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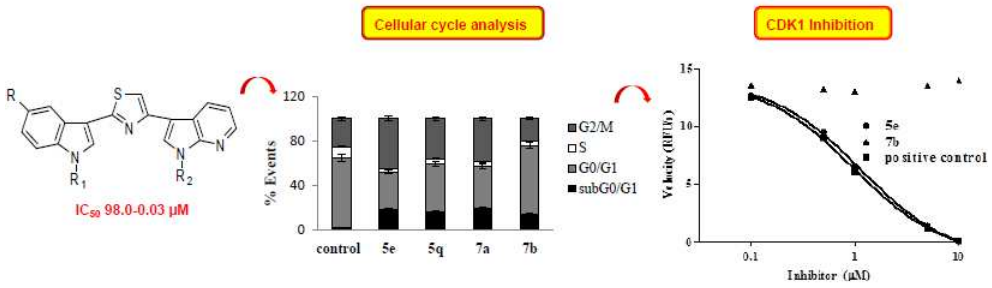

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Synthesis, antitumor activity and CDK1 inhibiton of new thiazole nortopsentin analogues

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Abstract

A new series of thiazole nortopsentin analogues was conveniently synthesized with fair overall yields. The antiproliferative activity of the new derivatives was tested against different human tumor cell lines of the NCI full panel. Four of them showed good antitumor activity with GI_{50} values from micro to nanomolar level. The mechanism of the antiproliferative effect of these derivatives, was pro-apoptotic, being associated with externalization of plasma membrane phosphatidylserine and DNA fragmentation. The most active and selective of the new thiazoles confined viable cells in G2/M phase and markedly inhibited *in vitro* CDK1 activity.

Keywords: Marine alkaloids, nortopsentin analogues, antiproliferative activity, apoptosis, CDK1 inhibitors, thiazolyl-1*H*-pyrrolo[2,3-*b*]pyridines.

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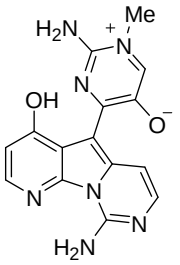
1. Introduction

In recent years, many efforts have been done to develop new small molecules active as antitumor agents [1-7] as well as to discovery new targets for cancer treatment. Human kinases remain interesting targets in oncology [8] and, among 518 human kinases, the family of cyclin-dependent kinases (CDKs) has attracted considerable interest due to their involvement in several pathological processes such as cancer [9]. Marine environment is an important source of secondary metabolites, among them numerous alkaloids acting as CDK inhibitors.

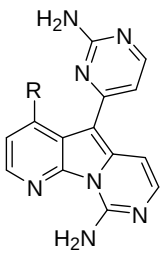
Variolins, isolated from the rare Antarctic sponge *Kirkpatrickia variolosa*, are marine alkaloids with an unique pyrido[3',2':4,5]pyrrolo[1,2-*c*]pyrimidine core (Chart 1). Four variolins were isolated: variolin A, variolin B, N(3')-methyltetrahydrovariolin B and variolin D [10,11]. Variolin B and its synthetic analogue, deoxyvariolin B, showed *in vitro* activity against P388 cell line with IC_{50} values of 210 nM and 157 nM respectively [12]. The latter compounds caused cell cycle perturbations, induced apoptosis and inhibited different cyclin-dependent kinases, in particular CDK1 and CDK2, in the micromolar range [13].

Meridianins A-G are a family of brominated and/or hydroxylated 3-(2-aminopyrimidine)indoles, which are purified from *Aplidium meridianum*, an ascidian collected in the South Atlantic [14] (Chart 1). Also these alkaloids prevented cell proliferation, induced apoptosis and resulted potent inhibitors of several protein kinases. In particular, meridianin E showed potent and selective inhibiton of CDK1 and CDK5 in the low micromolar range [15,16].

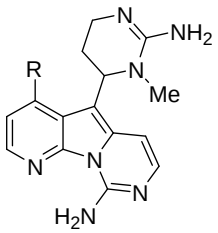
A new class of 3-(pyrimidin-4-yl)-7-azaindoles, meriolins (Chart 1), was synthesized as a chemical hybrid between the natural compounds variolins and meridianins. These new derivatives showed better antitumor and proapoptotic properties in human tumor cell cultures than their parent compounds and a relative selectivity against CDKs, especially CDK1, CDK2 and CDK9 [17,18].



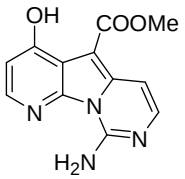
Variolin A



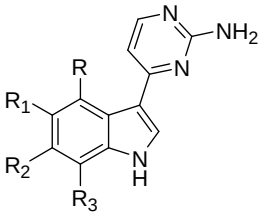
Variolin B R = OH
Deoxyvariolin B R = H



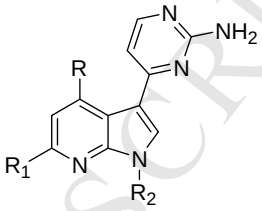
N(3')-methyltetrahydrovariolin B



Variolin D



Meridianin A R = OH; $R_1 = R_2 = R_3 = H$
Meridianin B R = OH; $R_1 = R_3 = H$; $R_2 = Br$
Meridianin C $R = R_2 = R_3 = H$; $R_1 = Br$
Meridianin D $R = R_1 = R_3 = H$; $R_2 = Br$
Meridianin E R = OH; $R_1 = R_2 = H$; $R_3 = Br$
Meridianin F $R = R_3 = H$; $R_1 = R_2 = Br$
Meridianin G $R = R_1 = R_2 = R_3 = H$



Meriolins

R = H, Cl, OMe, OEt, OPr, OiPr, $O(CH_2)_2OMe$
R_1 = H, Br
R_2 = H, Me, SO_2Ph

Chart 1. Marine alkaloids active as CDK1 inhibitors.

Nortopsentins A-C (Chart 2), isolated from the Caribbean deep sea *Spongosorites ruetzleri*, are bis-indolyl alkaloids, having an imidazole as a spacer between the two indole units, with an unknown mechanism of action. They displayed *in vitro* cytotoxicity against P388 cells (IC_{50} 4.5-20.7 µM) and their *N*-methylated derivatives showed a significant improvement in P388 activity compared to that of the parent compounds (IC_{50} 0.8-2.1 µM) [19,20]. Due to its interesting antitumor activity, nortopsentin has been considered an important lead compound for the design of new biologically active derivatives. Analogues in which the imidazole ring of the alkaloid was replaced by other five membered heterocycles such as bis-indolyl-thiophenes [21], -thiazoles [22], -pyrazoles [23], -furans and isoxazoles [24], -pyrroles [25], and -1,2,4-thiadiazoles [26] were reported. Most of them showed antiproliferative activity against a wide range of human tumor cell lines with GI_{50} in the micromolar to sub-micromolar range. In literature are reported many analogues, in which beside the imidazole core, one or both indole units were replaced by phenyl, azaindole or naphthyl moieties [27-33]. In particular, 3-[(2-indolyl)-5-phenyl]-pyridines **1** [27] and phenyl-thiazolyl-7-azaindoles **2** (Chart 2) [28] showed cytotoxic activity in the micro-nanomolar range and inhibited CDK1 with IC_{50} values of 0.31-0.90 and 0.41-0.85 µM, respectively. Indolyl-thiazolyl-4-azaindole **3** and indolyl-thiazolyl-7-azaindole **4** derivatives (Chart 2) were active against a wide range of human

tumor cell lines in the micro-submicromolar range, including STO and Meso II cell lines derived from human diffuse malignant peritoneal mesothelioma, a rapidly lethal human malignancy generally refractory to currently available therapies. These azaindole analogues were also able to inhibit CDK1 with IC_{50} values of 0.64-0.89 μM, comparable to those reported for roscovitine and purvalanol A, two well-known CDK1 inhibitors [29,30]. Moreover, in the mouse model, intraperitoneal administration of some derivatives of type **4** resulted in a significant tumor volume inhibition of DMPM xenografts (58-75%) at well-tolerated doses, and two complete responses were observed in each treatment group [30].

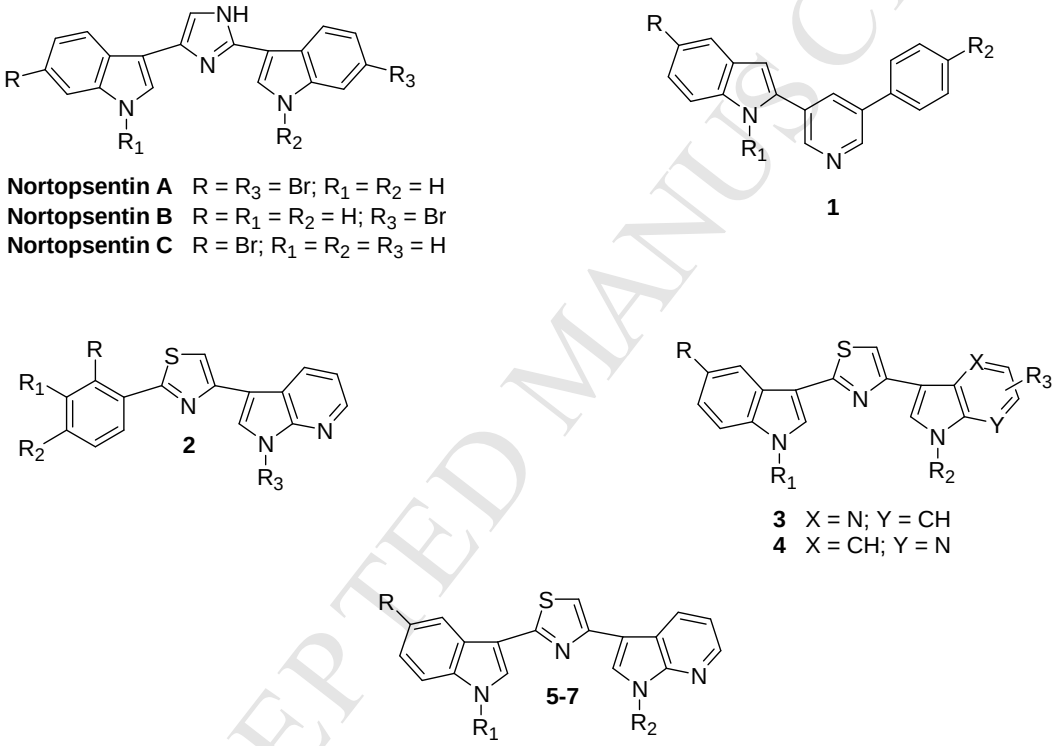


Chart 2. Nortopsentins and their analogues able to inhibit CDK1.

Continuing our studies on nitrogen heterocyclic systems endowed with antitumor activity [34-41], herein we report a new series of thiazoles **5-7** (Chart 2), analogues of derivatives **4**, in which the nitrogen atom of the indole and/or 7-azaindole moiety is decorated with a 2-methoxyethyl chain with the aim of increasing the water solubility. We also describe the cytotoxic activity of the new derivatives against different human tumor cell lines as well as their capacity to inhibit CDK1.

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2. Chemistry

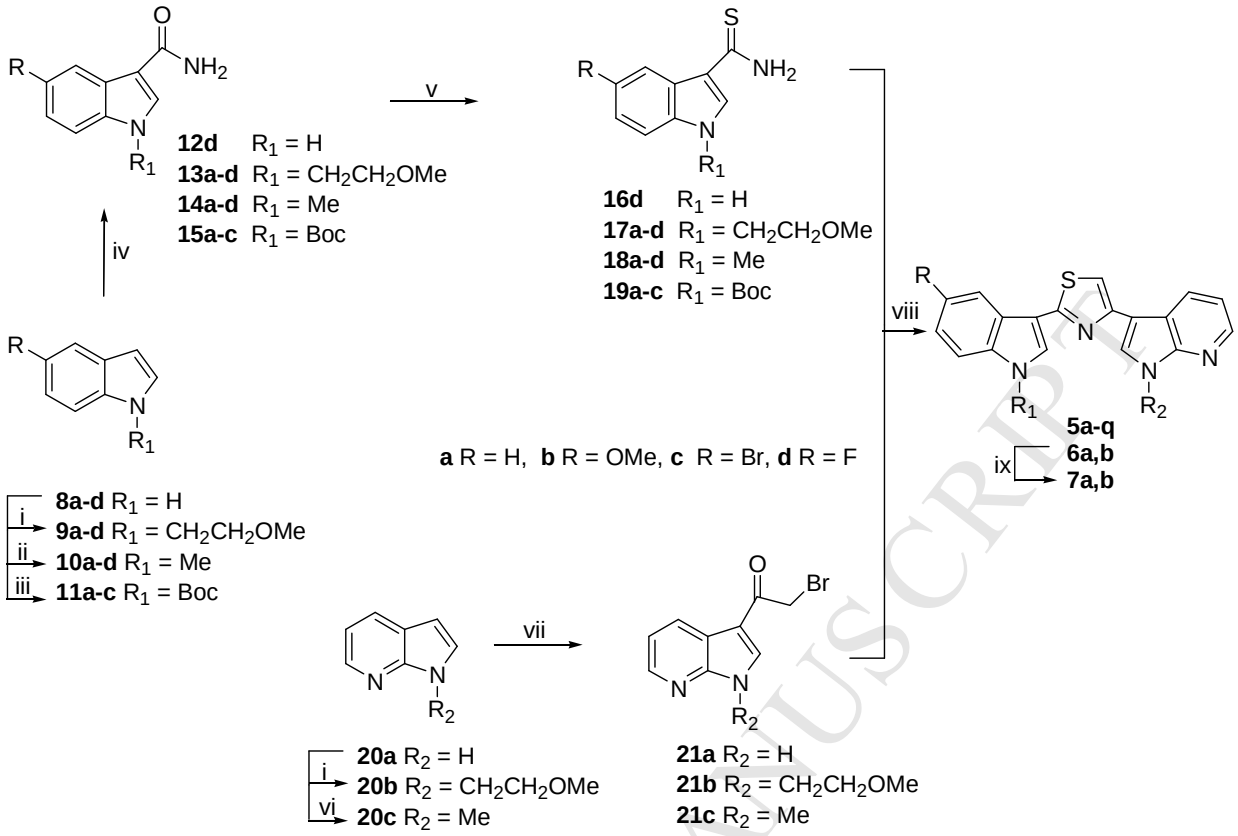
Our plan for the synthesis of new substituted thiazoles **5a-q** and **6a**,**b** was based on the Hantzsch reaction between thioamides **16d**, **17a-d**, **18a-d** and **19a-c** and *α*-bromoacetyl derivatives **21a-c** (Scheme 1). Indole-3-carbothioamides **16d**, **18a-d** and **19a-c** were obtained from the corresponding indoles **8d**, **10a-d**, and **11a-c** through the formation of indole-3-carboxamides **12d**, **14a-d** and **15a-c** as previously reported [30].

The new thioamides **17a-d** were conveniently synthesized from the corresponding amides **13a-d** in turn prepared (65-98% yields) by reaction of 1-(2-methoxyethyl)-1*H*-indoles **9a-d** with chlorosulfonyl isocyanate (CSI) in acetonitrile at 0 °C followed by alkaline hydrolysis of the chlorosulfonyl group. The subsequent reaction of new amides **13a-d** with Lawesson's reagent under reflux in tetrahydrofuran (THF) gave the desired thioamides **17a-d** in very good yields (70-99%) (Scheme 1).

3-Bromoacetyl-7-azaindoles **21a**,**c** were obtained from the corresponding 7-azaindoles **20a**,**c** as previously reported [28]. The new 2-bromo-1-[1-(2-methoxyethyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]ethanone **21b** was efficiently synthesized (70% yield) by reaction of 1-(2-methoxyethyl)-1*H*-pyrrolo[2,3-*b*]pyridine **20b** with bromoacetyl bromide in presence of aluminum chloride in dichloromethane (Scheme 1).

1-(2-Methoxyethyl)-1*H*-indoles **9a-d** and 1-(2-methoxyethyl)-1*H*-pyrrolo[2,3-*b*]pyridine **20b** were obtained in excellent yields (81-99%) from the corresponding indoles **8a-d** or azaindole **20a** by alkylation with 2-bromoethyl methyl ether using *N*,*N*-dimethylformamide (DMF) as a solvent and sodium hydride as base. [42,43]

The final reaction between the appropriate thioamides **16d**, **17a-d**, **18a-d** and **19a**,**c** and the suitable *α*-bromoacetyl compounds **21a-c** in ethanol under reflux gave the desired new thiazoles **5a-q** and **6a**,**b** (Table 1) in good to excellent yields (56-98%). The subsequent deprotection of *N*-*tert*-butylcarboxylate derivatives **6a**,**b** by using trifluoroacetic acid (TFA) in dichloromethane (DCM) under reflux afforded, after neutralization, the corresponding *N*-deprotected thiazoles **7a**,**b** in good yields (60,72%) (Table 1). The reaction of thioamide **19b** with the 3-bromoacetyl derivative **21b** gave a very instable compound unsuitable to undergo biological assay. The crude of this latter compound was used for the next *N*-Boc deprotection but also in this case an impure deprotected derivative was obtained.



Scheme 1. Synthesis of new thiazole derivatives **5a-q**, **6a,b** and **7a,b**.

Reagents: (i) (a), NaH, DMF, 0 °C-rt, 30 min; (b) $BrCH_2CH_2OMe$, 60 °C, 24 h, 81-99%; (ii) and (iii) for compounds **10a-d** and **11a-c**: see reference [30]; (iv) for compounds **12d**, **14a-d** and **15a-c**: see reference [30]; for compounds **13a-d**: (a) CSI, MeCN, 0 °C, 30 min; (b) 10% KOH, aq acetone, 65-98%; (v) for compounds **16d**, **18a-d** and **19a-c**: see reference [30]; for compounds **17a-d**: Lawesson's reagent, THF, reflux, 30 min, 70-99%; (vi) for compound **20c**: see reference [28]; (vii) for compounds **21a**,**c**: see reference [28]; for compound **21b**: $AlCl_3$, DCM, $BrCH_2COBr$, reflux, 40 min, 70%; (viii) EtOH, reflux, 30 min, 56-98%; (ix) (a) TFA, DCM, reflux, 24 h; (b) aq $NaHCO_3$, 60,72%.

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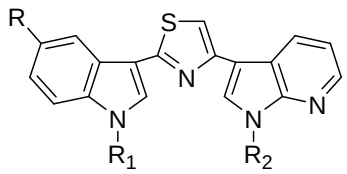
Table 1. New thiazole derivatives **5a-q**, **6a**,**b** and **7a**,**b**.



Compd	R	R_1	R_2	Compd	R	R_1	R_2
5a	H	CH_2CH_2OMe	H	**5l**	Br	Me	CH_2CH_2OMe
5b	H	CH_2CH_2OMe	Me	**5m**	F	CH_2CH_2OMe	H
5c	H	CH_2CH_2OMe	CH_2CH_2OMe	**5n**	F	CH_2CH_2OMe	Me
5d	H	Me	CH_2CH_2OMe	**5o**	F	CH_2CH_2OMe	CH_2CH_2OMe
5e	OMe	CH_2CH_2OMe	H	**5p**	F	Me	CH_2CH_2OMe
5f	OMe	CH_2CH_2OMe	Me	**5q**	F	H	CH_2CH_2OMe
5g	OMe	CH_2CH_2OMe	CH_2CH_2OMe	**6a**	H	Boc	CH_2CH_2OMe
5h	OMe	Me	CH_2CH_2OMe	**6b**	Br	Boc	CH_2CH_2OMe
5i	Br	CH_2CH_2OMe	H	**7a**	H	H	CH_2CH_2OMe
5j	Br	CH_2CH_2OMe	Me	**7b**	Br	H	CH_2CH_2OMe
5k	Br	CH_2CH_2OMe	CH_2CH_2OMe				

3. Results and Discussion

3.1 Cytotoxic activity

All the synthesized thiazoles **5a-q**, **6a**,**b** and **7a**,**b** were submitted to the National Cancer Institute (NCI; Bethesda, MD), and derivatives **5a-h**, **l**, **o-q**, **6a**,**b** and **7a**,**b** were prescreened according to the NCI protocol at a 10^{-5} M dose (data not shown) on the full panel of approximately 60 human cancer cell lines derived from 9 human cancer cell types that have been grouped into disease subpanels including leukemia, non-small cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast tumor cell lines. Compounds **5e**,**q** and **7a**,**b** satisfied the criteria set by the NCI for activity in this assay and were selected for further screenings at five concentrations at 10-fold dilution (10^{-4}−10^{-8} M) on the full panel. The growth inhibition activity of compounds was defined in terms of the GI_{50} value (which represents the molar concentration of the compound that inhibits 50% net cell growth). The average values of the mean graph midpoint (MG_MID) was calculated.

Data reported in Table 2 revealed that thiazoles **5e**,**q** and **7a**,**b** showed antiproliferative activity in the micromolar to nanomolar range (GI_{50} 0.03-98.0 µM). Compounds **5e** and **7b** were active against the total number of cell lines investigated, whereas compounds **5q** and **7a** were cytotoxic against a very high percentage of the tested cell lines (96% and 93% respectively). The most active compound was thiazole **5e** (MG_MD 3.6 µM) followed by thiazoles **7b**, **7a** and **5q** (MG_MD 4.5, 5.4 and 6.3 µM respectively).

Table 2. Overview of the in vitro antitumor screening[a] results for compounds **5e**,**q** and **7a**,**b**.

Compd	N_{cl}[c]	N[d]	GI_{50}[b] Range	GI_{50}[b] MG_MID[e]
5e	57	57	0.03-12.6	3.6
5q	53	51	0.65-98.0	6.3
7a	55	51	0.42-28.2	5.4
7b	54	54	1.79-13.9	4.5

[a] Data obtained from the NCI in vitro disease-oriented human tumor cell line screen. [b] Concentration (μM) that inhibits 50% net cell growth. [c] Number of cell lines investigated. [d] Number of cell lines giving positive GI_{50} values. [e] MG_MID: mean graph midpoint; this is the arithmetic mean value for all tested cancer cell lines.

Derivative **5e** was particularly efficacious against the breast cancer sub-panel (Table 3) having GI_{50} in the range 0.03-2.38 μM. The most sensitive breast cancer cell lines were MDA-MB-468 (GI_{50} 0.03 μM), T-47D (GI_{50} 0.04 μM) and MCF-7 (GI_{50} 0.05 μM). Moreover, thiazole **5e** exhibited good selectivity with respect to the non-small cell lung cancer (GI_{50} 0.07-5.18 μM) and ovarian cancer (GI_{50} 0.03-4.66 μM) sub-panels and to the KM12 (GI_{50} 0.16 μM) of the colon cancer sub-panel.

Thiazoles **5q** and **7a** (Table 3) showed good selectivity against KM12 cell line (GI_{50} 1.77 and 0.76 μM respectively) of the colon cancer sub-panel, MDA-MB-435 cell line (GI_{50} 0.90 and 0.86 μM, respectively) of the melanoma sub-panel and T-47D cell line (GI_{50} 0.65 and 0.42 μM, respectively) of the breast cancer sub-panel.

In spite of the low number of derivatives that underwent the test on the NCI full panel of human cell lines, it is possible to outline some SAR. The most active compound is **5e** (MG_MD 3.6 μM) which bears an unsubstituted azaindole portion, a methoxy group at position 5 and a methyloxyethyl substituent at the imino nitrogen of the indole moiety. The introduction of a methyl or a methyloxyethyl group at the imino nitrogen of the azaindole moiety as well as the replacement of the methoxy group with a hydrogen led to inactive compounds (**5f**, **5g** and **5a** did not satisfied criteria to be tested on the full panel). Moving the methyloxyethyl substituent from the indole to the azaindole moiety maintained the activity, although slightly lower. However, also in this series the introduction of a methyl group on the imino nitrogen of the indole moiety dropped the activity (**5d**, **5l** and **5p** did not satisfied criteria to be tested on the full panel).

Table 3. *In vitro* inhibition (μM) of cancer cell line growth by compounds **5e**,**q** and **7a**,**b**.

Cell line	**GI_{50}[a]**				***Cell line***	**GI_{50}[a]**			
	5e	**5q**	**7a**	**7b**		**5e**	**5q**	**7a**	**7b**
Leukemia					***Melanoma***				
CCRF-CM	6.70	-	>100	-	LOX IMVI	3.69	3.44	3.96	2.67
K-562	4.79	2.12	2.80	3.60	MALME-3M	12.6	4.54	3.58	3.66
MOLT-4	6.02	5.56	-	3.87	M14	5.82	3.14	3.22	3.27
RPMI-8226	7.09	-	-	5.14	MDA-MB-435	6.41	0.90	0.86	4.12
SR	1.28	3.24	3.02	3.25	SK-MEL-2	3.56	3.89	7.38	6.82
Non-Small Cell Lung Cancer					SK-MEL-28	7.39	7.72	8.19	4.79
A549/ATCC	4.41	4.51	7.10	6.89	SK-MEL-5	5.53	2.69	3.22	3.24
EKVX	0.07	1.98	2.00	2.05	UACC-257	4.26	-	>100	8.69
HOP-62	2.12	2.02	2.82	7.98	UACC-62	5.98	6.78	4.58	4.20
HOP-92	0.99	6.29	14.9	4.39	***Ovarian Cancer***				
NCI-H23	1.63	3.88	4.58	3.21	IGROV1	0.55	3.49	2.53	4.06
NCI-H322M	1.95	>100	>100	11.8	OVCAR-3	4.66	3.34	2.94	3.90
NCI-H460	1.32	3.38	3.40	3.07	OVCAR-4	0.03	2.59	1.76	2.74
NCI-H522	5.18	2.19	2.07	2.17	OVCAR-5	0.61	10.1	28.2	4.83
Colon Cancer					OVCAR-8	4.11	4.10	8.00	5.35
COLO-205	5.03	2.69	2.66	3.22	NCI/ADR-RES	3.07	2.17	1.76	2.86
HCC-2998	4.81	4.54	4.08	3.80	SK-OV-3	3.49	-	14.8	13.9
HCT-116	4.55	3.77	3.63	3.84	***Renal Cancer***				
HCT-15	2.15	2.35	2.34	3.14	786-0	3.44	5.12	6.25	4.03
HT29	4.31	2.90	3.19	3.36	A498	1.87	3.26	3.03	3.02
KM12	0.16	1.77	0.76	2.74	ACHN	3.08	5.27	5.60	3.04
SW-620	4.50	3.63	3.52	3.30	CAKI-1	4.36	9.54	8.36	5.21
CNS Cancer					RXF 393	1.18	5.91	5.76	3.30
SF-268	4.42	7.67	6.98	-	SN12C	5.51	>100	>100	7.72
SF-295	2.70	3.20	3.35	2.83	UO-31	2.31	4.41	4.15	3.10
SF-539	3.99	8.86	5.91	7.34	***Breast Cancer***				
SNB-19	9.71	98.0	16.2	12.2	MCF-7	0.05	2.09	1.74	1.79
SNB-75	1.10	2.97	1.43	4.72	MDA-MB-231/ATCC	2.02	2.59	5.05	2.65
U251	4.43	5.14	6.12	6.18	HS 578T	2.07	8.82	9.14	4.44
Prostate Cancer					BT-549	2.38	4.26	4.45	4.80
					T-47D	0.04	0.65	0.42	1.97
PC-3	4.22	5.06	6.81	3.68	MDA-MB-468	0.03	2.44	0.97	2.34
DU-145	4.28	18.8	15.4	-					

[a] Concentration (μM) that inhibits 50% net cell growth.

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3.2 Cell apoptosis

To gain insight into the mechanism of growth inhibitory effects (necrosis or apoptosis) of the synthesized thiazoles **5e**,**q** and **7a**,**b**, flow cytometry analysis of annexin V-FITC and propidium iodide (PI) stained MCF-7 cells was carried out to evaluate externalization of plasma membrane phosphatidylserine, a reliable marker of cell apoptosis. The chosen concentrations were selected taking into account the GI_{50} values measured at 72 h for this cell line, i.e. $5x10^{-8}$ M for **5e** and $2x10^{-6}$ M for **5q**,**7a** and **7b** (Table 3). As shown in Figure 1, none of the nortopsentin analogues exerted necrotic effects, while induced a clear shift of viable cells towards early apoptosis. In addition, the percentage of apoptotic cells after treatment with compound **5e** (54.2%) was higher than that displayed by the other analogues.

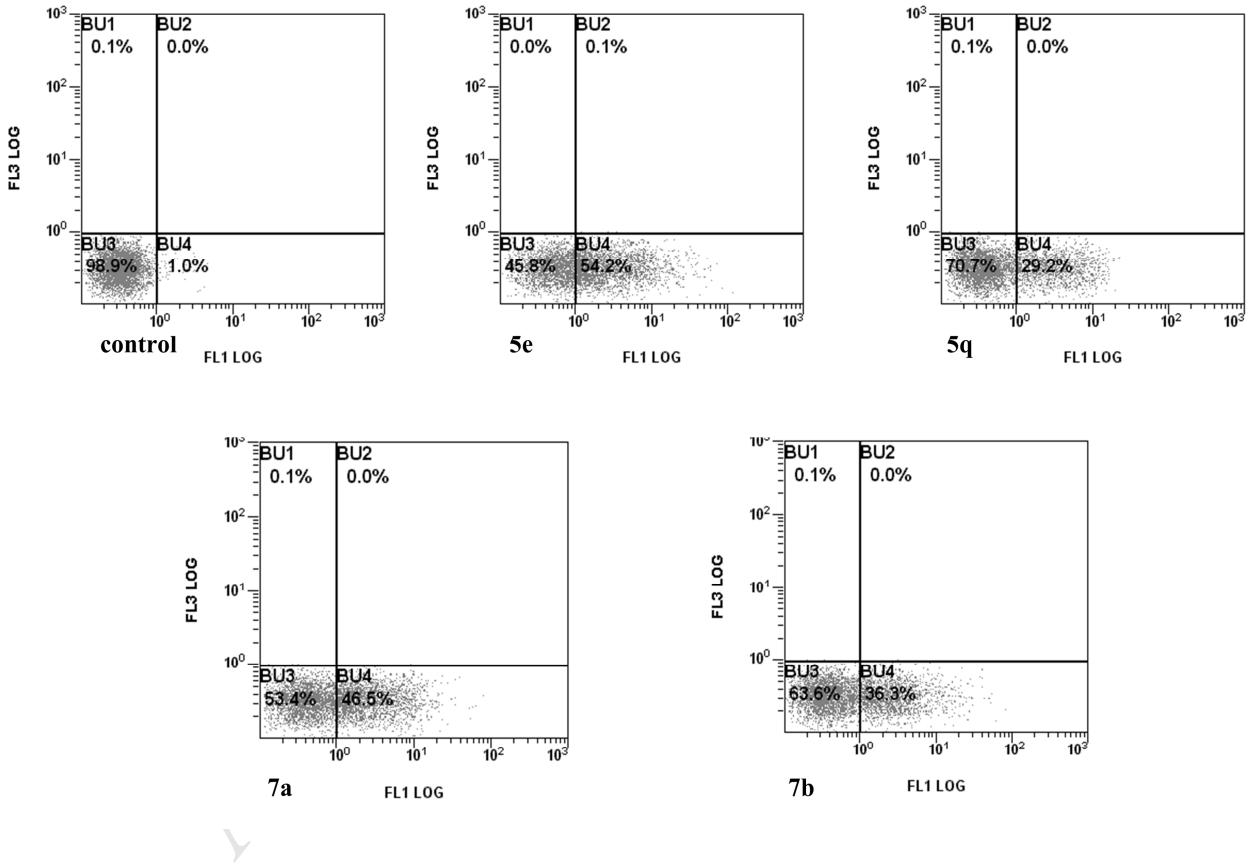

Figure 1. Flow cytometric analysis for the quantification, by annexin V/PI double staining, of nortopsentin analogues induced apoptosis in MCF-7 cells. Cell monolayers were incubated for 72 h in the absence (control) or in the presence of the synthesized compounds at their relevant GI_{50} values and submitted to double staining with annexin V/PI as reported in *Paragraph 4.2.2.* BU3, viable cells (annexin V-/P-); BU 4, cells in early apoptosis (annexin V+/PI-); BU 2, cells in tardive apoptosis (annexin V+/PI+); BU E1, necrotic cells (annexin V-/PI+). Representative images of three experiments with comparable results.

3.3 Cellular cycle analysis

One main feature of cancer cells is uncontrollable replication, and then cell cycle arrest become an important target in oncotherapy. Cell cycle distribution in PI stained MCF-7 cells was evaluated by flow cytometry. The analysis was performed after 48 h of incubation with tested compounds in order to detect the shifts in cell cycle distribution before a significant amount of cells underwent apoptosis. Appearance of a sub-G1-cell population, which is representative of cells with fragmented DNA, was evident after treatment with each of the compounds and it is consistent with their apoptotic activity (Figure 2). Moreover, derivatives **5e**,**q** and **7a** caused a net accumulation of cells in G2/M phase with a concomitant percentage reduction of cells in both the S and G0/G1 phase. Among all derivatives, **5e** appeared the most effective, confining more than 50% of the viable cells in G2/M phase. Compound **7b**, on the contrary, produced a moderate increase (9%) of viable cells in G0/G1 compared to the control (63.5%). These data suggested that **5e**,**q** and **7a** produced mitotic failure on MCF-7 cells, while thiazole **7b** weakly affected the G1/S transition of cell cycle.

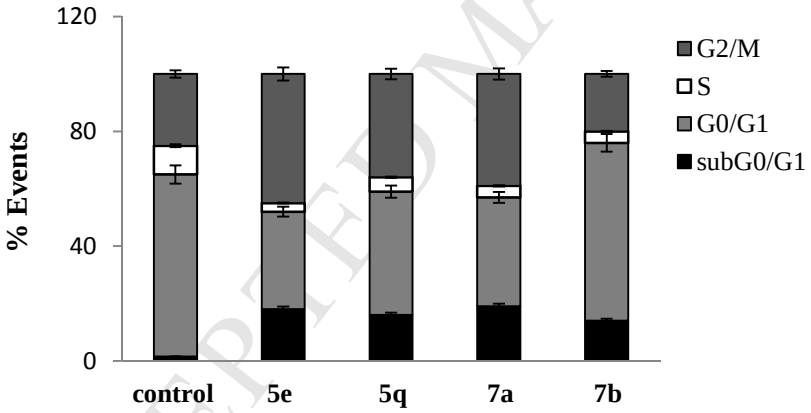

Figure 2. Cell cycle analysis of MCF-7 cells treated with compounds 5e,q and 7a,b. Cell monolayers were incubated in the absence (control) or in the presence of the compounds at their relevant GI_{50} values. After 48 h incubation, propidium iodide-stained cells were submitted to flow cytometric analysis as reported in *Paragraph 4.2.3*. The percentage of cells in the different phases of the cycle was calculated by Expo32 software. Values are the mean±SD of three separate experiments in triplicate.

3.4 Selective cytotoxicity

In order to determine the selective cytotoxicity of derivatives **5e**,**q** and **7a**,**b**, additional experiments were performed on intestinal normal-like differentiated Caco-2 cells. While thiazole **5e** did not

affect the cell viability at any assayed concentration, a reduction of the cell survival was detected after treatment with compounds **5q** and **7a**,**b** in the 25-100 μM range (Figure 3).

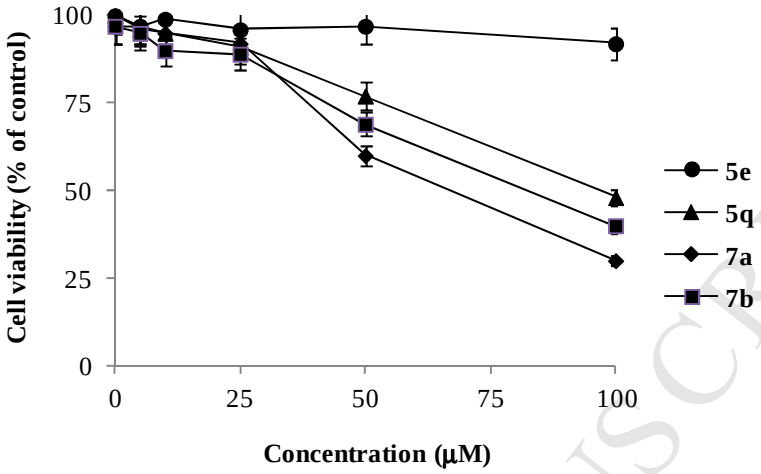

Figure 3. Effect of compunds 5e,q and 7a,b on the viability of human intestinal normal-like differentiated Caco-2 cells. Cells were treated with the compounds and cell viability was measured after 72 h by MTT assay in comparison to cells treated with vehicle alone (control). Values are the mean±SD of three separate experiments in triplicate.

Table 4 reports the calculated LC_{50} values and the selectivity index (SI). The high values of SI indicated that the synthesized compounds are selectively cytotoxic to cancer cells, suggesting their potential use as antiproliferative agents.

Table 4. Cytotoxic activity and the selectivity index (SI) of the synthesized compounds **5e,q** and **7a,b** against MCF-7 cancer cell line.

	Normal-like intestinal cells LC_{50} (μM)	MCF-7 GI_{50} (μM)[a]	SI
5e	<100	0.05	nd
5q	96.1±3.1	2.09	46
7a	71.4±2.1	1.74	41
7b	83.3±2.5	1.79	46

LC_{50} concentration which is lethal to 50% of the normal cells compared to untreated controls; GI_{50} concentration required to inhibit the tumor cell growth by 50% compared to untreated controls; nd, not determined.
[a]Data obtained from the NCI.

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3.5 CDK1 activity inhibition

Since azaindole nortopsentin analogues have been reported to effectively inhibit CDK1 activity and consequently induce cell cycle arrest at the G2/M phase [28-30], we assessed whether the thiazole derivative **5e**, the new most active and highly selective derivative against human cancer cells, could inhibit the *in vitro* catalytic activity of CDK1. For comparison, we decided to use an indolyl-thiazolyl-7-azaindole derivative of type **4** (Chart 2), i.e. (3-[2-(5-fluoro-1-methyl-1*H*-indol-3-yl)-1,3-thiazol-4-yl]-1-methyl-1*H*-pyrrolo[2,3-b] pyridine (positive control), previously synthesized by our research group and known to act as a potent inhibitor of CDK1 activity [30]. We also evaluated compound **7b** as an expected negative control. Nortopsentin derivative **5e** in the range 0.1-10 μM markedly inhibited CDK1 activity, with IC_{50} value of 1.14±0.09 μM, not significantly different ($p>0.05$, Student's *t*-test) from that calculated, in the same condition, for the positive control (0.97±0.01) (Figure 4). On the other hand, derivative **7b** did not affect the enzyme activity (Figure 4).

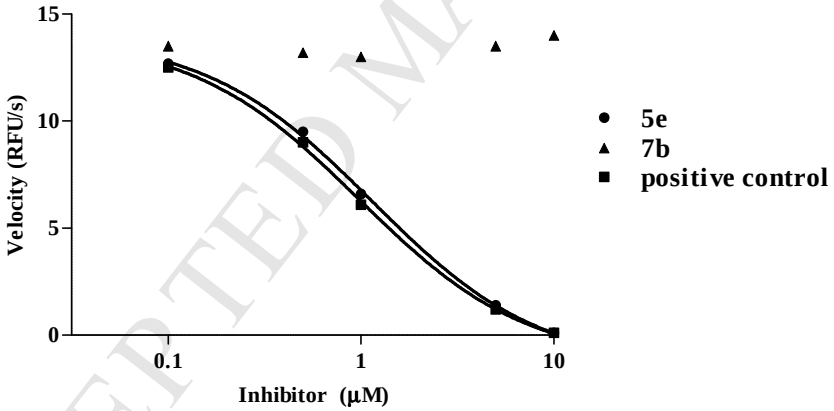

Figure 4. Inhibition of CDK1/cyclin B activity. The activity of recombinant human CDK1/cyclin B was assayed using Omnia® Kinase Assay Kit as reported in *Paragraph 4.2.4*, in the presence of increasing concentration of compounds either **5e**, **7b** and **positive control**. Initial velocity of the reaction (RCFU/s) was determined and plotted against compound concentration to calculate IC_{50} value. Values are the mean±SD of two determinations.

4. Conclusions

A new series of thiazole nortopsentin analogues of type **5-7** in which the nitrogen atom of the indole and/or 7-azaindole moiety is decorated with a 2-methoxyethyl chain was efficiently synthesized. Four of the new nortopsentin derivatives, **5e**,**q** and **7a**,**b** showed good antiproliferative activity against almost the totality of the about 60 human tumor cell lines of NCI full panel. The mechanism

of their antiproliferative effect, investigated on human breast cancer MCF-7 cells, was pro-apoptotic, being associated with externalization of plasma membrane phosphatidylserine and DNA fragmentation, accompanied to perturbation of the cell cycle progression. With the exception of compound **7b**, the newly synthesized nortopsentin derivatives confined viable cells in G2/M phase. Derivative **5e** showed the most interesting *in vitro* anticancer activity, expressing lower GI_{50} values, ranging from low micromolar to nanomolar level (12.6-0.03 μM), high selectivity towards tumor cells and high efficacy in causing mitotic failure. Importantly, compound **5e** markedly *in vitro* inhibited CDK1 activity with IC_{50} value comparable to that reported for other indolyl-thiazolyl-7-azaindole derivative or well-known CDK1 inhibitors, roscovitine and purvalanol A [30]. Overall, our data indicated that a possible growth inhibitory mechanism induced on MCF-7 cells by **5e**, was CDK1/cyclin B inhibition-induced G2/M phase arrest. Decoration of the nitrogen atom of the indole and/or 7-azaindole moiety with 2-methoxyethyl chain led to interesting biological results. For this reason, further developments of this class of compounds through decoration with other suitable chains should be encourage.

5. Experimental

5.1 Chemistry

All melting point were taken on a Büchi-Tottoly capillary apparatus and are uncorrected. IR spectra were determined in bromoform with a Shimadzu FT/IR 8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.0 MHz, respectively, in DMSO-d_6 solution, using a Bruker Avance II series 200 MHz spectrometer. Column chromatography was performed with Merk silica gel 230-400 mesh ASTM or with Büchi Sepacor chromatography module (prepacked cartridge system). Elemental analyses (C, H, N) were within ± 0.4% of theoretical values and were performed with a VARIO EL III elemental analyzer. Purity of all the tested compounds was greater than 95%, determined by HPLC (Agilent 1100 Series). Mass spectra were obtained using a Mariner^{TM} mass spectrometer, Applied Biosystems (Foster City, CA). A Harvard model 11 syringe pump (Holliston, MA) was used to infuse the sample solutions. The ESI source was operated in positive ion mode with an electrospray voltage of 4.5 kV.

^{1}H and ^{13}C NMR spectra of compounds **9b, 20b, 13b, 17b, 21b, 5e, 5q, 6a, 6b, 7a and 7b** are reported as examples in the supporting informations.

General procedures, analytical and spectroscopic data for compounds ***(10a-d)***, ***(11a-c)***, ***(12d)***, ***(14a-d)***, ***(15a-c)***, ***(16d)***, ***(18a-d)***, ***(19a-c)***, ***(20c)*** and ***(21a,c)*** were previously reported [28,30].

*5.1.1 General Procedure for the Synthesis of 1-(2-methoxyethyl)-1H-indoles (**9a-d**) and 1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**20b**)*

To a cold solution of the appropriate indole **8a-d** or azaindole **20a** (2.6 mmol) in anhydrous DMF (7.8 mL) NaH (60% suspension in mineral oil, 3.9 mmol, 0.16 g) was added. After 30 min stirring at room temperature, 2-bromoethyl methyl ether (3.1 mmol, 0.3 mL) was added. The reaction mixture was heated at 60 °C for 24 h. After cooling, the mixture was poured into ice-water and extracted with ethyl acetate (3 x 20 mL). The organic phases were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was used in the next step without any further purification (for compounds **9b-d**) or purified by column chromatography using petroleum ether/dichloromethane (65/35) (for compound **9a**) or petroleum ether/ethyl acetate (30/70) (for compound **20b**) as eluent.

*5.1.1.1 1-(2-Methoxyethyl)-1H-indole (**9a**)*

Yellow oil; yield: 83%; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.21 (s, 3H, CH_3), 3.64 (t, 2H, *J* = 5.3 Hz, CH_2), 4.33 (t, 2H, *J* = 5.3 Hz, CH_2), 6.43 (d, 1H, *J* = 3.1 Hz, H-3), 6.99-7.17 (m, 2H, H-5 and H-6), 7.35 (d, 1H, *J* = 3.1 Hz, H-2), 7.47-7.57 (m, 2H, H-4 and H-7); ^{13}C (50 MHz, DMSO-d_6) δ: 45.2 (t), 58.0 (q), 71.0 (t), 100.4 (d), 109.8 (d), 118.8 (d), 120.3 (d), 120.9 (d), 128.0 (s), 129.0 (d), 135.8 (s). Anal. Calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.70; H, 7.33; N, 7.82. MS *m/z* 176 (MH^+). HRMS: $[MH]^+$, found 176.1089 $C_{11}H_{13}NO$ requires 176.1070.

*5.1.1.2 5-Methoxy-1-(2-methoxyethyl)-1H-indole (**9b**)*

Yellow oil; yield: 99%; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.21 (s, 3H, CH_3), 3.62 (t, 2H, *J* = 5.0 Hz, CH_2), 3.75 (s, 3H, CH_3), 4.28 (t, 2H, *J* = 5.0 Hz, CH_2), 6.34 (d, 1H, *J* = 2.2 Hz, H-3), 6.78 (dd, 1H, *J* = 8.8, 2.0 Hz, H-6), 7.05 (d, 1H, *J* = 2.0 Hz, H-4), 7.30 (d, 1H, *J* = 2.2 Hz, H-2), 7.38 (d, 1H, *J* = 8.8 Hz, H-7); ^{13}C (50 MHz, DMSO-d_6) δ: 45.4 (t), 55.2 (q), 58.0 (q), 71.1 (t), 100.1 (d), 101.9 (d), 110.5 (d), 111.0 (d), 128.4 (s), 129.4 (d), 131.1 (s), 153.3 (s). Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 69.96; H, 7.52; N, 7.03. MS *m/z* 206 (MH^+). HRMS: $[MH]^+$, found 206.1152 $C_{12}H_{15}NO_2$ requires 206.1176.

*5.1.1.3 5-Bromo-1-(2-methoxyethyl)-1H-indole (**9c**)*

Yellow oil; yield: 99%; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.20 (s, 3H, CH_3), 3.63 (t, 2H, *J* = 5.0 Hz, CH_2), 4.33 (t, 2H, *J* = 5.0 Hz, CH_2), 6.43 (d, 1H, *J* = 2.3 Hz, H-3), 7.23 (d, 1H, *J* = 8.7 Hz, H-6), 7.71 (d, 1H, *J* = 2.3 Hz, H-2), 7.48 (d, 1H, *J* = 8.7 Hz, H-7), 7.73 (s, 1H, H-4); ^{13}C (50 MHz, DMSO-d_6) δ: 46.4 (t), 59.0 (q), 71.9 (t), 101.1 (d), 112.4 (s), 112.9 (d), 123.3 (d), 124.2 (d), 130.8 (s), 131.5 (d), 135.5 (s). Anal. Calcd for $C_{11}H_{12}BrNO$: C, 51.99; H, 4.76; N, 5.51. Found: C, 51.75;

H, 4.62; N, 5.70. MS *m/z* 254 (MH^+). HRMS: $[MH]^+$, found 254.0148 $C_{11}H_{12}BrNO$ requires 254.0175.

*5.1.1.4 5-Fluoro-1-(2-methoxyethyl)-1H-indole (**9d**)*

Yellow oil; yield: 98%; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.21 (s, 3H, CH_3), 3.63 (t, 2H, J = 4.9 Hz, CH_2), 4.33 (t, 2H, J = 4.9 Hz, CH_2), 6.41 (d, 1H, J = 2.0 Hz, H-3), 6.97-7.02 (m, 1H, H-6), 7.31 (d, 1H, J = 9.1 Hz, H-7), 7.43 (d, 1H, J = 2.0 Hz, H-2), 7.73 (m, 1H, H-4); ^{13}C (50 MHz, DMSO-d_6) δ: 45.5 (t), 58.0 (q), 71.0 (t), 100.5 (d, $J_{C3\text{-}F}$ = 4.7 Hz), 104.8 (d, $J_{C6\text{-}F}$ = 23.0 Hz), 109.0 (d, $J_{C4\text{-}F}$ = 26.1 Hz), 110.1 (d, $J_{C7\text{-}F}$ = 9.9 Hz), 128.2 (d, $J_{C3a\text{-}F}$ = 10.3 Hz), 130.9 (d), 131.6 (s), 157.0 (d, $J_{C5\text{-}F}$ = 232.0 Hz). Anal. Calcd for $C_{11}H_{12}FNO$: C, 68.38; H, 6.26; N, 7.25. Found: C, 68.57; H, 6.06; N, 7.07. MS *m/z* 194 (MH^+). HRMS: $[MH]^+$, found 194.0966 $C_{11}H_{12}FNO$ requires 194.0976.

*5.1.1.5 1-(2-Methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**20b**)*

Yellow oil; yield: 81%; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.23 (s, 3H, CH_3), 3.70 (t, 2H, J = 5.5 Hz, CH_2), 4.43 (t, 2H, J = 5.5 Hz, CH_2), 6.47 (d, 1H, J = 3.5 Hz, H-3), 7.09 (dd, 1H, J = 7.8, 4.7 Hz, H-5), 7.55 (d, 1H, J = 3.5 Hz, H-2), 7.97 (dd, 1H, J = 4.7, 1.5 Hz, H-6), 8.26 (dd, 1H, J = 7.8, 1.5 Hz, H-4); ^{13}C (50 MHz, DMSO-d_6) δ: 43.3 (t), 57.9 (q), 70.6 (t), 98.8 (d), 115.5 (d), 120.0 (s), 128.4 (d), 129.4 (d), 142.2 (d), 147.0 (s). Anal. Calcd for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86; N, 15.90. Found: C, 67.99; H, 6.70; N, 15.64. MS *m/z* 177 (MH^+). HRMS: $[MH]^+$, found 177.1046 $C_{10}H_{12}N_2O$ requires 177.1022.

*5.1.2 General Procedure for the Synthesis of 1-(2-methoxyethyl)-1H-indole-3-carboxamides (**13a-d**)*

To a solution of suitable indole **9a-d** (1.2 mmol) in anhydrous acetonitrile (1.5 mL) chlorosulfonyl isocyanate (2.4 mmol, 0.21 mL) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 30 min then a solution of acetone (8 mL) and water (1 mL) was added. The solution was basified with 10% aqueous solution of potassium hydroxide and the obtained precipitate was filtered off, dried and purified by column chromatography using dichloromethane/methanol (95/5) (for compounds **13a**,**b**) or dichloromethane/methanol (90/10) (for compounds **13c**,**d**) as eluent.

*5.1.2.1 1-(2-Methoxyethyl)-1H-indole-3-carboxamide (**13a**)*

White solid; yield: 65%; mp: 147-148 °C; IR cm^{-1}: 3373, 3184 (NH_2), 1609 (CO); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.23 (s, 3H, CH_3), 3.67 (t, 2H, J = 5.2 Hz, CH_2), 4.36 (t, 2H, J = 5.2 Hz, CH_2), 6.88-7.24 (m, 4H, H-5, H-6 and NH_2), 7.54 (dd, 1H, J = 6.8, 1.3 Hz, H-7), 8.04 (s, 1H, H-2), 8.15-8.20 (m, 1H, H-4); ^{13}C (50 MHz, DMSO-d_6) δ: 45.8 (t), 58.1 (q), 70.5 (t), 109.6 (s), 110.3 (d), 120.5

(d), 121.3 (d), 121.8 (d), 126.6 (s), 132.0 (d), 136.2 (s), 166.1 (s). Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.83; H, 6.32; N, 12.70. MS *m/z* 219 (MH^+). HRMS: $[MH]^+$, found 219.1116 $C_{12}H_{14}N_2O_2$ requires 219.1128.

*5.1.2.2 5-Methoxy-1-(2-methoxyethyl)-1H-indole-3-carboxamide (**13b**)*

White solid; yield: 71%; mp: 120-121 °C; IR cm^{-1}: 3380, 3182 (NH_2), 1601 (CO); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.22 (s, 3H, CH_3), 3.65 (t, 2H, *J* = 5.0 Hz, CH_2), 3.77 (s, 3H, CH_3), 4.31 (t, 2H, *J* = 5.0 Hz, CH_2), 6.82 (dd, 1H, *J* = 8.9, 2.3 Hz, H-6), 7.27 (bs, 2H, NH_2), 7.44 (d, 1H, *J* = 8.9 Hz, H-7), 7.67 (d, 1H, *J* = 2.3 Hz, H-4), 7.98 (s, 1H, H-2); ^{13}C (50 MHz, DMSO-d_6) δ: 46.0 (t), 55.2 (q), 58.1 (q), 70.5 (t), 102.7 (d), 109.1 (s), 111.1 (d), 111.8 (d), 127.3 (s), 131.3 (s), 132.2 (d), 154.5 (s), 166.3 (s). Anal. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.75; H, 6.40; N, 11.04. MS *m/z* 249 (MH^+). HRMS: $[MH]^+$, found 249.1248 $C_{13}H_{16}N_2O_3$ requires 249.1234.

*5.1.2.3 5-Bromo-1-(2-methoxyethyl)-1H-indole-3-carboxamide (**13c**)*

White solid; yield: 93%; mp: 185 °C; IR cm^{-1}: 3369, 3167 (NH_2), 1609 (CO); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.22 (s, 3H, CH_3), 3.65 (t, 2H, *J* = 5.0 Hz, CH_2), 4.36 (t, 2H, *J* = 5.0 Hz, CH_2), 6.95 (bs, 2H, NH_2), 7.32 (dd, 1H, *J* = 8.7, 2.0 Hz, H-6), 7.56 (d, 1H, *J* = 8.7 Hz, H-7), 8.09 (s, 1H, H-2), 8.33 (d, 1H, *J* = 2.0 Hz, H-4); ^{13}C (50 MHz, DMSO-d_6) δ: 46.1 (t), 58.1 (q), 70.5 (t), 109.2 (s), 112.6 (d), 113.5 (s), 123.4 (d), 124.3 (d), 128.4 (s), 133.1 (d), 135.0 (s), 165.7 (s). Anal. Calcd for $C_{12}H_{13}BrN_2O_2$: C, 48.50; H, 4.41; N, 9.43. Found: C, 48.36; H, 4.24; N, 9.67. MS *m/z* 297 (MH^+). HRMS: $[MH]^+$, found 297.0219 $C_{12}H_{13}BrN_2O_2$ requires 297.0233.

*5.1.2.4 5-Fluoro-1-(2-methoxyethyl)-1H-indole-3-carboxamide (**13d**)*

White solid; yield: 98%; mp: 148 °C; IR cm^{-1}: 3406, 3200 (NH_2), 1606 (CO); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.22 (s, 3H, CH_3), 3.66 (t, 2H, *J* = 5.2 Hz, CH_2), 4.37 (t, 2H, *J* = 5.2 Hz, CH_2), 6.92 (bs, 2H, NH_2), 7.05 (td, 1H, *J* = 11.8, 9.2, 2.7 Hz, H-6), 7.59 (dd, 1H, *J* = 9.2, 4.5 Hz, H-7), 7.85 (dd, 1H, *J* = 11.8, 2.7, Hz, H-4), 8.11 (s, 1H, H-2); ^{13}C (50 MHz, DMSO-d_6) δ: 46.1 (t), 58.1 (q), 70.5 (t), 105.9.(d, $J_{C6\text{-}F}$ = 24.5 Hz), 110.0.(d, $J_{C4\text{-}F}$ = 26.2 Hz), 111.7(d, $J_{C7\text{-}F}$ = 9.9 Hz), 127.0 (s), 127.2 (s), 132.9 (s), 133.5 (d), 163.0 (d, $J_{C5\text{-}F}$ = 282.0 Hz), 165.9 (s). Anal. Calcd for $C_{12}H_{13}FN_2O_2$: C, 61.01; H, 5.55; N, 11.86. Found: C, 61.25; H, 5.41; N, 11.67. MS *m/z* 237 (MH^+). HRMS: $[MH]^+$, found 237.1051 $C_{12}H_{13}FN_2O_2$ requires 237.1034.

*5.1.3 General Procedure for the Synthesis of 1-(2-methoxyethyl)-1H-indole-3-carbothioamides (**17a-d**)*

To a solution of the appropriate carboxamide **13a-d** (0.81 mmol) in THF (8 mL) Lawesson's reagent (0.41 mmol, 0.17 g) was added and the mixture was heated under reflux for 30 min. After cooling the solvent was evaporated under reduce pressure and the residue purified by column chromatography using petroleum ether/ethyl acetate (30/70) (for compounds **17a**,**b**) or ethyl acetate (for compounds **17c**,**d**) as eluent.

*5.1.3.1 1-(2-Methoxyethyl)-1H-indole-3-carbothioamide (**17a**)*

Yellow solid; yield: 70%; mp: 151-152 °C; IR cm^{-1}: 3377, 3182 (NH_2), 1595 (CS); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.23 (s, 3H, CH_3), 3.68 (t, 2H, J = 5.2 Hz, CH_2), 4.39 (t, 2H, J = 5.2 Hz, CH_2), 7.15-7.26 (m, 2H, H-5 and H-6), 7.55-7.59 (m, 1H, H-7), 8.12 (s, 1H, H-2), 8.57-8.62 (m, 1H, H-4), 8.83 (bs, 1H, SH), 9.02 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 45.8 (t), 58.1 (q), 70.4 (t), 110.6 (d), 115.5 (s), 121.0 (d), 121.8 (d), 122.1 (d), 126.0 (s), 132.0 (d), 136.7 (s), 193.0 (s). Anal. Calcd for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96. Found: C, 61.75; H, 6.16; N, 11.68. MS *m/z* 235 (MH^+). HRMS: $[MH]^+$, found 235.0923 $C_{12}H_{14}N_2OS$ requires 235.0900.

*5.1.3.2 5-Methoxy-1-(2-methoxyethyl)-1H-indole-3-carbothioamide (**17b**)*

Yellow solid; yield: 98%; mp: 133-134 °C; IR cm^{-1}: 3371, 3160 (NH_2), 1619 (CS); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.22 (s, 3H, CH_3), 3.66 (t, 2H, J = 5.1 Hz, CH_2), 3.79 (s, 3H, CH_3), 4.34 (t, 2H, J = 5.1 Hz, CH_2), 6.86 (dd, 1H, J = 8.9, 2.5 Hz, H-6), 7.47 (d, 1H, J = 8.9 Hz, H-7), 8.09 (s, 1H, H-2), 8.19 (d, 1H, J = 2.5 Hz, H-4), 8.77 (bs, 1H, SH), 8.93 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 46.0 (t), 55.3 (q), 58.1 (q), 70.4 (t), 103.9 (d), 111.4 (d), 111.8 (d), 115.0 (s), 126.8 (s), 131.8 (s), 132.4 (d), 154.9 (s), 192.9 (s). Anal. Calcd for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60. Found: C, 59.31; H, 5.99; N, 10.78. MS *m/z* 265 (MH^+). HRMS: $[MH]^+$, found 265.0994 $C_{13}H_{16}N_2O_2S$ requires 265.1005.

*5.1.3.3 5-Bromo-1-(2-methoxyethyl)-1H-indole-3-carbothioamide (**17c**)*

Yellow solid; yield: 92%; mp: 181-182 °C; IR cm^{-1}: 3365, 3181 (NH_2), 1629 (CS); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.22 (s, 3H, CH_3), 3.66 (t, 2H, J = 5.0 Hz, CH_2), 4.38 (t, 2H, J = 5.0 Hz, CH_2), 7.36 (dd, 1H, J = 8.8, 2.0 Hz, H-6), 7.58 (d, 1H, J = 8.8 Hz, H-7), 8.16 (s, 1H, H-2), 8.89 (d, 1H, J = 2.0 Hz, H-4), 8.96 (bs, 1H, SH), 9.10 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 46.1 (t), 58.1 (q), 70.3 (t), 112.8 (d), 114.1 (s), 114.7 (s), 124.1 (d), 124.6 (d), 128.1 (s), 132.4 (d), 135.6 (s), 192.5 (s). Anal. Calcd for $C_{12}H_{13}BrN_2OS$: C, 46.02; H, 4.18; N, 8.94. Found: C, 45.88; H, 4.35; N, 8.78. MS *m/z* 313 (MH^+). HRMS: $[MH]^+$, found 313.0021 $C_{12}H_{13}BrN_2OS$ requires 313.0005.

*5.1.3.4 5-Fluoro-1-(2-methoxyethyl)-1H-indole-3-carbothioamide (**17d**)*

Yellow solid; yield: 99%; mp: 119-120 °C; IR cm^{-1}: 3371, 3160 (NH_2), 1647 (CS); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.23 (s, 3H, CH_3), 3.67 (t, 2H, *J* = 4.9 Hz, CH_2), 4.39 (t, 2H, *J* = 4.9 Hz, CH_2), 7.09 (td, 1H, *J* = 11.7, 9.1, 2.6 Hz, H-6), 7.62 (d, 1H, *J* = 9.1, 4.6 Hz, H-7), 8.20 (s, 1H, H-2), 8.44 (dd, 1H, *J* = 11.7, 2.6 Hz, H-4), 8.83 (s, 1H, SH), 9.02 (s, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 46.2 (t), 58.1 (q), 70.3 (t) , 106.8 (d, $J_{C6\text{-}F}$ = 25.9 Hz), 110.2 (d, $J_{C4\text{-}F}$ = 26.3 Hz), 112.0 (d, $J_{C7\text{-}F}$ = 10.0 Hz), 115.1 (d, $J_{C7a\text{-}F}$ = 4.7 Hz), 126.9 (d, $J_{C3a\text{-}F}$ = 11.0 Hz), 133.0 (d), 133.5 (s), 158.1 (d, $J_{C5\text{-}F}$ = 233.0 Hz), 192.6 (s). Anal. Calcd for $C_{12}H_{13}FN_2OS$: C, 57.12; H, 5.19; N, 11.10. Found: C, 56.94; H, 5.43; N, 10.94. MS *m/z* 253 (MH^+). HRMS: $[MH]^+$, found 253.0817 $C_{12}H_{13}FN_2OS$ requires 253.0805.

*5.1.4 Synthesis of 2-bromo-1-[1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]ethanone (**21b**)*

Anhydrous $AlCl_3$ (20.2 mmo,l 2.70 g) was slowly added to a solution of 1-(2-methoxyethyl)-1*H*-pyrrolo[2,3-*b*]pyridine **20b** (5.67 mmol, 1.0 g) in anhydrous DCM (15 mL). The reaction mixture was heated under reflux and a solution of bromoacetyl bromide (5.67 mmol, 0.5 mL) in anhydrous DCM (3 mL) was added dropwise. The resulting mixture was stirred under reflux for 40 min. After cooling, water and ice were slowly added and the obtained precipitate was filtered off, dried and purified by column chromatography using dichloromethane/ethyl acetate (85/15) as eluent. White solid, yield: 70%; mp: 83-84 °C; IR cm^{-1}: 1653 (CO); ^1H NMR (200 MHz, DMSO-d_6) δ: 3.25 (s, 3H, CH_3), 3.79 (t, 2H, *J* = 5.2 Hz, CH_2), 4.50 (t, 2H, *J* = 5.2 Hz, CH_2), 4.68 (s, 2H, CH_2), 7.34 (dd, 1H, *J* = 7.9, 4.7 Hz, H-5), 8.41 (dd, 1H, *J* = 4.7, 1.6 Hz, H-6), 8.48 (dd, 1H, *J* = 7.9, 1.6 Hz, H-4), 8.71 (s, 1H, H-2); ^{13}C (50 MHz, DMSO-d_6) δ: 33.0 (t), 44.4 (t), 58.0 (q), 69.7 (t), 111.1 (s), 118.2 (s), 118.8 (d), 129.9 (d), 138.2 (d), 144.3 (d), 147.8 (s), 186.3 (s). Anal. Calcd for $C_{12}H_{13}BrN_2O_2$: C, 48.50; H, 4.41; N, 9.43. Found: C, 48.34; H, 4.26; N, 9.59. MS *m/z* 297 (MH^+). HRMS: $[MH]^+$, found 297.0207 $C_{12}H_{13}BrN_2O_2$ requires 297.0233.

*5.1.5 General Procedure for the Synthesis of thiazoles (**5a-q** and **6a**,**b**)*

A suspension of the proper thioamides **16d**, **17a-d**, **18a-d** and **19a-c** (2 mmol) and bromoacetyl derivatives **21a-c** (2 mmol) in anhydrous ethanol (8 mL) was refluxed for 30 min. After cooling, the precipitate obtained, was filtered off, dried, and recrystallized from ethanol to give the desidered thiazoles **5a-q** and **6a**,**b**.

*5.1.5.1 3-{2-[1-(2-Methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1H-pyrrolo[2,3-b]pyridine (**5a**)*

Yellow solid; yield: 66%; mp: 238-239 °C; IR cm^{-1}: 3300 (NH); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.25 (s, 3H, CH_3), 3.74 (t, 2H, *J* = 5.1 Hz, CH_2), 4.48 (t, 2H, *J* = 5.1 Hz, CH_2), 7.26-7.35 (m, 2H, H-5' and H-6'), 7.49 (dd, 1H, *J* = 7.9, 5.3 Hz, H-5''), 7.64-7.69 (m, 1H, H-7'), 7.87 (s, 1H, H-5), 8.24-8.33 (m, 3H, H-2', H-2'' and H-4'), 8.48 (dd, 1H, *J* = 5.3, 1.2 Hz, H-6''), 9.00 (d, 1H, *J* = 7.9 Hz, H-4''), 12.60 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 45.8 (t), 58.1 (q), 70.7 (t), 108.9 (d), 109.5 (s), 111.0 (d), 111.3 (s), 116.2 (d), 120.3 (d), 121.2 (d), 121.5 (s), 122.5 (d), 124.6 (s), 126.9 (d), 130.3 (d), 135.2 (d), 135.6 (s), 136.6 (s), 136.9 (d), 147.8 (s), 162.3 (s). Anal. Calcd for $C_{21}H_{18}N_4OS$: C, 67.36; H, 4.85; N, 14.96. Found: C, 67.24; H, 4.69; N, 14.79. MS *m/z* 375 (MH^+). HRMS: $[MH]^+$, found 375.1260 $C_{21}H_{18}N_4OS$ requires 375.1274.

*5.1.5.2 3-{2-[1-(2-Methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1-methyl-1H-pyrrolo[2,3-b]pyridine (**5b**)*

Orange solid; yield: 70%; mp: 248-249 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.24 (s, 3H, CH_3), 3.73 (t, 2H, *J* = 5.0 Hz, CH_2), 4.00 (s, 3H, CH_3), 4.47 (t, 2H, *J* = 5.0 Hz, CH_2), 7.28-7.32 (m, 2H, H-5' and H-6'), 7.47 (dd, 1H, *J* = 7.9, 5.2 Hz, H-5''), 7.63-7.67 (m, 1H, H-7'), 7.82 (s, 1H, H-5), 8.25-8.33 (m, 3H, H-2', H-2'' and H-4'), 8.50 (d, 1H, *J* = 5.2 Hz, H-6''), 8.90 (d, 1H, *J* = 7.9 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 31.6 (q), 45.8 (t), 58.1 (q), 70.6 (t), 109.3 (s), 109.6 (s), 110.9 (s), 111.1 (d), 116.1 (d), 120.0 (d), 120.4 (s), 121.1 (d), 122.5 (d), 124.6 (s), 129.7 (d), 130.4 (s), 130.5 (d), 130.6 (d), 131.5 (d), 136.6 (s), 141.8 (d), 162.1 (s). Anal. Calcd for $C_{22}H_{20}N_4OS$: C, 68.02; H, 5.19; N, 14.42. Found: C, 68.17; H, 5.39; N, 14.30. MS *m/z* 389 (MH^+). HRMS: $[MH]^+$, found 389.1450 $C_{22}H_{20}N_4OS$ requires 389.1431.

*5.1.5.3 1-(2-Methoxyethyl)-3-{2-[1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1H-pyrrolo[2,3-b]pyridine (**5c**)*

Orange solid; yield: 91%; mp: 219-220 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.24 (s, 3H, CH_3), 3.26 (s, 3H, CH_3), 3.71-3.82 (m, 4H, 2 x CH_2), 4.48 (t, 2H, *J* = 5.0 Hz, CH_2), 4.59 (t, 2H, *J* = 5.0 Hz, CH_2), 7.26-7.35 (m, 2H, H-5' and H-6'), 7.42 (dd, 1H, *J* = 7.9, 5.0 Hz, H-5''), 7.62-7.70 (m, 1H, H-7'), 7.82 (s, 1H, H-5), 8.24-8.31 (m, 3H, H-2', H-2'' and H-4'), 8.46 (d, 1H, *J* = 5.0, 1.2 Hz, H-6''), 8.83 (dd, 1H, *J* = 7.9, 1.2 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 44.4 (t), 45.8 (t), 58.1 (2 x q), 70.5 (t), 70.7 (t), 107.9 (d), 109.4 (s), 109.5 (s), 111.0 (d), 116.4 (d), 119.0 (s), 120.4 (d), 121.1 (d), 122.4 (d), 124.6 (s), 129.2 (d), 130.3 (d), 131.5 (d), 136.6 (s), 140.4 (d), 144.8 (s), 148.1 (s), 162.1 (s). Anal. Calcd for $C_{24}H_{24}N_4O_2S$: C, 66.64; H, 5.59; N, 12.95. Found: C, 66.80; H, 5.44; N, 12.74. MS *m/z* 433 (MH^+). HRMS: $[MH]^+$, found 433.1675 $C_{24}H_{24}N_4O_2S$ requires 433.1693.

*5.1.5.4 1-(2-Methoxyethyl)-3-[2-(1-methyl-1H-indol-3-yl)-1,3-thiazol-4-yl]-1H-pyrrolo[2,3-b]pyridine (**5d**)*

Yellow solid; yield: 98%; mp: 242-243 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.14 (s, 3H, CH_3), 3.79 (t, 2H, J = 5.3 Hz, CH_2), 3.92 (s, 3H, CH_3), 4.56 (t, 2H, J = 5.3 Hz, CH_2), 7.30-7.37 (m, 3H, H-5', H-5'' and H-6'), 7.56-7.64 (m, 1H, H-7'), 7.76 (s, 1H, H-5), 8.23 (s, 1H, H-2'), 8.25 (s, 1H, H-2''), 8.30-8.42 (m, 2H, H-4' and H-6''), 8.73 (dd, 1H, J = 8.0, 1.5 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 32.9 (q), 44.4 (t), 58.1 (q), 70.5 (t), 107.8 (d), 109.2 (s), 109.5 (s), 110.7 (d), 116.3 (d), 118.9 (s), 120.4 (d), 121.1 (d), 122.5 (d), 124.5 (s), 129.1 (d), 130.8 (d), 131.3 (d), 137.1 (s), 140.5 (d), 144.9 (s), 148.2 (s), 162.1 (s). Anal. Calcd for $C_{22}H_{20}N_4OS$: C, 68.02; H, 5.19; N, 14.42. C, 68.17; H, 5.00; N, 14.31. MS *m/z* 389 (MH^+). HRMS: $[MH]^+$, found 389.1416 $C_{22}H_{20}N_4OS$ requires 389.1431.

*5.1.5.5 3-{2-[5-Methoxy-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1H-pyrrolo[2,3-b]pyridine (**5e**)*

Yellow solid; yield: 73%; mp: 237-238 °C; IR cm^{-1}: 3299 (NH), ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.24 (s, 3H, CH_3), 3.72 (t, 2H, J = 5.0 Hz, CH_2), 3.90 (s, 3H, CH_3), 4.43 (t, 2H, J = 5.0 Hz, CH_2), 6.94 (dd, 1H, J = 8.9, 2.4 Hz, H-6'), 7.44 (dd, 1H, J = 7.9, 5.2 Hz, H-5''), 7.57 (d, 1H, J = 8.9 Hz, H-7'), 7.82 (s, 1H, H-5), 7.88 (d, 1H, J = 2.4 Hz, H-4'), 8.17 (s, 1H, H-2'), 8.26 (d, 1H, J = 2.2 Hz, H-2''), 8.47 (dd, 1H, J = 5.2, 1.2 Hz, H-6''), 9.04 (d, 1H, J = 7.9 Hz, H-4''), 12.54 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 45.9 (t), 55.2 (q), 58.1 (q), 70.7 (t), 101.9 (d), 108.2 (d), 109.2 (s), 111.1 (s), 111.8 (d), 112.5 (d), 116.0 (d), 120.7 (s), 125.1 (s), 126.4 (d), 130.5 (d), 131.6 (s), 134.3 (d), 137.8 (d), 142.5 (s), 148.0 (s), 154.9 (s), 162.5 (s). Anal. Calcd for $C_{22}H_{20}N_4O_2S$: C, 65.33; H, 4.98; N, 13.85. Found: C, 65.55; H, 4.82; N, 13.70. MS *m/z* 405 (MH^+). HRMS: $[MH]^+$, found 405.1408 $C_{22}H_{20}N_4O_2S$ requires 405.1380.

*5.1.5.6 3-{2-[5-Methoxy-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1-methyl-1H-pyrrolo[2,3-b]pyridine (**5f**)*

Yellow solid; yield: 70%; mp: 223-224 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.24 (s, 3H, CH_3), 3.71 (t, 2H, J = 5.1 Hz, CH_2), 3.89 (s, 3H, CH_3), 3.98 (s, 3H, CH_3), 4.42 (t, 2H, J = 5.1 Hz, CH_2), 6.94 (dd, 1H, J = 8.9, 2.5 Hz, H-6'), 7.42 (dd, 1H, J = 7.9, 5.0 Hz, H-5''), 7.56 (d, 1H, J = 8.9 Hz, H-7'), 7.76 (s, 1H, H-5), 7.82 (d, 1H, J = 2.5 Hz, H-4'), 8.18 (s, 1H, H-2'), 8.27 (s, 1H, H-2''), 8.49 (dd, 1H, J = 5.0, 1.2 Hz, H-6''), 8.94 (dd, 1H, J = 7.9, 1.2 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 32.1 (q), 46.0 (t), 55.3 (q), 58.1 (q), 70.7 (t), 102.1 (d), 107.7 (d), 109.0 (s), 109.5 (s), 111.8 (d),

112.4 (d), 116.0 (d), 116.1 (s), 122.1 (d), 124.9 (s), 125.1 (s), 129.7 (d), 130.6 (d), 131.7 (s), 132.3 (d), 154.9 (s), 155.1 (s), 162.4 (s). Anal. Calcd for $C_{23}H_{22}N_4O_2S$: C, 66.01; H, 5.30; N, 13.39. Found: C, 66.14; H, 5.05; N, 13.23. MS *m/z* 419 (MH^+). HRMS: $[MH]^+$, found 419.1552 $C_{23}H_{22}N_4O_2S$ requires 419.1536.

*5.1.5.7 1-(2-Methoxyethyl)-3-{2-[5-methoxy-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1H-pyrrolo[2,3-b]pyridine (**5g**)*

Yellow solid; yield: 60%; mp: 214-215 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.25 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 3.69-3.81 (m, 4H, 2 x CH_2), 3.91 (s, 3H, CH_3), 4.43 (t, 2H, *J* = 5.1 Hz, CH_2), 4.55 (t, 2H, *J* = 5.1 Hz, CH_2), 6.95 (dd, 1H, *J* = 8.9, 2.5 Hz, H-6'), 7.33 (dd, 1H, *J* = 7.9, 4.9 Hz, H-5''), 7.57 (d, 1H, *J* = 8.9 Hz, H-7'), 7.74 (s, 1H, H-5), 7.88 (d, 1H, *J* = 2.5 Hz, H-4'), 8.15 (s, 1H, H-2'), 8.23 (s, 1H, H-2''), 8.41 (dd, 1H, *J* = 4.9, 1.4, Hz H-6''), 8.81 (dd, 1H, *J* = 7.9, 1.4 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 44.2 (t), 46.0 (t), 55.1 (q), 58.1 (2 x q), 70.5 (t), 70.7 (t), 102.0 (d), 107.8 (s), 108.9 (s), 109.5 (s), 111.9 (d), 112.6 (d), 116.2 (d), 116.8 (d), 119.6 (s), 125.1 (s), 129.1 (d), 130.6 (d), 131.6 (d), 141.6 (d), 144.1 (s), 147.7 (s), 155.0 (s), 162.5 (s). Anal. Calcd for $C_{25}H_{26}N_4O_3S$: C, 64.91; H, 5.67; N, 12.11. Found: C, 64.78; H, 5.48; N, 12.01. MS *m/z* 463 (MH^+). HRMS: $[MH]^+$, found 463.1783 $C_{25}H_{26}N_4O_3S$ requires 463.1798.

*5.1.5.8 1-(2-Methoxyethyl)-3-[2-(5-methoxy-1-methyl-1H-indol-3-yl)-1,3-thiazol-4-yl]-1H-pyrrolo[2,3-b]pyridine (**5h**)*

Orange solid; yield: 96%; mp: 238-239 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.27 (s, 3H, CH_3), 3.78 (t, 2H, *J* = 5.1 Hz, CH_2), 3.88 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 4.55 (t, 2H, *J* = 5.1 Hz, CH_2), 6.96 (dd, 1H, *J* = 8.9, 2.5 Hz, H-6'), 7.33 (dd, 1H, *J* = 7.9, 4.9 Hz, H-5''), 7.50 (d, 1H, *J* = 8.9 Hz, H-7'), 7.72 (s, 1H, H-5), 7.90 (d, 1H, *J* = 2.5 Hz, H-4'), 8.16 (s, 1H, H-2'), 8.22 (s, 1H, H-2''), 8.42 (dd, 1H, *J* = 4.9, 1.4 Hz, H-6''), 8.81 (dd, 1H, *J* = 7.9, 1.4 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 33.05 (q), 44.1 (t), 55.2 (q), 58.0 (q), 70.4 (t), 101.9 (d), 107.4 (d), 108.8 (s), 109.5 (s), 110.6 (d), 111.6 (d), 112.5 (d), 116.2 (d), 119.0 (s), 125.1 (s), 131.0 (d), 131.2 (s), 131.5 (d), 140.5 (d), 144.8 (s), 148.1 (s), 155.0 (s), 162.4 (s). Anal. Calcd for $C_{23}H_{22}N_4O_2S$: C, 66.01; H, 5.30; N, 13.39. Found: C, 65.75; H, 5.06; N, 13.25. MS *m/z* 419 (MH^+). HRMS: $[MH]^+$, found 419.1549 $C_{23}H_{22}N_4O_2S$ requires 419.1536.

*5.1.5.9 3-{2-[5-Bromo-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1H-pyrrolo[2,3-b]pyridine (**5i**)*

Orange solid; yield: 75%; mp: 232 °C; IR cm^{-1}: 3355 (NH) ;^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.24 (s, 3H, CH_3), 3.73 (t, 2H, *J* = 5.0 Hz, CH_2), 4.48 (t, 2H, *J* = 5.0 Hz, CH_2), 7.38-7.46 (m, 2H, H-5'' and H-6'), 7.67 (d, 1H, *J* = 8.8 Hz, H-7'), 7.84 (s, 1H, H-5), 8.21 (d, 1H, *J* = 2.3 Hz, H-2''), 8.28 (s, 1H, H-2'), 8.45 (dd, 1H, *J* = 5.1, 1.2 Hz, H-6''), 8.52 (d, 1H, *J* = 1.8, Hz, H-4'),8.89 (d, 1H, *J* = 7.9 Hz, H-4''), 12.42 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 46.0 (t), 58.1 (q), 70.7 (t), 109.2 (d), 111.2 (s), 113.1 (s), 113.2 (d), 113.7 (s), 116.1 (d), 120.8 (s), 122.6 (d), 123.7 (s), 124.9 (d), 126.2 (s), 126.6 (d), 131.4 (d), 134.6 (d), 135.4 (s), 137.4 (d), 148.2 (s), 161.6 (s). Anal. Calcd for $C_{21}H_{17}BrN_4OS$: C, 55.64; H, 3.78; N, 12.36. Found: C, 55.43; H, 3.68; N, 12.51. MS *m/z* 453 (MH^+). HRMS: $[MH]^+$, found 453.0395 $C_{21}H_{17}BrN_4OS$ requires 453.0379.

*5.1.5.10 3-{2-[5-Bromo-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1-methyl-1H-pyrrolo[2,3-b]pyridine (**5j**)*

Orange solid; yield: 84%; mp: 250-251 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.24 (s, 3H, CH_3), 3.72 (t, 2H, *J* = 5.0 Hz, CH_2), 3.96 (s, 3H, CH_3), 4.47 (t, 2H, *J* = 5.0 Hz, CH_2), 7.34 (dd, 1H, *J* = 7.9, 4.9 Hz, H-5''), 7.44 (dd, 1H, *J* = 8.8, 1.9 Hz, H-6'), 7.67 (d, 1H, *J* = 8.8 Hz, H-7'), 7.77 (s, 1H, H-5), 8.21-8.26 (m, 2H, H-2' and H-2''), 8.44 (dd, 1H, *J* = 4.9, 1.3 Hz, H-6''), 8.50 (d, 1H, *J* = 1.9, Hz, H-4'),8.75 (dd, 1H, *J* = 7.9, 1.3 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 31.9 (q), 46.0 (t), 58.1 (q), 70.7 (t), 108.1 (d), 109.2 (s), 109.5 (s), 113.2 (d), 113.7 (s), 116.0 (d), 122.6 (d), 125.0 (d), 126.2 (s), 129.5 (d), 131.4 (d), 131.6 (d), 135.4 (s), 140.1 (d), 140.3 (s), 145.2 (s), 148.6 (s), 161.4 (s). Anal. Calcd for $C_{22}H_{19}BrN_4OS$: C, 56.54; H, 4.10; N, 11.99. Found: C, 56.30; H, 3.95; N, 11.89. MS *m/z* 467 (MH^+). HRMS: $[MH]^+$, found 467.0525 $C_{22}H_{19}BrN_4OS$ requires 467.0536.

*5.1.5.11 3-{2-[5-Bromo-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**5k**)*

Orange solid; yield: 64%; mp: 149-150 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.24 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 3.70-3.72 (m, 4H, 2 x CH_2), 4.45-4.58 (m, 4H, 2 x CH_2), 7.31 (dd, 1H, *J* = 7.9, 4.8 Hz, H-5''), 7.44 (dd, 1H, *J* = 8.8, 1.9 Hz, H-6'), 7.67 (d, 1H, *J* = 8.8 Hz, H-7'), 7.77 (s, 1H, H-5), 8.20-8.26 (m, 2H, H-2' and H-2''), 8.39 (dd, 1H, *J* = 4.8, 1.2 Hz, H-6''), 8.52 (d, 1H, *J* = 1.9, Hz, H-4'), 8.69 (dd, 1H, *J* = 7.9, 1.2 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 44.2 (t), 46.0 (t), 58.1 (2 x q). 70.5 (t), 70.7 (t), 108.0 (d), 109.2 (s), 109.5 (s), 113.2 (d), 113.7 (s), 116.3 (d), 118.7 (s), 122.7 (d), 125.0 (d), 126.2 (s), 128.8 (d), 130.9 (s), 131.0 (d), 131.4 (d), 135.4 (s), 140.8 (d), 148.7 (s), 161.4 (s). Anal. Calcd for $C_{24}H_{23}BrN_4O_2S$: C, 56.36; H, 4.53; N, 10.95. Found: C, 56.21; H, 4.27; N, 10.81. MS *m/z* 511 (MH^+). HRMS: $[MH]^+$, found 511.0811 $C_{24}H_{23}BrN_4O_2S$ requires 511.0798.

*5.1.5.12 3-[2-(5-Bromo-1-methyl-1H-indol-3-yl)-1,3-thiazol-4-yl]-1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**5l**)*

Yellow solid; yield: 84%; mp: 240-241 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.29 (s, 3H, CH_3), 3.79 (t, 2H, J = 5.1 Hz, CH_2), 3.89 (s, 3H, CH_3), 4.57 (t, 2H, J = 5.1 Hz, CH_2), 7.36-7.45 (m, 2H, H-5'' and H-6'), 7.56 (d, 1H, J = 8.7 Hz, H-7'), 7.78 (s, 1H, H-5), 8.24-8.26 (m, 2H, H-2' and H-2''), 8.45-8.47 (m, 2H, H-4' and H-6''), 8.82 (dd, 1H, J = 7.9, 1.3 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 33.1 (q), 44.4 (t), 58.1 (q), 70.5 (t), 108.1 (d), 108.9 (s), 109.7 (s), 112.8 (d), 113.8 (s), 116.2 (d), 119.1 (s), 122.7 (d), 124.9 (d), 126.1 (s), 128.9 (d), 131.5 (d), 132.0 (d), 135.8 (s), 140.2 (d), 144.6 (s), 148.4 (s), 161.5 (s). Anal. Calcd for $C_{22}H_{19}BrN_4OS$: C, 56.54; H, 4.10; N, 11.99. Found: C, 56.75; H, 3.95; N, 11.85. MS *m/z* 467 (MH^+). HRMS: $[MH]^+$, found 467.0552 $C_{22}H_{19}BrN_4OS$ requires 467.0536.

*5.1.5.13 3-{2-[5-Fluoro-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1H-pyrrolo[2,3-b]pyridine (**5m**)*

Orange solid; yield: 70%; mp: 203-204 °C; IR cm^{-1}: 3237 (NH); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.25 (s, 3H, CH_3), 3.73 (t, 2H, J = 5.0 Hz, CH_2), 4.48 (t, 2H, J = 5.0 Hz, CH_2), 7.17 (td, 1H, J = 11.7, 9.2, 2.5 Hz, H-6'), 7.46 (dd, 1H, J = 7.9, 5.2 Hz, H-5''), 7.70 (dd, 1H, J = 9.2, 4.5 Hz, H-7'), 7.85 (s, 1H, H-5), 8.03 (dd, 1H, J = 11.7, 2.5 Hz, H-4'), 8.26 (d, 1H, J = 2.3 Hz, H-2''), 8.30 (s, 1H, H-2'), 8.47 (dd, 1H, J = 5.2, 1.2 Hz, H-6''), 8.92 (d, 1H, J = 7.9 Hz, H-4''), 12.53 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 46.1 (t), 58.1 (q), 70.7 (t), 105.2 (d, $J_{C6'\text{-}F}$ = 25.5 Hz), 109.0 (d), 109.6 (d, $J_{C7'a\text{-}F}$ = 4.9 Hz), 110.7 (d, $J_{C4'\text{-}F}$ = 25.8 Hz), 111.3 (s), 112.4 (d, $J_{C7'\text{-}F}$ = 9.6 Hz), 116.2 (d), 121.0 (s), 124.8 (d, $J_{C3'a\text{-}F}$ = 10.8 Hz), 126.9 (d), 131.9 (d), 133.3 (s), 134.8 (d), 137.1 (d), 141.7 (s), 148.0 (s), 158.1 (d, $J_{C5'\text{-}F}$ = 234 Hz), 161.9 (s). Anal. Calcd for $C_{21}H_{17}FN_4OS$: C, 64.27; H, 4.37; N, 14.28. Found: C, 64.01; H, 4.26; N, 14.57. MS *m/z* 393 (MH^+). HRMS: $[MH]^+$, found 393.1199 $C_{21}H_{17}FN_4OS$ requires 393.1180.

*5.1.5.14 3-{2-[5-Fluoro-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1-methyl-1H-pyrrolo[2,3-b]pyridine (**5n**)*

Yellow solid; yield: 75%; mp: 252-253 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.25 (s, 3H, CH_3), 3.72 (t, 2H, J = 5.1 Hz, CH_2), 3.97 (s, 3H, CH_3), 4.47 (t, 2H, J = 5.1 Hz, CH_2), 7.17 (td, 1H, J = 11.7, 9.2, 2.5, Hz, H-6'), 7.37 (dd, 1H, J = 7.9, 4.9 Hz, H-5''), 7.69 (dd, 1H, J = 9.2, 4.5 Hz, H-7'), 7.77 (s, 1H, H-5), 8.07(dd, 1H, J = 11.7, 2.5 Hz, H-4'), 8.27 (bs, 2H, H-2' and H-2''), 8.44 (dd, 1H, J = 4.9, 1.3 Hz, H-6''), 8.74 (dd, 1H, J = 7.9, 1.3 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 31.7 (q), 46.0 (t), 58.1 (q), 70.7 (t), 105.3 (d, $J_{C6'\text{-}F}$ = 24.9 Hz), 107.5 (s), 107.6 (d), 109.3 (s), 109.7 (s), 110.7

(d, $J_{C4'-F}$ = 26.2 Hz), 112.3 (d, $J_{C7'-F}$ = 10.2 Hz) 116.1 (d), 116.3 (s), 124.8 (d, $J_{C3'a-F}$ = 10.9 Hz), 129.7 (d), 130.9 (d), 131.8 (d), 133.3 (s), 140.5 (d), 148.7 (s), 158.2 (d, $J_{C5'-F}$ = 235 Hz), 161.6 (s). Anal. Calcd for $C_{22}H_{19}FN_4OS$ C, 65.01; H, 4.71; N, 13.78. Found: C, 65.22; H, 4.56; N, 14.05. MS *m/z* 407 (MH^+). HRMS: $[MH]^+$, found 407.1324 $C_{22}H_{19}FN_4OS$ requires 407.1336.

*5.1.5.15 3-{2-[5-Fluoro-1-(2-methoxyethyl)-1H-indol-3-yl]-1,3-thiazol-4-yl}-1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**5o**)*

Orange solid; yield: 72%; mp: 173°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.25 (s, 3H, CH_3), 3.28 (s, 3H, CH_3), 3.71-3.82 (m, 4H, 2 x CH_2), 4.45-4.59 (m, 4H, 2 x CH_2), 7.17 (td, 1H, *J* = 11.7, 9.2, 2.5 Hz, H-6'), 7.32 (dd, 1H, *J* = 7.9, 4.8 Hz, H-5''), 7.70 (dd, 1H, *J* = 9.2, 4.5 Hz, H-7'), 7.76 (s, 1H, H-5), 8.06 (dd, 1H, *J* = 11.7, 2.5 Hz, H-4'), 8.25 (s, 1H, ArH), 8.27 (s, 1H, ArH), 8.40 (dd, 1H, *J* = 4.8, 1.4 Hz, H-6''), 8.67 (dd, 1H, *J* = 7.9, 1.4 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 44.1 (t), 46.0 (t), 58.0 (q), 58.1 (q), 70.5 (t), 70.7 (t), 105.4 (d, $J_{C6'-F}$ = 24.1 Hz), 107.6 (d), 109.4 (s), 109.7 (d, $J_{C7'a-F}$ = 4.2 Hz), 110.6 (d, $J_{C4'-F}$ = 26.4 Hz), 112.4 (d, $J_{C7'-F}$ = 10.0Hz), 116.3 (d), 118.4. (s), 125.0 (d, $J_{C3'a-F}$ = 10.7 Hz), 128.9 (d), 130.3 (d), 131.8 (d), 133.3 (s), 141.3 (d), 145.7 (s), 148.7 (s), 158.2 (d, $J_{C5'-F}$ = 234 Hz), 161.6 (s). Anal. Calcd for $C_{24}H_{23}FN_4O_2S$: C, 63.98; H, 5.15; N, 12.44. Found: C, 64.22; H, 5.02; N, 12.30. MS *m/z* 451 (MH^+). HRMS: $[MH]^+$, found 451.1623 $C_{24}H_{23}FN_4O_2S$ requires 451.1599.

*5.1.5.16 3-[2-(5-Fluoro-1-methyl-1H-indol-3-yl)-1,3-thiazol-4-yl]-1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**5p**)*

Yellow solid; yield: 89%; mp: 209-210°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.28 (s, 3H, CH_3), 3.79 (t, 2H, *J* = 5.3 Hz, CH_2), 3.92 (s, 3H, CH_3), 4.56 (t, 2H, *J* = 5.3 Hz, CH_2), 7.19 (td, 1H, *J* = 11.6, 9.2, 2.5 Hz, H-6'), 7.33 (dd, 1H, *J* = 7.9, 4.8 Hz, H-5''), 7.63 (dd, 1H, *J* = 9.2, 4.4 Hz, H-7'), 7.76 (s, 1H, H-5), 8.07 (dd, 1H, *J* = 11.6, 2.5 Hz, H-4'), 8.25 (s, 1H, ArH), 8.29 (s, 1H, ArH), 8.40 (dd, 1H, *J* = 4.8, 1.4 Hz, H-6''), 8.69 (d, 1H, *J* = 7.9, 1.4 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 33.2 (q), 44.4 (t), 58.1 (q), 70.5 (t), 105.4 (d, $J_{C6'-F}$ = 24.4 Hz), 107.9 (d), 109.4 (d, $_{JC7'a-F}$ = 4.6 Hz), 109.6 (s), 110.7 (d, $J_{C4'-F}$ = 26.0 Hz), 112.1 (d, $J_{C7'-F}$ = 9.9 Hz), 116.3 (d), 119.0 (s), 124.8 (d, $J_{C3'a-F}$ = 10.8 Hz), 129.2 (d), 131.4 (d), 132.4 (d), 133.8 (s), 140.3 (d), 144.7 (s), 148.3 (s), 158.2 (d, $J_{C5'-F}$ = 234 Hz), 161.8 (s). Anal. Calcd for $C_{22}H_{19}FN_4OS$: C, 65.01; H, 4.71; N, 13.78. Found: C, 64.77; H, 4.57; N, 13.52. MS *m/z* 407 (MH^+). HRMS: $[MH]^+$, found 407.1312 $C_{22}H_{19}FN_4OS$ requires 407.1336.

*5.1.5.17 3-[2-(5-Fluoro-1H-indol-3-yl)-1,3-thiazol-4-yl]-1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**5q**)*

Green solid; yield: 56%; mp: 185-186°C; IR cm^{-1}: 3454 (NH); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.27 (s, 3H, CH_3), 3.79 (t, 2H, *J* = 5.1 Hz, CH_2), 4.59 (t, 2H, *J* = 5.1 Hz, CH_2), 7.12 (td, 1H, *J* = 11.6, 9.2, 2.5 Hz, H-6'), 7.39-7.46 (m, 1H, H-7'), 7.55 (dd, 1H, *J* = 8.9, 4.6 Hz, H-5''), 7.82 (s, 1H, H-5), 8.04 (dd, 1H, *J* = 11.6, 2.5 Hz, H-4'), 8.29 (d, 1H, *J* = 2.8 Hz, H-2'), 8.32 (s, 1H, H-2''), 8.47 (d, 1H, *J* = 4.6 Hz, H-6''), 8.83 (d, 1H, *J* = 8.9 Hz, H-4''), 11.98 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 44.3 (t), 58.1 (q), 70.5 (t), 105.2 (d, $J_{C6'-F}$ = 24.0 Hz), 107.9 (d), 109.6 (s), 110.4 (s), 110.7 (d, $J_{C4'-F}$ = 26.3 Hz), 113.5 (d, $J_{C7'-F}$ = 10.0Hz), 116.3 (d), 119.0. (s), 124.5 (d, $J_{C3'a-F}$ = 10.8 Hz), 128.8 (d), 129.2 (d), 131.4 (d), 133.2 (s), 140.4 (d), 144.7 (s), 148.2 (s), 158.0 (d, $J_{C5'-F}$ = 233 Hz), 162.2 (s). Anal. Calcd for $C_{21}H_{17}FN_4OS$: C, 64.27; H, 4.37; N, 14.28. Found: C, 64.17; H, 4.62; N, 14.12. MS *m/z* 393 (MH^+). HRMS: $[MH]^+$, found 393.1197 $C_{21}H_{17}FN_4OS$ requires 393.1180.

*5.1.5.18 Tert-butyl 3-{4-[1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1,3-thiazol-2-yl}-1H-indole-1-carboxylate (**6a**)*

Yellow solid; yield: 94%; mp: 179-180 °C; IR cm^{-1}: 1738 (CO); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.68 (s, 9H, 3 x CH_3), 3.27 (s, 3H, CH_3), 3.79 (t, 2H, *J* = 5.2 Hz, CH_2), 4.58 (t, 2H, *J* = 5.2 Hz, CH_2), 7.36-7.50 (m, 3H, H-5', H-5'' and H-6'), 7.97 (s, 1H, H-5), 8.15-8.20 (m, 1H, H-7'), 8.32-8.49 (m, 4H, H-2', H-2'', H-4' and H-6''), 8.76 (dd, 1H, *J* = 8.0, 1.3 Hz, H-4''); ^{13}C (50 MHz, DMSO-d_6) δ: 27.6 (3 x q), 44.3 (t), 58.1 (q), 70.5 (t), 84.9 (s), 99.6 (s), 109.3 (s), 110.0 (d), 115.0 (d), 116.4 (d), 118.6 (s), 121.3 (d), 123.9 (d), 125.5 (d), 125.6 (d), 126.6 (s), 129.2 (d), 131.0 (d), 135.0 (s), 140.7 (d), 145.1 (s), 148.6 (s), 149.3 (s), 159.8 (s). Anal. Calcd for $C_{26}H_{26}N_4O_3S$: C, 65.80; H, 5.52; N, 11.81. Found: C, 65.69; H, 5.28; N, 11.62. MS *m/z* 475 (MH^+). HRMS: $[MH]^+$, found 475.1776 $C_{26}H_{26}N_4O_3S$ requires 475.1798.

*5.1.5.19 Tert-butyl 5-bromo-3-{4-[1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1,3-thiazol-2-yl}-1H-indole-1-carboxylate (**6b**)*

Yellow solid; yield: 71%; mp: 157-158 °C; IR cm^{-1}: 1742 (CO); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.69 (s, 9H, 3 x CH_3), 3.30 (s, 3H, CH_3), 3.79 (t, 2H, *J* = 5.1 Hz, CH_2), 4.55 (t, 2H, *J* = 5.1 Hz, CH_2), 7.29 (dd, 1H, *J* = 7.9, 4.7 Hz, H-5''), 7.65 (dd, 1H, *J* = 8.9, 2.0 Hz, H-6'), 7.94 (s, 1H, H-5), 8.10 (d, 1H, *J* = 8.9 Hz, H-7'), 8.21 (s, 1H, H-2''), 8.37-8.39 (m, 2H, H-2' and H-6''), 8.63 (dd, 1H, *J* = 7.9, 1.5 Hz, H-4''), 8.68 (d, 1H, *J* = 2.0 Hz, H-4'); ^{13}C (50 MHz, DMSO-d_6) δ: 27.6 (3 x q), 43.9 (t), 58.0 (q), 70.7 (t), 85.3 (s), 99.5 (s), 108.9 (s), 109.8 (d), 114.1 (s), 116.3 (d), 116.8 (d),

118.0 (s), 123.8 (d), 126.7 (d), 127.9 (d), 128.4 (s), 128.6 (d), 129.7 (d), 133.8 (s), 141.8 (d), 146.2 (s), 148.3 (s), 149.6 (s), 159.3 (s). Anal. Calcd for $C_{26}H_{25}BrN_4O_3S$: C, 56.42; H, 4.55; N, 10.12. Found: C, 56.19; H, 4.44; N, 9.89. MS *m/z* 553 (MH^+). HRMS: $[MH]^+$, found 553.0892 $C_{26}H_{25}BrN_4O_3S$ requires 553.0903.

*5.1.6 General Procedure for the Synthesis of thiazoles (**7a,b**)*

To a suspension of appropriate thiazole **6a**,**b** (0.38 mL) in DCM (5 mL) trifluoroacetic acid (7.0 mmol, 0.54 mL) was added and the mixture was heated under reflux for 24 h. After cooling, the mixture was neutralized with saturated aqueous sodium hydrogen carbonate solution and extracted with dichloromethane (3 x 20). The organic phases was dried (Na_2SO_4), evaporated under reduced pressure, and the residue was recrystallized with ethanol to afford the desidered thiazoles **7a**,**b**.

*5.1.6.1 3-[2-(1H-Indol-3-yl)-1,3-thiazol-4-yl]-1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**7a**)*

Green solid; yield: 72%; mp: 152 °C; IR cm^{-1}: 3408 (NH); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.28 (s, 3H, CH_3), 3.79 (t, 2H, *J* = 5.4 Hz, CH_2), 4.55 (t, 2H, *J* = 5.4 Hz, CH_2), 7.22-7.32 (m, 3H, H-5', H-5'' and H-6'), 7.48-7.56 (m, 1H, H-7'), 7.73 (s, 1H, H-5), 8.18 (d, 1H, *J* = 2.8 Hz, H-2'), 8.21 (s, 1H, H-2''), 8.32-8.38 (m, 2H, H-4' and H-6''), 8.66 (dd, 1H, *J* = 7.9, 1.5 Hz, H-4''), 11.80 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 43.9 (t), 58.0 (q), 70.5 (t), 107.2 (d), 109.2 (s), 110.4 (s), 112.2 (d), 116.3 (d), 118.0 (s), 120.3 (d), 120.8 (d), 122.4 (d), 124.2 (s), 126.8 (d), 128.6 (d), 129.8 (d), 136.6 (s), 141.9 (d), 146.4 (s), 148.8 (s), 162.3 (s). Anal. Calcd for $C_{21}H_{18}N_4OS$: C, 67.36; H, 4.85; N, 14.96. Found: C, 67.21; H, 4.69; N, 14.74. MS *m/z* 375 (MH^+). HRMS: $[MH]^+$, found 375.1260 $C_{21}H_{18}N_4OS$ requires 375.1274.

*5.1.6.2 3-[2-(5-Bromo-1H-indol-3-yl)-1,3-thiazol-4-yl]-1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridine (**7b**)*

Green solid; yield: 60%; mp: 128-129 °C; IR cm^{-1}: 3377 (NH); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.31 (s, 3H, CH_3), 3.79 (t, 2H, *J* = 5.2 Hz, CH_2), 4.55 (t, 2H, *J* = 5.2 Hz, CH_2), 7.28 (dd, 1H, *J* = 7.9, 4.7 Hz, H-5''), 7.39 (dd, 1H, *J* = 8.7, 1.9 Hz, H-6'), 7.50 (d, 1H, *J* = 8.7 Hz, H-7'), 7.74 (s, 1H, H-5), 8.18 (s, 1H, H-2''), 8.24 (s, 1H, *J* = 2.8 Hz, H-2'), 8.37 (dd, 1H, *J* = 4.7, 1.4 Hz, H-6''), 8.54 (d, 1H, *J* = 1.9 Hz, H-4'); 8.66 (dd, 1H, *J* = 7.9, 1.4 Hz, H-4''), 11.98 (bs, 1H, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 43.7 (t), 58.0 (q), 70.6 (t), 107.4 (d), 109.1 (s), 110.1 (s), 113.3 (s), 114.3 (d), 116.3 (d), 117.9 (s), 122.7 (d), 124.9 (d), 126.0 (s), 128.1 (d), 128.3 (d), 129.4 (d), 135.3 (s), 142.2 (d), 146.6 (s), 149.2 (s), 161.7 (s). Anal. Calcd for $C_{21}H_{17}BrN_4OS$: C, 55.64; H, 3.78; N, 12.36. Found: C, 55.52; H, 3.68; N, 12.60. MS *m/z* 453 (MH^+). HRMS: $[MH]^+$, found 453.0398

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$C_{21}H_{17}BrN_4OS$ requires 453.0379.

5.2 Biology

Nortopsentin analogues **5e**,**q** and **7a**,**b** were dissolved in DMSO and then diluted in culture medium so that the effective DMSO concentration did not exceed 0.1%. MCF-7 (breast cancer) and Caco-2 (colorectal cancer) cell lines were purchased from American Type Culture Collection, Rockville, MD, USA and grown in RPMI medium supplemented with L-glutamine (2 mM), 10% fetal bovine serum (FBS), penicillin (100 U/mL), streptomycin (100 µg/mL) and gentamicin (5 µg/mL). Cells were maintained in log phase by seeding twice a week at a density of 3×10^8 cells/L in humidified 5% CO_2 atmosphere, at 37 °C. MCF-7 cells were left to incubate overnight to allow adhesion before treatment with the compounds or vehicle alone (control cells), while Caco-2 cells were treated 15 days after confluence, at which time the cells are differentiated in normal intestinal-like cells [44]. In all experiments, no differences were found between cells treated with DMSO 0.1% and untreated cells in terms of cell number and viability.

5.2.1 Viability Assay in Vitro

Cytotoxic activity of the nortopsentin derivatives **5e**,**q** and **7a**,**b** on differentiated Caco-2 cells was determined by the colorimetric assay based on the reduction of 3-(4,5-dimethyl-2-thiazolyl)bromide-2,5-diphenyl-2*H*-tetrazolium (MTT) to purple formazan by mitochondrial dehydrogenases. Briefly, Caco-2 lines cells were seeded at 2×10^4 cells/well in 96-well plates containing 200 µL RPMI. The appropriated, monolayer cultures were treated for 72 h with various concentrations (5–100 µM) of the tested compounds. Then cells were washed with fresh medium and 50 µL FBS-free medium containing 5 mg/mL MTT was added. Cells were incubated 2 h at 37 °C, then medium was discarded by centrifugation, formazan blue formed in the cells dissolved in DMSO, and absorbance measured at 570 nm in a microplate reader (Bio-RAD, Hercules, CA, USA). Formazan of control cells was taken as 100% viability and the 50% lethal concentrations (LC_{50}) were determined by plotting the graph of cell viability versus the concentrations. The selectivity index (SI) values were calculated for each compound by dividing the LC_{50} of normal differentiated Caco2 cells by the GI_{50} of MCF-7 tumor cells. Each experiment was repeated at least three times in triplicate.

5.2.2 Measurement of phosphatidylserine exposure

The externalization of phosphatidylserine to the cell surface was detected by flow cytometry by double staining with annexin V/PI. MCF-7 cells were seeded in triplicate in 24-wells culture plates

at a density of 2.0 x 10^5 cells/cm^2. After an overnight incubation, cells were washed with fresh medium and incubated with the compounds prepared as described above. After 72 h, cells were harvested by trypsinization and adjusted at 2.0 x 10^5 cells/mL with combining buffer. One hundred μL of cell suspended solution was added to a new tube, and incubated with 5 μL annexin V and 10 μL of a 20 μg/mL propidium iodide (PI) solution at room temperature in the dark for 15 min. Then samples of at least 1.0 x10^4 cells were subjected to fluorescence-activated cell sorting (FACS) analysis by Epics XL™ flow cytometer using Expo32 software (Beckman Coulter, Fullerton, CA), using appropriate 2-bidimensional gating method.

5.2.3 Cell cycle analysis

Cell cycle stage was analyzed by flow cytometry. After 48 h treatment as described above, the cell layer was trypsinized and washed with cold phosphate buffered saline (PBS). Aliquots of 1 x 10^6 cells were incubated in the dark in a PBS solution containing 20 μg/ml PI and 200 μg/ml RNase, for 30 min, at room temperature. Then samples were immediately subjected to FACS analysis. At least 1x 10^4 cells were analyzed for each sample.

5.2.4 In vitro CDK1/cyclin B assay

The effect of the tested compounds on CDK1/cyclin B activity was evaluated using the Omnia® Kinase Assay Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's protocols. Briefly, increasing concentrations of **5e** and **7b** (0.1−10 μM) were mixed in a Corning ® 384-well, low volume plate with recombinant CDK1/cyclin B protein (Invitrogen) in 1× kinase buffer containing Omnia® peptide substrate, ATP, and dithiothreitol, and the kinase reaction was performed at 30 °C for 60 min. As positive control an indolyl-thiazolyl-7-azaindole derivative of type **4** (Chart 2), i.e. (3-[2-(5-fluoro-1-methyl-1*H*-indol-3-yl)-1,3-thiazol-4-yl]-1-methyl-1*H*-pyrrolo[2,3-*b*] pyridine, was used in the same concentration range. Fluorescences were measured upon excitation at 360 nm and emission at 485 nm in a plate reader (AF2200, Eppendorf AG, Hamburg,Germany) and the progress curves from each well were registered. Initial velocity was determined from the slope of the plot of relative fluorescence units versus time and then plotted against inhibitor concentration to estimate IC_{50} using the dose-response inhibition model in GraphPad Prism 5.02 from GraphPad Software (San Diego, CA).

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Figure 1. Flow cytometric analysis for the quantification, by annexin V/PI double staining, of nortopsentin analogues induced apoptosis in MCF-7 cells. Cell monolayers were incubated for 72 h in the absence (control) or in the presence of the synthesized compounds at their relevant GI_{50} values and submitted to double staining with annexin V/PI as reported in *Paragraph 4.2.2*. BU3, viable cells (annexin V-/P-); BU 4, cells in early apoptosis (annexin V+/PI-); BU 2, cells in tardive apoptosis (annexin V+/PI+); BU E1, necrotic cells (annexin V-/PI+). Representative images of three experiments with comparable results.

Figure 2. Cell cycle analysis of MCF-7 cells treated with compounds 5e,q and 7a,b. Cell monolayers were incubated in the absence (control) or in the presence of the compounds at their relevant GI_{50} values. After 48 h incubation, propidium iodide-stained cells were submitted to flow cytometric analysis as reported in *Paragraph 4.2.3*. The percentage of cells in the different phases of the cycle was calculated by Expo32 software. Values are the mean±SD of three separate experiments in triplicate.

Figure 3. Effect of 5e,**q** and **7a**,**b on the viability of human intestinal normal-like differentiated Caco-2 cells**. Cells were treated with the compounds and cell viability was measured after 72 h by MTT assay in comparison to cells treated with vehicle alone (control). Values are the mean±SD of three separate experiments in triplicate.

Figure 4. Inhibition of CDK1/cyclin B activity. The activity of recombinant human CDK1/cyclin B was assayed using Omnia® Kinase Assay Kit as reported in *Paragraph 4.2.4*, in the presence of increasing concentration of compounds either **5e**, **7b** and **positive control**. Initial velocity of the reaction (RCFU/s) was determined and plotted against compound concentration to calculate IC_{50} value. Values are the mean±SD of two determinations.

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HIGHLIGHTS

- A new series of thiazole nortopsentin analogues was conveniently synthesized.
- Four derivatives showed interesting activity with GI_{50} values from micro to nanomolar level.
- The mechanism of their antiproliferative effect was pro-apoptotic.
- The most potent compound markedly inhibited *in vitro* CDK1 activity.

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