, J = 7.29 Hz), 134.12 (C), 140.7 (C), 142.0 (C), 154.12 (q, J = 77.3 Hz), 157.45 (d, J_{CF} = 246.75 Hz), 189.82 (C). ^{19}F NMR (282.2 MHz, DMSO-d_6) δ = −117.28 (td, F, J = 2.0, 7 and 8.5 Hz), −72.0 (CF_3). $C_{17}H_{11}F_4N_3O_2$, MW calcd. 365.28. Mass (m/z, %): 365 (M^+, 100), 296 (35), 256 (20), 137 (22). IR (KBr, cm^{-1}): 3258, 3093, 1718, 1615, 1564, 1212, 1147.

*4.1.9. 2,2,2-Trifluoro-N-(6-(2-fluoro-5-(trifluoromethyl)benzoyl)-imidazo[1,2-a]pyridin-2-yl)acetamide (**6d**)*

Compound **6d** was prepared starting from 2-fluoro-5-trifluoromethylbenzaldehyde as electrophile and isolated as a yellow solid (126 mg, 30% yield); m.p. 145–147 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.25 (dd, 1H, J = 1.5 and 8.5 Hz), 7.39 (dd, 1H, J = 2.0 and 4.5 Hz), 7.46 (qddd, 1H, J = 0.5, 2.0, 7 and 8.5 Hz), 7.53 (t, 1H, J = 1 and 2 Hz), 7.69 (dd, 1H, J = 2 and 7.5 Hz), 8.41 (s, 1H), 9.12 (dd, 1H, J = 1 and 1.5 Hz), 12.48 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 104.19 (CH), 115.26 (CH), 115.80 (d, CH, J = 22.12 Hz), 123.10 (C), 123.8 (CH), 124.10 (q, J = 246.75 Hz) 126.6 (C), 131.4 (CH), 133.48 (CH), 133.9 (d, CH, J = 7.29 Hz), 134.12 (C), 141.2 (C), 142.2 (C), 153.9 (q, J = 246.75 Hz), 157.45 (d, J = 77.3 Hz), 188.61 (C). $C_{17}H_8F_7N_3O_2$, MW calcd. 419.25. Mass (m/z, %): 419 (M^+, 100), 400 (15), 350 (95). IR (KBr, cm^{-1}): 3014, 2836, 1725, 1670, 1492, 1217.

*4.1.10. 2,2,2-Trifluoro-N-(6-(2-fluoro-4-(trifluoromethyl)benzoyl)-imidazo[1,2-a]pyridin-2-yl)acetamide (**6e**)*

Compound **6e** was prepared starting from 2-fluoro-4-trifluoromethylbenzaldehyde as electrophile and isolated as a yellow solid (134 mg, 32% yield); m.p. 100–102 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.53 (d, 1H, *J* = 9 Hz), 7.55 (d, 1H, *J* = 9 Hz), 7.63 (d, 1H, *J* = 8.5 Hz), 7.73 (t, 1H, *J* = 7 Hz), 7.77 (dt, 1H, *J* = 1 and 9 Hz), 8.26 (s, 1H), 11.20 (bs, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 103.73 (CH), 114.19 (CH), 115.57 (q, CF_3, *J* = 289.9 Hz), 116 (CH), 121.85 (CH), 122.5 (q, CF_3, *J* = 289.9 Hz), 124 (C), 125 (CH), 129.18 (C), 131.30 (CH), 131.38 (CH), 135.6 (C), 140.8 (C), 142.70 (C), 154.7 (CO), 159.30 (d, J_{CF} = 254.4 MHz), 188.3 (CO). $C_{17}H_8F_7N_3O_2$, MW calcd. 419.25. Mass (*m*/*z*, %): 419 (M^+, 100), 400 (15), 350 (95), 191 (63). IR (KBr, cm^{-1}): 3033, 2866, 2832, 1726, 1630, 1572, 1332, 1217, 1137.

4.1.11. (6-Chloropyridin-3-yl)(1-methyl-1H-indol-3-yl) methanone (**7a**)

A suspension of aluminium(III)chloride (10.67 g, 80 mmol) in anhydrous dichloromethane (15 mL) was cooled to 0 °C and 6-chloronicotinyl chloride (7.74 g, 44 mmol) was slowly added over 5 min. The resulting mixture was then allowed to warm to r.t. over 30 min. The resulting suspension was then re-cooled to 0 °C and a solution of *N*-methylindole (5 g, 40 mmol) in dichloromethane (25 mL) was added dropwise over 15 min and then heated to reflux for 4 h. The reaction mixture was poured onto ethyl acetate (300 mL) and NaOH 2 N (100 mL) was added. Resulting precipitate was filtered and the organic phase was dried over $NaSO_4$. Solvents were removed *in vacuo* and the solid was purified by silica gel column chromatography (9.7 g, 90% yield), m.p. 89 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.88 (s, 3H, CH_3), 7.37 (t, 1H, *J* = 4.8 Hz), 7.40 (t, 1H, *J* = 5.2 Hz), 7.45 (d, 1H, *J* = 0.8 Hz), 7.49 (d, 1H, *J* = 0.8 Hz), 7.53 (s, 1H), 8.10 (dd, 1H, *J* = 2.4 and 5.8 Hz), 8.39 (dt, 1H, *J* = 1.6 Hz), 8.81 (d, 1H, *J* = 2.4 Hz). $C_{15}H_{11}ClN_2O$, MW calcd. 270.71. Mass (*m*/*z*, %): 270 (M^+, 78), 158 (100). IR (KBr, cm^{-1}): 3048, 1631, 1524, 1364, 1100.

4.1.12. (6-Chloropyridin-3-yl)(1-methyl-1H-imidazol-2-yl)methanone (**7b**)

1-Methyl-1H-pyrrole (2.07 g, 25.21 mmol) was dissolved in anhydrous dichloromethane (50 mL) and cooled to −60 °C under argon atmosphere. To the stirred solution was added dropwise *n*-Butyllithium 1.6 M (18.9 mL, 30.25 mmol) over a 15 min period. After 1 h at −60 °C a solution of Weinreb amide 6-chloro-N-methoxy-N-methylnicotinamide (6.06 g, 30.25 mmol) in dichloromethane (20 mL) was added dropwise maintaining the temperature below −50 °C. The mixture was stirred for 4 h at r.t. before quenching with a saturated ammonium chloride solution (20 mL). Organic layer was dried over Na_2SO_4 and purified by silica gel column chromatography to yield **7b** (4.6 g, 82% yield) m.p. 104–105 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 4.11 (s, 3H, CH_3), 7.17 (s, 1H), 7.26 (d, 1H, *J* = 1.8 Hz), 7.44 (dd, 1H, *J* = 0.6 and 8.2 Hz), 8.64 (dd, 1H, *J* = 2.6, 5.8 Hz), 9.29 (dd, 1H, *J* = 0.6 and 1.8 Hz). $C_{10}H_8ClN_3O$, MW calcd. 221.64. Mass (*m*/*z*, %): 220 (M^+ − 1, 100), 192 (85), 186 (55). IR (KBr, cm^{-1}): 3106, 1642, 1406, 1262, 1180, 900, 776.

4.1.13. (6-Chloropyridin-3-yl)(1H-imidazol-2-yl)methanone (**7c**)

6-Chloronicotinyl chloride (544.6 mg, 8 mmol) is added dropwise to a solution of imidazole (2.82 g, 16 mmol) and triethylamine (1.62 g, 16 mmol) in pyridine (5 mL) at 0 °C under Nitrogen. The mixture was stirred for 4 h at r.t. and then treated with 2 N NaOH (80 mL). Subsequent refluxing for 1 h, and addition of water (10 mL), and cooling leads to precipitation of the product which was purified by column chromatography. White solid (997 mg, 60% yield), m.p. 192–193 °C.

^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ = 7.46 (d, 1H, *J* = 0.6), 7.48 (s, 1H), 7.51 (d, 1H, *J* = 0.6), 8.88 (dd, 1H, *J* = 2.4 and 6.0 Hz), 9.45 (dd, 1H, *J* = 0.6 and 1.8). $C_9H_6ClN_3O$, MW calcd. 207.62. Mass (*m*/*z*, %): 207 (M^+, 20), 179 (100), 144 (35). IR (KBr, cm^{-1}): 3275, 3114, 1639, 1409, 1135, 776.

4.1.14. (6-Aminopyridin-3-yl)(1-methyl-1H-indol-3-yl)methanone (**8a**)

Anhydrous NH_3 (15 mL) was added to a suspension of **7a** (541.5 mg, 2 mmol) in ethanol (15 mL) in a Parr reactor, the mixture was stirred at 150 °C for 8 h. Excess ammonia was permitted to evaporated and the product was purified by silica gel column chromatography (422 mg, 84% yield) m.p. 222–224 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 3.87 (s, 3H, CH_3), 6.52 (d, 1H, *J* = 8.8 Hz), 6.65 (bs, 2H, NH_2), 7.22 (t, 1H, *J* = 5.8 Hz), 7.29 (t, 1H, *J* = 5.2 Hz), 7.54 (dd, 1H, *J* = 1.8 and 5.4 Hz), 7.84 (dd, 1H, *J* = 2.4 and 6.2 Hz), 8.07 (s, 1H), 8.19 (dd, 1 H, *J* = 1.6 and 4.6 Hz), 8.45 (d, 1H, *J* = 2.2). $C_{15}H_{13}N_3O$, MW calcd. 251.28. Mass (*m*/*z*, %): 251 (M^+, 100), 158 (70). IR (KBr, cm^{-1}): 3213, 3044, 1586, 1398, 1371.

4.1.15. (6-Aminopyridin-3-yl)(1-methyl-1H-imidazol-2-yl)-methanone (**8b**)

Compound **8b** was prepared as described for **8a**, starting from (6-chloropyridin-3-yl)(1-methyl-1H-imidazol-2-yl)methanone **7b**, and purified by silica gel column chromatography (323 mg, 80% yield), m.p. 210–211 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 3.93 (s, 3H, CH_3), 6.46 (dd, 1H, *J* = 0.8, 8.2 Hz), 6.94 (s, 2H, NH_2), 7.14 (d, 1H, *J* = 1.0 Hz), 7.49 (d, 1H, *J* = 0.8 Hz), 8.21 (dd, 1H, *J* = 2.4 and 6.4 Hz), 9.02 (dd, 1H, *J* = 0.4 and 1.8 Hz). $C_{10}H_{10}N_4O$, MW calcd. 202.1.

4.1.16. (6-Aminopyridin-3-yl)(1H-imidazol-2-yl)methanone (**8c**)

Compound **8c** was prepared as described for **8a**, starting from (6-chloropyridin-3-yl)(1H-imidazol-2-yl)methanone **7c**, and purified by silica gel column chromatography (320 mg, 85% yield), m.p. 225 °C.

^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ = 5.56 (bs, 2H), 6.56 (dd, 1H, *J* = 0.6 and 8.4 Hz), 7.28 (bs, 2H), 8.66 (dd, 1H, *J* = 2.2 and 6.4 Hz), 9.34 (d, 1H, *J* = 1.8 Hz), 12.49 (bs, 1H). $C_9H_8N_4O$, MW calcd. 188.19. Mass (*m*/*z*, %): 188 (M^+, 55), 160 (70), 121 (35). IR (KBr, cm^{-1}): 3447, 3303, 2919, 1604, 1411, 1314, 1107.

4.1.17. 4-Methyl-N-(5-(1-methyl-1H-indole-3-carbonyl)pyridin-2-yl) benzenesulfonamide (**9a**)

(6-Aminopyridin-3-yl)(1-methyl-1H-indol-3-yl)methanone **8a** (628.2 mg, 2.5 mmol) was dissolved in anhydrous pyridine (10 mL). *p*-Toluensulphonyl chloride (567.4 mg, 3 mmol) was added and the solution was heated at 90 °C under Nitrogen overnight. Pyridine was removed in vacuum to yield a brown solid. Water (20 mL) was added and the mixture was stirred for 1 h. The solid was collected and dried in vacuum to give **9a** as a white solid (882 mg, 87% yield), m.p. 205 °C.

^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ = 2.40 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 7.26–7.41 (m, 6H), 7.62 (s, 1H), 7.89 (dd, 2H, *J* = 1.6 and 4.8 Hz), 8.03 (dd, 1H, *J* = 2.4 and 6.4 Hz), 8.37 (m, 1H), 8.59 (bs, 1H). $C_{22}H_{19}N_3O_3S$, MW calcd. 405.47.

4.1.18. 4-Methyl-N-(5-(1-methyl-1H-imidazole-2-carbonyl)-pyridin-2-yl)benzenesulfonamide (**9b**)

Compound **9b** was prepared as described for **9a**, starting from **8b** and was obtained as a brown solid (731 mg, 82% yield) m.p. 180 °C.

^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ = 2.39 (s, 3H, CH_3), 4.04 (s, 3H, NCH_3), 7.12 (s, 1H), 7.18 (s, 1H), 7.26 (d, 2H, *J* = 8 Hz), 7.35 (bs, 1H, NH), 7.86 (d, 2H, *J* = 8.2 Hz), 8.47 (dd, 1H, *J* = 2.2 and 7 Hz), 9.30 (d, 1H, *J* = 2.2). $C_{17}H_{16}N_4O_3S$, MW calcd. 356.4.

4.1.19. N-(5-(1H-imidazole-2-carbonyl)pyridin-2-yl)-4-methyl-benzenesulfonamide (**9c**)

Compound **9c** was prepared as described for **9a**, starting from **8c** and was obtained as a white solid (642 mg, 75% yield), m.p. 176 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.34 (s, 3H, CH_3), 7.27 (dd, 1H, J = 0.8 and 7.2 Hz), 7.28 (d, 1H, J = 7.2), 7.35 (d, 2H, J = 8), 7.52 (dd, 1H, J = 0.8, 1.8 Hz), 7.80 (d, 2H, J = 8.2 Hz), 8.49 (dd, 1H, J = 2.2 and 7.2 Hz), 9.40 (d, 1H, J = 1.6 Hz), 13.50 (bs, 1H). $C_{16}H_{14}N_4O_3S$, MW calcd. 342.37. Mass (*m*/*z*, %): 342 (M^+ + 1, 5), 277 (100).

4.1.20. (Z)-2-(5-(1-methyl-1H-indole-3-carbonyl)-2-(tosylimino)-pyridin-1(2H)-yl)acetamide (**10a**)

To a suspension of **9a** (446 mg, 1.1 mmol) in anhydride DMF (5 mL) was added *i*-Pr_2Net (2.1 mL, 1.21 mmol) under argon. To the solution was added 2-iodoacetamide (219 mg, 1.2 mmol) and the mixture was stirred at r.t. overnight. The solution was poured onto water (20 mL), filtered, washed with water (50 mL) and dried in vacuum to give **10a** as a yellow solid (382 mg, 75% yield), m.p. 275 °C.

^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ = 2.39 (s, 3H, CH_3), 3.88 (s, 3H, NCH_3), 4.97 (s, 2H, NH_2), 6.47 (bs, 1H, R_2NH), 7.23–7.42 (m, 5H), 7.60 (d, 1H, J = 9.4), 7.74 (bs, 1H), 7.81 (d, 1H, J = 8.2), 7.84 (s, 1H), 8.0 (dd, 1H, J = 2.2 and 7.2 Hz), 8.27 (d, 1H, J = 2.4), 8.31 (d, 1H, J = 1.8). $C_{24}H_{22}N_4O_4S$, MW calcd. 462.52.

4.1.21. (Z)-2-(5-(1-methyl-1H-imidazole-2-carbonyl)-2-(tosylimino)pyridin-1(2H)-yl)acetamide (**10b**)

Compound **10b** was prepared as described for **10a**, starting from **9b** and was obtained as a white solid (355 mg, 78% yield), m.p. 218–220 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.34 (s, 3H, CH_3), 3.96 (s, 3H, NCH_3), 4.93 (bs, 2H), 7.21 (d, 1H, J = 0.8 Hz), 7.29 (d, 1H, J = 0.4 Hz), 7.37 (s, 1H), 7.42 (bs, 1H, NH), 7.61 (d, 1H, J = 0.4), 7.7 (d, 2H, J = 8.4 Hz), 7.82 (bs, 1H), 8.46 (dd, 1H, J = 2.2 and 9.6 Hz), 9.18 (d, 1H, J = 2.0 Hz). $C_{19}H_{19}N_5O_4S$, MW calcd. 413.45.

4.1.22. (Z)-2-(5-(1H-imidazole-2-carbonyl)-2-(tosylimino)pyridin-1(2H)-yl)acetamide (**10c**)

Compound **10c** was prepared as described for **10a**, starting from **9c** and was obtained as a white solid (294 mg, 67% yield) m.p. 214–215 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.34 (s, 3H, CH_3), 4.94 (s, 2H), 7.29 (d, 2H, J = 8.4 Hz), 7.43 (d, 1H, J = 4.6 Hz), 7.44 (bs, 2H), 7.45 (d, 1H, J = 5 Hz), 7.70 (d, 2H, J = 8.2), 7.84 (bs, 1H), 8.60 (dd, 1H, J = 2.2 and 7.4 Hz), 9.40 (d, 1H, J = 2.0 Hz), 13.50 (bs, 1H). $C_{18}H_{17}N_5O_4S$, MW calcd. 399.42.

4.1.23. 2,2,2-Trifluoro-N-(6-(1-methyl-1H-indole-3-carbonyl)-imidazo[1,2-a]pyridin-2-yl)acetamide (**11a**)

To a suspension of **10a** (463.5 mg, 1 mmol) in anhydrous dichloromethane (10 mL) was added trifluoroacetic anhydride until dissolution and the solution was refluxed for 6 h. Solvents were removed in vacuum and the solid was purified by silica gel column chromatography (278 mg, 72% yield), m.p. 235–237 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.90 (s, 3H, CH_3), 7.29 (td, 1H, J = 1.0, 1.5 and 7.0 Hz), 7.34 (td, 1H, J = 1.5, 7 and 7 Hz), 7.59 (dd, 1H, J = 1.5 and 7 Hz), 7.61 (d, 1H, J = 9.5 Hz), 7.67 (dd, 1H, J = 1.5 and 9.5 Hz), 8.26 (td, 1H, J = 0.5, 1.5 and 7.0 Hz), 8.29 (s, 1H), 8.36 (s, 1H), 9.22 (dd, 1H, J = 1.0, 1.5 Hz), 12.55 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 33.23 (CH_3), 103.85 (CH), 110.67 (CH), 113.51 (C), 115.36 (CH), 115.65 (q, J_{CF} = 288.25), 121.50 (CH), 122.32 (CH), 123.26 (CH), 125.16 (CH), 125.38 (C), 126.64 (C), 129.61 (CH), 137.36 (C), 139.55 (CH), 140.13 (C), 141.58 (C), 154.00 (q, J_{CF} = 37.83), 185.54 (CO). $C_{19}H_{13}F_3N_4O_2$, MW calcd. 386.33.

4.1.24. 2,2,2-Trifluoro-N-(6-(1-methyl-1H-imidazole-2-carbonyl)-imidazo[1,2-a]pyridin-2-yl)acetamide (**11b**)

To a suspension of **10b** (415 mg, 1 mmol) in anhydrous dichloromethane (10 mL) was added trifluoroacetic anhydride until dissolution and the solution was refluxed for 5 h. Solvents were removed in vacuum and the solid was purified by silica gel column chromatography (202 mg, 60% yield) m.p. 218–220 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.0 (s, 3H, CH_3), 7.25 (s, 1H), 7.57 (d, 1H, J = 9.5 HZ), 7.63 (s, 1H), 7.98 (dd, 1H, J = 1.5 and 9.5 Hz), 8.44 (s, 1H), 9.83 (t,), 12.54 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 36.5 (CH_3), 104.60 (CH), 114.98 (CH), 116.1 (q, J_{CF} = 287.25), 123.0 (C), 141.1 (C), 142.25 (C), 142.52 (C), 154.54 (d, J_{CF} = 38.38), 179.84 (CO). $C_{14}H_{10}F_3N_5O_2$, MW calcd. 337.26. Mass (*m*/*z*, %): 337 (M^+, 100), 268 (20), 240 (15). IR (KBr, cm^{-1}): 3432, 3296, 2925, 2852, 1724, 1637, 1567, 1406, 1153.

4.1.25. N-(6-(1H-imidazole-2-carbonyl)imidazo[1,2-a]pyridin-2-yl) -2,2,2-trifluoroacetamide (**11c**)

To a suspension of **10c** (400 mg, 1 mmol) in anhydrous dichloromethane (10 mL) was added trifluoroacetic anhydride until dissolution and the solution was refluxed for 5 h. Solvents were removed in vacuum and the solid was purified by silica gel column chromatography (181 mg, 56% yield), m.p 302–304 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 7.47 (s, 2H), 7.62 (d, 1H, J = 9.6), 8.13 (dd, 1H, J = 1.8 and 7.8 Hz), 8.48 (s, 1H), 10.09 (bs, 1H), 12.60 (bs, 1H). $C_{13}H_8F_3N_5O_2$, MW calcd. 323.2. Mass (*m*/*z*, %): 323 (M^+, 100), 254 (75), 226 (40).

4.2. Biology

4.2.1. Cell culture and cytotoxicity assays

All tested substances were dissolved in DMSO and diluted to tested concentrations. Cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine at 37 °C under a 5% CO_2 atmosphere. For each cell line, 70% confluent cell culture flask was trypsinized and cells were seeded in 96 well plates at a density of 5000 cells/well; 24 h after seeding, the cells were treated with the different compounds for 48 h and viability was accessed by sulphorhodamine B (SRB) method.

When incubation with tested compounds was finished, adherent cell cultures were fixed out in situ by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid and incubated at 4 °C for 1 h. The supernatant was discarded and the plates were washed with water and left dry to the air. The fixed cells were stained with 100 μL of 0.4% SRB solution. Protein-bonded dye was solubilized with 10 mM unbuffered Tris base and the optical density was achieved on a microplate reader (EI_x 808; Bio-Tek Instruments, Inc., Winooski, VT, USA) using a test wavelength of 515 nm. Preliminary screening was made at 50 μM for tested compounds using 5-Fluorouracil as a positive control. A dose response curve was plotted for each most active compound and the IC_{50} was estimated from non-linear regression using JMP software (version 3.2.1; SAS Institute Inc., Cary, NC, USA).

4.2.2. Cell cycle analysis

Log phase MCF7 and SK-LU-1 cells were seeded out using RPMI-1640 + 10% FBS at 1×10^6 cells per 10 cm petri dish, and the cells were allowed to attach for 24 h at 37 °C. The compounds were added to the cells and incubated for an additional 24 h. After incubation, the cells were harvested with PBS-EDTA and then centrifuged at 3000 r.p.m. for 5 min. The PBS-EDTA was removed, and the cell pellet was washed with 1× PBS, followed by centrifugation at 3000 r.p.m. for 5 min. The supernatant was discarded, and the pellet was resuspended in 5 mL of ice-cold 70% ethanol. The cells were then held at −20 °C for 24–48 h. The ethanol-fixed cells were centrifuged at 3000 r.p.m. for 5 min, the supernatant was removed, and the cell pellet was washed with 1× PBS. Following another centrifugation at 3000 r.p.m. for 5 min, the PBS was removed, and each sample pellet was mixed with 1 mL of staining

solution (0.1% Triton X-100, 0.2 mg/mL DNase-free RNase A, 20 μg/mL propidium iodide in PBS). The cells DNA content of cells was measured on a FACScan Flow Cytometer (Becton Dickinson, USA) and analysis was made with Flowjo software V 7.2.5 (Tree Star Inc., Ashland, OR, USA).

4.2.3. Apoptosis

Apoptosis was performed by determining phosphatidylserine exposure on the cell membrane and DNA fragmentation. Cells were labeled with fluorescein isothiocyanate-conjugated annexin V and propidium iodide (PI) in binding buffer (Sigma–Aldrich, St. Louis, MO, USA), and analyzed using a FACScan Flow Cytometer (Becton Dickinson, USA) with Flowjo software V 7.2.5 (Tree Star Inc., Ashland, OR, USA).

4.2.4. Kinase assays

All reactions were run in 20 μL containing 2.5% DMSO for 30 min at 37 °C and 7 μM of each tested compound. The cyclin B/CDK-1 assay conditions were 50 mM Tris–HCl (pH = 7.4), 10 mM $MgCl_2$, 50 mM NaF, 500 μM Na_3VO_4, 8 mM β-glicerophosphate, 1 mM DTT, 400 μM. ATP (Sigma–Aldrich, St. Louis, MO, USA), 4 μCi $ATP^{\gamma 32}$, 250 ng recombinant Histone 1.2 (Calbiochem, San Diego, CA, USA), and 220 ng cyclin B/CDK-1 (Biofin, Kassel, Germany) enzyme. The cyclin E/CDK-2 assay conditions were 50 mM Tris–HCl (pH = 7.4), 10 mM $MgCl_2$, 50 mM NaF, 500 μM Na_3VO_4, 8 mM β-glicerophosphate, 1 mM DTT, 400 μM. ATP (Sigma–Aldrich, St. Louis, MO, USA), 4 μCi $ATP^{\gamma 32}$, 250 ng recombinant Histone 1.2 (Calbiochem, San Diego, CA, USA), and 135 ng cyclin E/CDK-2 (Biofin, Kassel, Germany) enzyme. All reactions were terminated with 5 μL β-Merchaptoethanol and the samples were resolved by 10% poliacrilamide gels and revealed in a Typhoon 9400 (Amersham Biosciences, Inc.). Band quantifications were made using a Kodak MI software.

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