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Original article

Synthesis and antiproliferative properties of $N_{3/8}$-disubstituted 3,8-diazabicyclo[3.2.1]octane analogues of 3,8-bis[2-(3,4,5-trimethoxyphenyl)pyridin-4-yl]methyl-piperazine

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Abstract

A series of novel $N_{3/8}$-disubstituted-3,8-diazabicyclo[3.2.1]octanes in order to improve the *in vitro* activity of the prototype 3,8-bis[2-(3,4,5-trimethoxyphenyl)pyridyl-4-yl)methylpiperazine (**1**) were synthesized and evaluated by assays of growth inhibition against several tumor cell lines. Compounds **2a**,**b**,**f** and **m** demonstrated not only growth-inhibitory activities against leukemia cancer cells, but also fairly good activities against the growth of certain solid tumors. Among them, **2a** is the most potent one with IC_{50} values in the low micromolar range. Moreover, compound **2a** has been selected for *in vitro* testing on MCF-7 cell to evaluate the mode of action of this lead compound.
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Keywords: Piperazine; 3,8-Diazabicyclo[3.2.1]octane

1. Introduction

The global burden of cancer at the beginning of the 21st century is immense. Cancer is a collection of over 100 devastating diseases characterized by malignant cells that are clearly distinguished from normal cells by an out-of-control growth.

Clinically, weapons in the fight against cancer are surgery, radiotherapy and chemotherapy. Current chemotherapy consists of cytotoxic (cell-killing) agents and antihormonal drugs which reduce the chaotic proliferation of cancerous aberrant cells. Significant side effects such as nausea, vomiting, diarrhea, hair loss and serious infections are often encountered during chemotherapy.

Clinical strategies have been developed to address such issues, especially cycles of therapy, but unfortunately this approach may result, in the tumor cell population and in an increased selection pressure which would induce dangerous drug resistance. It is interesting to note that this general profile applies, to a different extent, to all cytotoxic agents even if they act with different mechanisms.

Because of this critical situation, the search for new drugs for antitumour chemotherapy continues at a steady pace [1].

The piperazine nucleus is often found embedded in chemotherapic agents exhibiting a wide range of biological activities (anthelmintic, antiprotozoal, bactericidal, fungicidal, antiviral, and antitumour properties [2,3]). A series of simple N,N'-bisarylpyridinylmethyl piperazines such as **1** have been shown to exhibit *in vitro* growth-inhibitory properties against MCF-7 and HCT-15 cells with IC_{50} values in the micromolar

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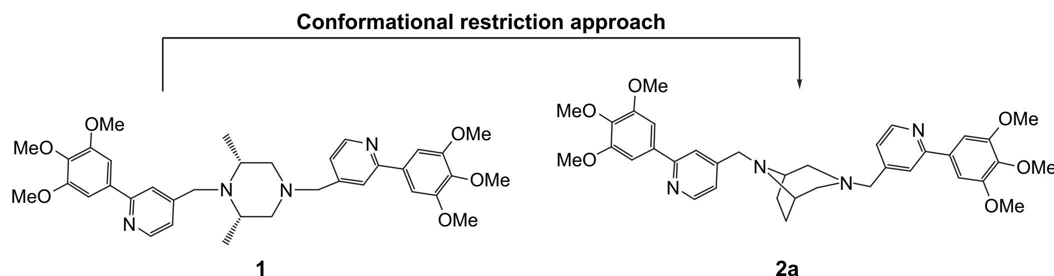


Chart 1. Structure modification of piperazine derivative **1**.

concentration range (Chart 1). HTC cell IC_{50} 6 μM, MCF-7 cell IC_{50} 6 μM [4].

In our effort to search for novel antitumour compounds we decided to assume **1** as a lead structure designing new analogue **2a** in which the *cis*-dimethyl piperazine moiety of **1** was replaced with a 3,8-diazabicyclo[3.2.1]octanyl ring system. Here, we wish to report on the synthesis of our target compounds and on the results of an initial *in vitro* testing for antitumour activity.

2. Chemistry

Compounds **2a–r** (Table 1) were retrosynthetically disconnected at $C-N_{3/8}$ bonds to give 3,8-diazabicyclo[3.2.1]octane (**3**) and appropriate halides (Fig. 1).

Table 1
3,8-Bisdiaza-bicyclo[3.2.1]octane derivatives

Compound		X	R
2			
	a	N	3-(3′,4′,5′ Trimethoxyphenyl)
	b	N	3-*para*-Methoxy phenyl
	c	N	3-*meta*-Methoxy phenyl
	d	N	3-*para*-Hydroxy phenyl
	e	N	3-*meta*-Hydroxy phenyl
	f	N	3-*para*-Methyl phenyl
	g	N	3-*meta*-Methyl phenyl
	h	N	3-*para*-Acetamido phenyl
	i	N	3-*meta*-Acetamido phenyl
	j	N	3-*para*-Chloro phenyl
	k	N	3-*para*-Nitro phenyl
	l	N	3-*meta*-Nitro phenyl
	m	N	3-Phenyl
	n	C	3-Phenyl
	o	C	4-Phenyl
	p	C	H
2q			
2r			

Synthesis of the intermediate **3** in Scheme 1 was accomplished in three steps from the known 3,8-dibenzyl-3,8-diazabyciclo[3.2.1]octane-2,4-dione [5,6] **4** as chloridrate by converting it to the N_8-dibenzyl-derivative **6** by a reaction with hydrogen and catalyst (Pd/C) in ethanol. This imide on reduction with sodium bis(2-methoxyethoxy)aluminium hydride (SMEAH) in toluene at room temperature produced diamine **6** which was then N_3-debenzylated by catalytic hydrogenation to afford **3** as dihydrochloride salt in 87% yield. Amine salt was then alkylated with various halides in the presence of a base under microwave irradiation to produce different title compounds.

The substituted 4-(chloromethyl)-2-phenylpyridine **10a, d–n** was readily prepared from 2-chloro-isonicotinic ethyl ester **7**. Suzuki reaction of **7** with designed arylboronic esters in the presence of catalytic amount of [1,1′-bis(diphenylphosphino)-ferrocene] dichloro palladium (II) ($PdCl_2$(dppf)) afforded arylpyridinyl esters **8** in moderate to good yield. Esters **8** were transformed into the required halides **10a, d–n** using standard procedure, that is, by the reaction with DIBALH to give the corresponding alcohols **9** which were treated with thionyl chloride (Scheme 2).

The analogues **10b** and **10c** were prepared using different starting material as outlined in Schemes 3 and 4.

Catalytic Grignard cross-coupling of commercially available 2-bromo-4-methyl pyridine **11** proceeded with high yields in the presence of nickel catalyst coordinated with 1,2-bis(diphenylphosphino) ethane (dppe). The methyl group in the cross-coupling product **12** was oxidized with potassium permanganate to afford compound **13**. Reduction of the corresponding methyl ester **14** furnished alcohol **15** and subsequent reaction with thionyl chloride yielded the desired intermediate 4-(chloromethyl)-2-(4-methoxyphenyl)pyridine **10b**.

Compound **10c** was prepared starting from (2-bromopyridin-4-yl)methanol **16** which was protected as silyl ether and under catalytic Grignard cross-coupling conditions gave **17** in 65% yield.

After removal of the protecting group, halogeno-substitution of hydroxyl function proceeded efficiently to give 4-(chloromethyl)-2-(3-methoxyphenyl)pyridine **10c**.

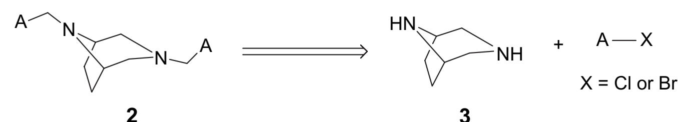
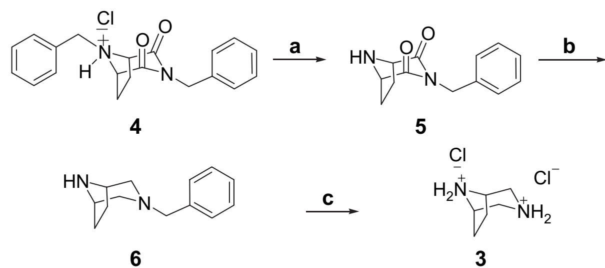
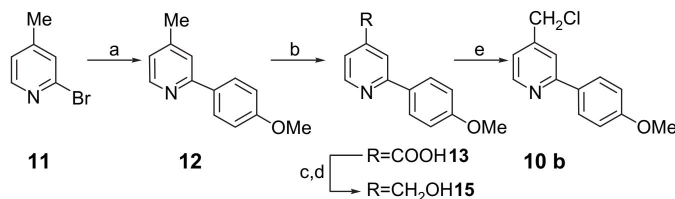


Fig. 1. Antithesis of title derivatives **2**.



Scheme 1. Reagents and conditions: (a) i: Pd/C 10%, ethanol, rt, 3 h, 3 atm; ii: 10% Na_2CO_3; (b) SMEAH, toluene, rt, 2 h; (c) Pd/C 10%, ethanol, 6 N HCl, rt, 24 h.



Scheme 3. Reagents and conditions: (a) 4-methoxyphenyl magnesium bromide, $NiCl_2$(dppe), THF, rt, 18 h; (b) $KMnO_4$, *tert*-BuOH/H_2O, reflux, 24 h; (c) i: $SOCl_2$, 60 °C, 4 h; ii: MeOH, rt, 2 h; (d) DIBALH, toluene, rt, 1 h; (e) $SOCl_2$, CH_2Cl_2, rt, 4 h.

In the case of analogues **2q** and **2r** the deviation from derivative **2a** was more pronounced. On the aromatic units, were carried out respectively, naphthyl and anthranyl moieties.

The final alkylation was performed using microwave program, which was composed by appropriate ramping and holding steps [7]. Identification of the optimum profile was reported.

3. Biological results

The antiproliferative activity of test compounds was evaluated against a panel of cell lines derived from haematological (CCRF-CEM, WIL-2NS and CCRF-SB) (Table 2) or solid (SKMEL28, MCF-7, SKMES-1, HepG2, and DU145) human tumors (Table 3).

They were also evaluated against cell lines derived from normal human tissues (CRL 7065).

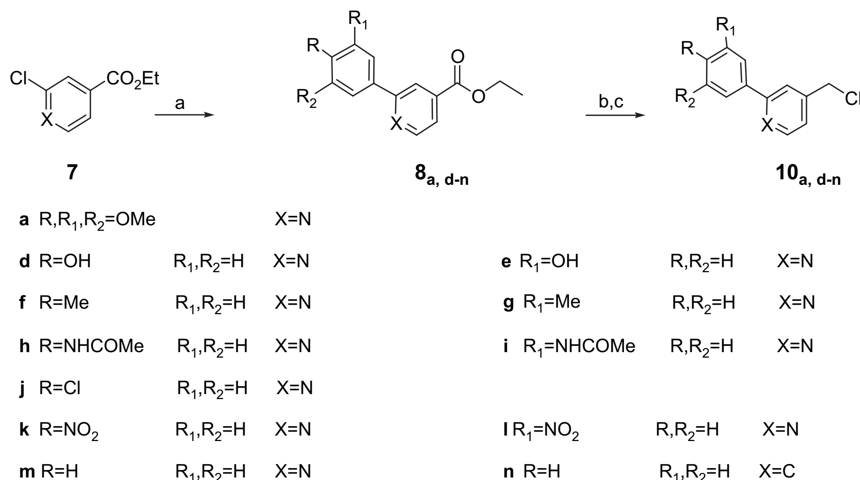
4. Results and discussion

The antiproliferative activity of test compounds was evaluated against a panel of cell lines derived from normal human tissues (CRL 7065) either haematological (CCRF-CEM, WIL-2NS and CCRF-SB) (Table 2) or solid (SKMEL28, MCF-7, SKMES-1, HepG2, and DU145) human tumors (Table 3).

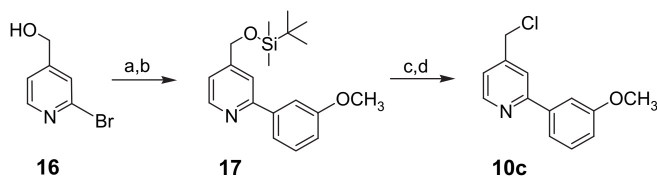
Initial substitution of the *cis*-dimethyl piperazine nucleus of **1** with 3,8-diazabicyclo[3.2.1]octane ring system provided **2a** as an active compound against leukemia cancer cells with IC_{50} in the low micromolar range. Moreover, **2a** showed a wide spectrum of inhibitory effect on cancer cell growth of solid tumors ranging from 4 to 14 μM.

The IC_{50} obtained for $N_{3/8}$-methoxypyridylmethyl-3,8-diazabicyclo[3.2.1]octane derivative **2b** showed that simple *para*-methoxy substitution of the side chain appendages favorably affected the antiproliferative activity being more potent than counterpart **2a** against certain solid tumor cell lines (SKMEL28, SKMES-1, HepG2, DU145). Moreover compound **2b** did have respectable antiproliferative activity against leukemia cell lines with IC_{50} values up to 3 μM. The lower IC_{50} values for *meta*-methoxy derivative **2c** clearly showed that substitution at this position was less tolerated. The *para*-hydroxy analogue **2d** displayed respectable cytotoxicity but it was less active than the ground term **2a**. Compound **2e** showed weak selective cytotoxicity against CCRF-CEM and CCRJ-Sb with IC_{50} values of 14 and 16 μM, respectively; however, **2e** was non-cytotoxic against almost all other tumor cells (Table 4).

These results suggest that the methoxy group at phenyl *para* position is important for anticancer response.



Scheme 2. Reagents and conditions: (a) arylboronic acid or ester, K_2CO_3, $PdCl_2$(dppf), EtOH, 60 W, 110 °C, 10 min; (b) DIBALH, toluene, rt, 15 min or THF, rt 18 h; (c) $SOCl_2$, benzene, reflux, 10 min.



Scheme 4. Reagents and conditions: (a) TBDSCl, imidazole, DMF, 25 °C, 13 h; (b) 3-methoxyphenyl magnesium bromide, $NiCl_2$(dppe), THF, rt, 18 h; (c) TBAF, THF, 0 °C, 25 °C, 3 h; (d) $SOCl_2$, CH_2Cl_2, rt, 4 h.

Compound **2f** containing a *para*-methyl group demonstrated good selective cytotoxicity against the solid tumor cell lines while the *meta*-methyl derivative exhibited marginal activity. A direct comparison of **2b**,**c** and **2h**,**i** allowed a determination of the effect of replacing the OCH_3 with $NHCOCH_3$, since the two pairs of structure were otherwise identical. The decreased IC_{50} values for acetylamino derivatives **2h**,**i** clearly showed that this type of substitution was less tolerated.

Turning to the analogues containing an electron withdrawing group such as Cl or NO_2, compounds **2j**–**k**, only the chloro derivative **2j** displayed significant cytotoxicity especially against CCRF-CEM and solid tumor cell lines.

Compound **2m**, the unsubstituted bisphenyl pyridylmethyl-3,8-diazabicyclo[3.2.1]octane derivative, showed moderate cytotoxicity toward all of the tested cell lines, except against MT-4 and WIL-2NS.

Table 2
Microwave program applied for final alkylation

Time (min)	Power (W)	Temperature (°C)
5	80	80
60	300	120
5	200	110

When the nitrogen atom of **2m** was replaced with a carbon atom, as seen in **2n**, cytotoxicity against solid tumor cell lines decreased, but **2n** still exhibited weak activity against leukemia cell lines.

Compound **2o**, having a phenyl group at C-4 pyridylmethyl segment showed moderate cytotoxicity against leukemia cell lines as well as against SH-MEL-28 and DU145. Furthermore, replacing the phenyl moiety of **2o** with a hydrogen atom yielded **2p** which was non-toxic against all tumor cell lines, suggesting that the phenyl pyridylmethyl side chain appendages are structural features that bestow antiproliferative activity. However, it is interesting to note that compounds **2q** and **2r** bearing a naphthylmethyl or an anthracenylmethyl moiety at $N_{3/8}$-diazabicycloalkane core displayed moderate to good anticellular activity.

In conclusion, we have designed and prepared a new series of $N_{3/8}$-disubstituted-diazabicyclo[3.2.1]octane analogues **2** of 3,8-bis [2(3,4,5-trimethoxyphenyl)pyridin-4yl)methylpiperazine (**1**) for evaluation in an *in vitro* preclinical screening against some human tumor cell lines. The results showed

Table 3
Cytotoxicity against leukemia/lymphoma

Compound	IC_{50} (μM)[a]				
	MT-4[b]	CCRF-CEM[c]	WIL-2NS[d]	CCRF-SB[e]	CRL 7065[f]
2a	2	2 ± 0.2	3 ± 0.05	3 ± 1	>100
2b	4	3 ± 0.05	6 ± 0.6	5 ± 1	>100
2c	N.T.	11 ± 0.05	13 ± 1	12 ± 1	>100
2d	N.T.	15 ± 2	18 ± 3	12 ± 1	80 ± 0.1
2e	N.T.	14 ± 0.2	28 ± 8	16 ± 3	>100
2f	N.T.	8 ± 0.5	26 ± 7	12 ± 2	>100
2g	>100	18 ± 5	25 ± 3	18 ± 4	>100
2h	18	19 ± 2	30 ± 2	25 ± 5	>100
2i	>100	16 ± 0.2	25 ± 0.7	17 ± 0.2	>100
2j	N.T.	7 ± 0.3	29 ± 2	13 ± 2	>100
2k	N.T.	91 ± 10	>100	>100	>100
2l	N.T.	36 ± 2	63 ± 19	27 ± 6	>100
2m	19	8 ± 2	15 ± 0.7	11 ± 0.05	>100
2n	N.T.	12 ± 0.5	12 ± 0.5	10 ± 2	41 ± 2
2o	>100	7 ± 1	11 ± 2	6 ± 0.4	>100
2p	>100	46 ± 0.2	60 ± 6	81 ± 19	>100
2q	N.T.	8 ± 0.3	7 ± 0.01	7 ± 0.7	20 ± 0.9
2r	N.T.	6 ± 0.1	6 ± 0.1	9 ± 0.1	69 ± 0.1
5	>100	85 ± 12	>100	88 ± 12	>100
6	>100	>100	>100	>100	>100
6MP	0.1 ± 0.02	1 ± 0.05	3 ± 0.05	1 ± 0.1	>100
CPT	0.004 ± 0.0004	0.003 ± 0.0005	0.005 ± 0.0005	0.003 ± 0.0005	0.5 ± 0.005

[a] Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (±SD) for three independent determinations.
[b] $CD4^+$ human T-cells containing an integrated HTLV-1 genome.
[c] $CD4^+$ human acute T-lymphoblastic leukemia.
[d] Human splenic B-lymphoblastoid cells.
[e] Human acute B-lymphoblastic leukemia.
[f] Human foreskin fibroblasts, 6MP: 6-mercapto purine, CPT: camptothecin.

Table 4
Cytotoxicity against solid tumor-derived cell lines

Compound	IC_{50} (μM) [a]				
	SK-MEL-28[b]	MCF-7[c]	SKMES-1[d]	HepG2[e]	DU145[f]
2a	4 ± 0.1	5 ± 0.9	10 ± 0.5	8 ± 0.2	14 ± 3
2b	2 ± 0.3	6 ± 2	3 ± 0.6	3 ± 0.2	2 ± 0.2
2c	8 ± 1	15 ± 2	8 ± 0.7	15 ± 1	9 ± 0.7
2d	19 ± 2	25 ± 0.3	23 ± 2	13 ± 2	15 ± 3
2e	>100	>100	>100	>100	>100
2f	7 ± 2	7 ± 0.6	8 ± 0.3	7 ± 0.3	7 ± 0.3
2g	15 ± 4	24 ± 7	18 ± 0.9	41 ± 7	34 ± 3
2h	20 ± 0.8	43 ± 2	22 ± 0.7	30 ± 1	42 ± 0.5
2i	34 ± 0.1	23 ± 5	33 ± 3	43 ± 5	24 ± 2
2j	10 ± 0.4	10 ± 0.5	12 ± 0.5	9 ± 0.1	9 ± 0.1
2k	>100	>100	>100	>100	>100
2l	31 ± 0.4	68 ± 0.1	30 ± 0.1	41 ± 1	85 ± 1.5
2m	8 ± 0.4	9 ± 3	11 ± 0.5	11 ± 0.3	8 ± 0.9
2n	15 ± 0.3	25 ± 3	10 ± 2	38 ± 2	14 ± 3
2o	6 ± 2	84 ± 17	43 ± 7	45 ± 14	7 ± 0.5
2p	>100	>100	>100	>100	>100
2q	13 ± 2	11 ± 3	17 ± 0.1	18 ± 0.1	80 ± 0.1
2r	30 ± 10	54 ± 1	40 ± 1	50 ± 0.2	41 ± 1.4
5	96 ± 4	86 ± 13	81 ± 20	92 ± 8	87 ± 13
6	>100	>100	>100	>100	>100
6MP	15 ± 1	3 ± 0.5	58 ± 2	8 ± 0.5	2 ± 0.05
CPT	0.04 ± 0.005	0.04 ± 0.01	0.01 ± 0.004	0.03 ± 0.005	0.01 ± 0.005

[a] Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (±SD) for three independent determinations.
[b] Human skin melanoma.
[c] Human breast adenocarcinoma.
[d] Human lung squamous carcinoma.
[e] Human hepatocellular carcinoma.
[f] Human prostate carcinoma.

that the antitumour activities of these derivatives were strongly related to the nature of the side chain appendages on 3,8-diazabicyclo[3.2.1]octane core with compounds **2a**,**b** showing significant activity against leukemia cell lines and some solid tumor cell lines.

Generally, they were less active against normal cells and cells derived from solid tumors.

Compound **2a**, bearing a trimethoxy group resulted as being the most potent, showing an IC_{50} against the various cell lines ranging between 2 and 14.0 μM, thus resulting as being equally potent as 6-MP against the haematological tumors. It is interesting to note that the above compound resulted as being six-fold more potent than 6-MP against lung squamous carcinoma, and equally potent as 6-MP against breast adenocarcinoma, hepatocellular and prostate carcinoma.

Despite the enormous variation in tumor types, a common theme in neoplasia is that normal cell proliferation controls are lost at the level of either cell signalling, cell-cycle arrest, differentiation, or apoptotic cell death [8].

Recently, increased attention has been focused on the role of apoptosis in mediating the cytotoxic effects of anticancer agents. The induction of apoptosis has been shown to be an important determinant of the response of most tumors to cytotoxic therapy.

For these reasons we investigate primarily the effects of **2a** on apoptosis and cellular cycle of MCF-7 human breast cancer cells.

This cellular line was selected to compare the analysis with the derivative **1** described in the literature.

First, we have checked the activity of **2a** on apoptosis using propidium iodide (PI) staining by flow cytometry.

MCF-7 cells were incubated with **2a** (10–50 μM) for 24 h. Results in Fig. 2 showed that there was no significant increase in apoptotic cells after treatment with **2a**.

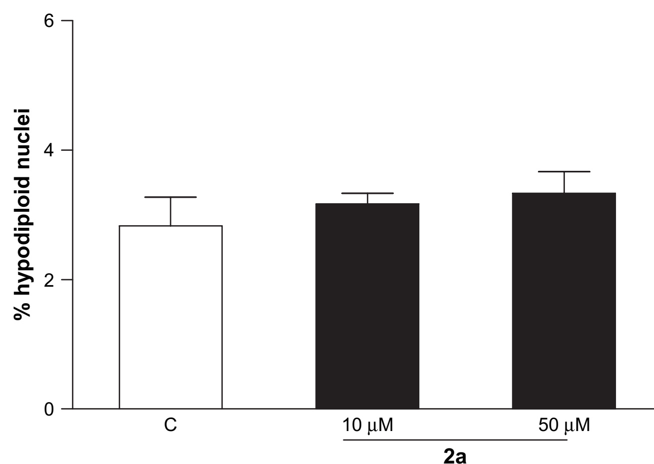


Fig. 2. Apoptosis detection by propidium iodide (PI) staining of hypodiploid nuclei. MCF-7 cells were incubated with **2a** (10–50 mM) for 24 h. Compound **2a** had no effects on MCF-7 apoptosis. Data shown are representative of three experiments performed in triplicate.

This allows us to speculate this derivative, bearing favourable R substituents, which could act with a cytostatic mechanism.

Furthermore we have studied effects of **2a** on cell cycle of MCF-7 cells.

In particular, cells were treated with **2a** (10 μM) for 24 h. At the end the incubation time cell cycle was analysed as DNA content of individual cells by flow cytometry. As shown in Fig. 3 treatment of cells with **2a** induces an accumulation of MCF-7 cells in G0/G1 phase of the cell cycle. Under control condition 58.2% of MCF-7 cells are in G0/G1 phase of cell cycle, and after **2a** treatment for 24 h the percentage of cells is increased to 87.

During this phase kinases developed a central role in the control of the cell cycle. For the kinases to be active it must be associated with another type of protein called cyclins. Hence they are called cyclin-dependent kinases or "Cdks". Cdks control the various checkpoints, they are the gatekeepers that decide whether to pass or fail cells as they go through the various quality control checkpoints in the cell cycle.

It has been shown that the cyclin-dependent kinase inhibitor p21CIP/WAF-1 is an important regulator gene of cell-cycle progression [9].

For this reason we analysed the expression of this protein after **2a** treatment. MCF-7 cells were treated with **2a** (10 μM) at different times and then total intracellular protein extracts were analysed by Western blotting using p21CIP-1/Waf-1 monoclonal antibody. Results in Fig. 4 show a significant increase of p21CIP1/Waf-1 protein after **2a** treatment for 24 h.

Cellular arrest in G0/G1 phase of cell cycle of MCF-7 cells was accompanied by an increase in p21CIP1/Waf-1 protein levels suggesting that antiproliferative effects of **2a** are mediated by p21CIP1/Waf-1 expression.

5. Conclusion

The precise mechanism of action of some of these derivatives is currently under investigation in our laboratory. The ability of **2a** to cause accumulation of p21 and their inactivity

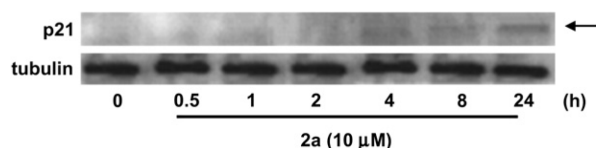


Fig. 4. Expression of p21CIP1/Waf-1 in MCF-7 cells after **2a** treatment at different times measured by western blotting analysis. Compound **2a** (10 mM) induced a time dependent increase of p21/Waf-1. Results are representative of three experiments.

with normal CRL 7065 line will be taken into account even though comparable structures in the literature have the ability to bind tightly but reversible to DNA by intercalation between the base pairs of the double helix [10,11].

Optimisation of the design of these compounds from a structure–activity relationship point of view, as well as the elucidation of their mechanisms of action, could very well lead to the development of novel types of antineoplastic drug.

6. Experimental

6.1. General

Microwave experiments were performed in a CEM Discover monomode reactor (CEM Corp., Matthews, NC). All reactions were conducted in a specially adapted cylindrical Pyrex vessel. All reagents were of analytical grade and purchased from Sigma–Aldrich (Milan, Italy). Flash chromatography was performed on Carlo Erba silica gel 60 (230–400 mesh; Carlo Erba, Milan, Italy). TLC was carried out using plates coated with silica gel $60F_{254}$ purchased from Merck (Darmstadt, Germany). Melting points were determined in open capillary tubes on an Electrothermal 9100 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were registered on a Bruker AC 300. Chemical shifts are reported in parts per million. The abbreviations used are as follows: s, singlet; d, doublet; dd, double doublet; br s, broad singlet. The analytical HPLC analyses were carried out on Beckman Coulter 125 S, equipped with two high pressure binary gradient delivery

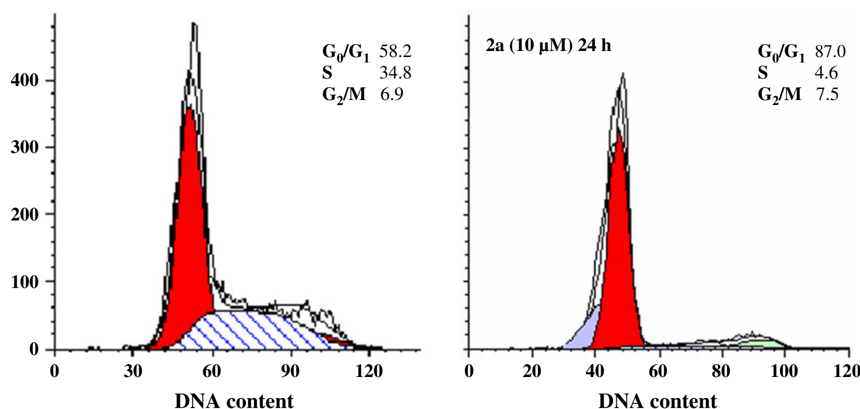


Fig. 3. Cell cycle analysis. Treatment of cells with **2a** (10 mM) for 24 h induces an accumulation of MCF-7 cells in the G0/G1 phase of the cell cycle. Data shown are representative of three experiments.

systems, a System Gold 166 variable-wavelength UV–vis detector and a Rheodyne 7725i injector (Rheodyne, Inc., Cotati, CA, USA) with a 20-μL stainless steel loop.

For the analytical tests, compounds were prepared dissolving in methanol (0.5 mg/mL). Each solution (20 μL) was injected in a Jupiter Phenomenex RP 18 (4.5 × 250 mm) analytical column. The mobile phase was a combination mixture of H_2O + 0.1% TFA (solvent A) and CH_3CN + 0.1% TFA (solvent B). The elution was made in gradient from 5% of B to 71% in 22 min, the flow rate was 1.0 mL/min. UV detection wavelength was set at 210 nm. Mass spectrometry analysis ESI-MS was carried out on a Finnigan LCQ Deca ion trap instrument.

*6.1.1. 3-Benzyl-3,8-diazabicyclo[3.2.1]octan-2,4-dione (**5**)*

A solution of **4** (3 g, 8.4 mmol) in absolute ethanol (70 mL) was hydrogenated in the presence of 10% palladium charcoal (0.3 g, 0.3 mmol), in Parr apparatus, at room temperature and at 3 atm for 3 h. The catalyst was filtered off and ethanol was removed under reduced pressure to give 1.98 g of **5** as hydrochloric salt, which was dissolved in a solution of 10% Na_2CO_3 (8 mL) and the aqueous solution was extracted with Et_2O. The organic layer was dried over Na_2SO_4 and evaporated affording **5** (85%) as free base. ^{1}H NMR ($CDCl_3$): 1.65–1.97 (m, 4H); 2.2 (d, 2H, J = 12 Hz); 6.45 (d, 2H, J = 9.2 Hz); 3.42 (m, 2H); 3.46 (s, 4H); 7.18–7.38 (m, 10H).

*6.1.2. 3-Benzyl-3,8-diazabicyclo[3.2.1]octane (**6**)*

A solution of SMEAH in toluene (5.74 mL, 19.1 mmol) was added dropwise to a, magnetically stirred, cold (0 °C) solution of **5** (1 g, 4.34 mmol) in dry toluene (50 mL) and kept under argon atmosphere. The reaction mixture was stirred at room temperature for 2 h. The solution was cooled to 0 °C and quenched with 20% Na_2CO_3. The aqueous layer was separated and extracted with Et_2O. The organic layer was dried over Na_2SO_4 and evaporated to give a residue which was then subjected to flash chromatography. Elution with $CHCl_3$/MeOH/NH_3 (95:5:0.05) afforded 0.92 g of **6** (70%) as a white solid. ^{1}H NMR ($CDCl_3$): 1.65–1.97 (m, 4H); 2.2 (d, 2H, J = 12 Hz); 6.45 (d, 2H, J = 9.2 Hz); 3.42 (m, 2H); 3.46 (s, 2H); 7.18–7.38 (m, 5H).

*6.1.3. 3,8-Diazabicyclo[3.2.1]octane (DBO) (**3**)*

A solution of **7** (1.31 g, 6.5 mmol) in absolute ethanol (55 mL) and 6 N HCl (3 mL) was hydrogenated in the presence of 10% palladium charcoal (0.3 g, 0.3 mmol), in Parr apparatus, at room temperature and at 3 atm for 12 h. The catalyst was filtered off and ethanol was removed under reduced pressure. The residue was dissolved in Et_2O and ethanol was added dropwise until the formation of a precipitate. The precipitate was collected affording 1 g of **3** (83%) as a white solid. ^{1}H NMR (CD_3OD): 2.35–2.45 (m, 4H); 3.55 (d, 2H, J = 13.59 Hz); 3.68 (d, 2H, J = 13.59 Hz); 4.30 (s, 2H).

*6.2. General procedures for **8a,d–n***

Methyl 2-chloropyridine-4-carboxylate (1.7 mmol), phenylboronic acid or ester (1.5 eq), K_2CO_3 (2 eq), $PdCl_2$(dppf) (5 mol%), 6 mL of 95% ethanol were taken in glass tube with a magnetic stir bar. The vessel was sealed with a septum and placed into the microwave cavity. Microwave irradiation of 60 W was used, the temperature being ramped from room temperature to 110 °C.

The reaction mixture was held at this temperature for 10 min. After the mixture was allowed to cool to room temperature, the reaction vessel was opened and the contents were poured in a flask.

The solvent was removed and saturated $NaHCO_3$ were added. After further extractions of the aqueous layer with CH_2Cl_2, the organic washings were combined, dried over Na_2SO_4 and concentrated *in vacuo*, left a residue which was then subjected to flash chromatography.

*6.2.1. Ethyl 2-(3,4,5-trimethoxyphenyl)pyridine-4-carboxylate (**8a**)*

Elution with *n*-hexane/EtOAc (50:10–45:15) afforded **8a** (85%) as a pale oil. ^{1}H NMR ($CDCl_3$): 1.48 (t, 3H, J = 7.23 Hz); 3.95 (s, 3H); 4.01 (s, 6H); 4.49 (q, 2H, J = 7.23 Hz); 7.34 (s, 2H); 7.79 (d, 1H, J = 4.82 Hz); 8.27 (s, 1H); 8.84 (d, 1H, J = 4.82 Hz).

*6.2.2. Ethyl-(4-hydroxyphenyl)-pyridine-4-carboxylate (**8d**)*

Elution with light petroleum/EtOAc (95:5) afforded **8d** (72%). ^{1}H NMR ($CDCl_3$): 1.47 (t, 3H, J = 7.02 Hz); 4.48 (q, 2H, J = 7.02 Hz); 6.97 (d, 2H, J = 8.55 Hz); 7.45 (d, 1H, J = 5.04 Hz); 7.99 (d, 1H, J = 58.55 Hz); 8.26 (s, 1H); 8.81 (d, 1H, J = 5.04 Hz).

*6.2.3. Ethyl-2(3-hydroxyphenyl)-pyridine-4-carboxylate (**8e**)*

Elution with light petroleum/EtOAc (9:1) afforded **8e** (62%). ^{1}H NMR ($CDCl_3$): 1.45 (t, 3H, J = 7.23 Hz); 4.46 (q, 2H, J = 7.23 Hz); 7.41 (d, 2H, J = 5.26); 7.81 (d, 1H, J = 5.26 Hz); 7.93 (t, 1H, J = 3.95 Hz); 8.07 (s, 1H); 8.26 (s, 1H); 8.82 (d, 1H, J = 5.26 Hz).

*6.2.4. Ethyl-(2-p-tolyl)-pyridine-4-carboxylate (**8f**)*

Elution with light petroleum/EtOAc (95:5) afforded **8f** (44%). ^{1}H NMR ($CDCl_3$): 1.45 (t, 3H, J = 7.02 Hz); 2.45 (s, 3H); 4.50 (q, 2H, J = 7.02 Hz); 7.33 (d, 2H, J = 8.55 Hz); 7.77 (d, 1H, J = 5.04 Hz); 7.98 (d, 2H, J = 8.55 Hz); 8.30 (s, 1H); 8.83 (d, 1H, J = 5.04 Hz).

*6.2.5. Ethyl-2(3-tolyl)-pyridine-4-carboxylate (**8g**)*

Elution with *n*-hexane/EtOAc (95:5) afforded **8g** (65%). ^{1}H NMR ($CDCl_3$): 1.45 (t, 3H, J = 7.02 Hz); 2.5 (s, 3H); 4.49 (q, 2H, J = 7.02 Hz); 7.31 (d, 1H, J = 7.67 Hz); 7.81 (d, 1H, J = 5.04 Hz); 7.87 (d, 1H, J = 7.67 Hz); 7.92 (s, 1H); 8.32 (s, 1H); 8.86 (d, 1H, J = 5.04 Hz).

*6.2.6. Ethyl-2(4-acetamidophenyl)-pyridine-4-carboxylate (**8h**)*

Elution with light petroleum/EtOAc (8:2–4:6) afforded **8h** (60%). ^{1}H NMR ($CDCl_3$): 1.47 (t, 3H, $J=7.02$ Hz); 2.25 (s, 3H); 4.48 (q, 2H, $J=7.02$ Hz); 7.68 (d, 2H, $J=8.55$ Hz); 7.78 (d, 1H, $J=5.04$ Hz); 8.08 (d, 2H, $J=8.55$ Hz); 8.29 (s, 1H); 8.83 (d, 2H, $J=5.04$ Hz).

*6.2.7. Ethyl-2(3-acetamidophenyl)-pyridine-4-carboxylate (**8i**)*

Elution with light petroleum/EtOAc (8:2–4:6) afforded **8i** (60%). ^{1}H NMR ($CDCl_3$): 1.47 (t, 3H, $J=7.02$ Hz); 2.25 (s, 3H); 4.48 (q, 2H, $J=7.02$ Hz); 7.74 (t, 1H, $J=7.89$ Hz); 7.9 (d, 1H, $J=5.04$ Hz); 8.33 (d, 1H, $J=7.89$ Hz); 8.39 (s, 1H); 8.46 (d, 1H, $J=7.89$ Hz); 8.91 (d, 1H, $J=5.04$ Hz); 8.98 (s, 1H).

*6.2.8. Ethyl-(4-chlorophenyl)-pyridine-4-carboxylate (**8j**)*

Elution with *n*-hexane/EtOAc (95:5) afforded **8j** (50%). ^{1}H NMR ($CDCl_3$): 1.43 (t, 3H, $J=7.1$ Hz); 4.44 (q, 2H, $J=7.1$ Hz); 7.44 (dd, 2H, $J=7.2$–1.7 Hz); 7.77 (dd, 1H, $J=5.1$–1.4 Hz); 7.98 (dd, 1H, $J=6.7$–1.7 Hz); 8.23 (s, 1H); 8.79 (d, 1H, $J=4.9$ Hz).

*6.2.9. Ethyl-2(4-nitrophenyl)-pyridine-4-carboxylate (**8k**)*

Elution with light petroleum/EtOAc (95:5) afforded **8k** (66%). ^{1}H NMR ($CDCl_3$): 1.47 (t, 3H, $J=7.02$ Hz); 4.46 (q, 2H, $J=7.02$ Hz); 7.97 (d, 1H, $J=5.04$ Hz); 8.28 (d, 2H, $J=8.11$ Hz); 8.38 (d, 3H, $J=8.11$ Hz); 8.93 (d, 1H, $J=5.04$ Hz).

*6.2.10. Ethyl-2(3-nitrophenyl)-pyridine-4-carboxylate (**8l**)*

Elution with hexane/EtOAc (85:15) afforded **8l** (87%). ^{1}H NMR ($CDCl_3$): 1.49 (t, 3H, $J=7.02$ Hz); 4.51 (q, 2H, $J=7.02$ Hz); 7.74 (t, 1H, $J=7.89$ Hz); 7.9 (d, 1H, $J=5.04$ Hz); 8.33 (d, 1H, $J=7.89$ Hz); 8.39 (s, 1H); 8.46 (d, 1H, $J=7.89$ Hz); 8.91 (d, 1H, $J=5.04$ Hz); 8.98 (s, 1H).

*6.2.11. Ethyl-(2-phenyl)-pyridine-4-carboxylate (**8m**)*

Elution with light petroleum/EtOAc (95:5) afforded **8m** (92%). ^{1}H NMR ($CDCl_3$): 1.48 (t, 3H, $J=7.23$ Hz); 4.49 (q, 2H, $J=7.23$ Hz); 7.43–7.59 (m, 3H); 7.82 (dd, 1H, $J=1.32$–4.82 Hz); 8.1 (dd, 1H, $J=8.55$–1.10 Hz); 8.34 (t, 1H, $J=1.10$ Hz); 8.87 (dd, 1H, $J=5.04$–0.66 Hz).

*6.2.12. Ethyl-3-phenylbenzoate (**8n**)*

The residue was chromatographed on silica gel using *n*-hexane/Et_2O (95:5) as an eluent yielding **8n** (77%) as a pale oil. ^{1}H NMR ($CDCl_3$): 1.47 (t, 3H, $J=7.02$ Hz); 4.48 (q, 2H, $J=7.02$ Hz); 7.29–7.58 (m, 4H); 7.68 (d, 1H, $J=5.04$ Hz); 7.82 (d, 1H, $J=4.82$ Hz); 8.09 (d, 1H, $J=4.82$ Hz); 8.36 (s, 1H).

*6.3. General procedures for **9a**,**d–n***

A solution of 1.5 M DIBALH in toluene or THF (3 eq) was added dropwise to a magnetically stirred solution of **10** (1.535 mmol) in toluene or THF (15 mL) and cooled at 0 °C. The reaction mixture was stirred at room temperature until disappearance of starting material. The reaction was quenched with a saturated solution of $NaHCO_3$. The mixture was filtered and the solvent was removed *in vacuo*.

*6.3.1. 2-(3,4,5-(Trimethoxyphenyl)pyridin-4-yl)methanol (**9a**)*

The residue was diluted with water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with hexane/EtOAc (7:3–4:6) afforded **9a** (58%) as a white powder. ^{1}H NMR ($CDCl_3$): 3.93 (s, 3H); 3.98 (s, 6H); 4.79 (s, 2H); 7.24 (d, 1H, $J=4.82$); 7.25 (s, 2H); 7.72 (s, 1H); 8.66 (d, 1H, $J=4.82$ Hz).

*6.3.2. 4-(4-(Hydroxymethyl)pyridine-2-yl)phenol (**9d**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (6:4) as an eluent yielding **9d** (71%) as a pale oil. ^{1}H NMR ($CDCl_3$): 4.80 (s, 2H); 6.95 (d, 2H, $J=8.77$ Hz); 7.19 (d, 1H, $J=5.04$ Hz); 7.68 (s, 1H); 7.88 (d, 2H, $J=8.77$ Hz); 8.61 (d, 1H, $J=5.04$ Hz).

*6.3.3. 3-(4-(Hydroxymethyl)pyridine-2-yl)phenol (**9e**)*

The residue was chromatographed on silica gel using light petroleum/Et_2O (3:7) as an eluent yielding **9e** (68%) as a pale oil. ^{1}H NMR ($CDCl_3$): 4.85 (s, 2H); 6.93 (d, 1H, $J=4.82$ Hz); 7.27 (d, 1H, $J=7.89$ Hz); 7.37 (t, 1H, $J=7.89$ Hz); 7.54 (s, 1H); 7.55 (d, 1H, $J=7.89$ Hz); 7.75 (s, 1H); 8.67 (d, 1H, $J=4.82$ Hz).

*6.3.4. (2-p-Tolylpyiridin-4-yl)methanol (**9f**)*

The residue was diluted with water and extracted with Et_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with hexane/EtOAc (8:2) afforded **9f** (68%), as a pale oil. ^{1}H NMR ($CDCl_3$): 2.43 (s, 3H); 4.69 (s, 2H); 7.33 (d, 2H, $J=8.11$ Hz); 7.37 (d, 2H, $J=5.04$ Hz); 7.82 (s, 1H); 7.84 (d, 2H, $J=8.11$ Hz).

*6.3.5. (2-m-Tolylpyridin-4-yl)methanol (**9g**)*

The residue was diluted with water and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with light petroleum/EtOAc (9:1) afforded **9g** (78%), as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 2.0 (br s, OH); 2.47 (s, 3H); 4.84 (s, 2H); 7.24–7.38 (m, 2H); 7.39 (t, 1H, $J=7.89$); 7.63 (s, 1H); 7.80 (d, 2H, $J=7.87$ Hz); 8.68 (d, 1H, $J=5.04$ Hz).

*6.3.6. N-4-(4-(Hydroxymethyl)pyridin-2-yl)phenyl-acetamide (**9h**)*

The residue was chromatographed on silica gel using light petroleum/Et_2O (6:4) as an eluent yielding **9h** (55%) as a pale oil. ^{1}H NMR ($CDCl_3$): 4.85 (s, 2H); 6.93 (d, 1H, $J=4.82$ Hz); 7.27 (d, 1H, $J=7.89$ Hz); 7.37 (t, 1H, $J=7.89$ Hz); 7.54 (s, 1H); 7.55 (d, 1H, $J=7.89$ Hz); 7.75 (s, 1H); 8.67 (d, 1H, $J=4.82$ Hz).

6.3.7. N-(3-(4-Hydroxymethyl)pyridine-2-yl)phenyl) acetamide (**9i**)

The residue was chromatographed on silica gel using light petroleum/EtOAc (6:4) as an eluent yielding **9i** (57%) as a pale oil. ^{1}H NMR ($CDCl_3$): 2.19 (s, 3H); 4.88 (s, 2H); 7.34 (d, 1H, $J = 5.04$ Hz); 7.68 (t, 1H, $J = 7.89$); 7.87 (s, 1H); 8.3 (d, 1H, $J = 7.89$ Hz); 8.42 (d, 1H, $J = 7.89$ Hz); 8.72 (d, 1H, $J = 5.04$ Hz); 8.91 (s, 1H).

6.3.8. (2-(4-Chlorophenyl)pyridine-4-yl)methanol (**9j**)

The residue was diluted with water and extracted with Et_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with light petroleum/EtOAc (8:2–1:1) afforded **9j** (84%), as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 1.93 (t, 1H, $J = 5.7$ Hz); 4.85 (d, 2H, $J = 5.7$ Hz); 7.27 (d, 2H, $J = 5.04$ Hz); 7.48 (d, 2H, $J = 8.55$ Hz); 7.75 (s, 1H); 7.99 (d, 2H, $J = 8.55$ Hz); 8.68 (d, 1H, $J = 5.04$ Hz).

6.3.9. (2-(4-Nitrophenyl)pyridine-4-yl)methanol (**9k**)

The residue was diluted with water and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with light petroleum/EtOAc (7:3) afforded **9k** (77%), as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 4.76 (s, 2H); 7.25 (d, 1H, $J = 5.04$ Hz); 7.9 (s, 1H); 8.25 (d, 2H, $J = 8.77$ Hz); 8.3 (d, 2H, $J = 8.77$ Hz); 8.75 (d, 1H, $J = 5.04$ Hz).

6.3.10. (2-(3-Nitrophenyl)pyridin-4-yl)methanol (**9l**)

The residue was diluted with water and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with light petroleum/EtOAc (7:3–2:8) afforded **9l** (69%), as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 4.88 (s, 2H); 7.34 (d, 1H, $J = 5.04$ Hz); 7.68 (t, 1H, $J = 7.89$); 7.87 (s, 1H); 8.3 (d, 1H, $J = 7.89$ Hz); 8.42 (d, 1H, $J = 7.89$ Hz); 8.72 (d, 1H, $J = 5.04$ Hz); 8.91 (s, 1H).

6.3.11. 2-(2-Phenylpyiridin-4-yl)methanol (**9m**)

The residue was diluted with water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated affording **9m** (96%), as a pale oil. ^{1}H NMR ($CDCl_3$): 4.85 (s, 2H); 7.27 (d, 1H,$J = 6.36$ Hz); 7.44–7.55 (m, 3H); 7.78 (s, 1H); 8.04 (d, 2H, $J = 7.02$ Hz); 8.70 (d, 1H, $J = 5.04$ Hz).

6.3.12. 3-Biphenylmethanol (**9n**)

The residue was diluted with water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated affording **9n** (98%). ^{1}H NMR ($CDCl_3$): 4.81 (s, 2H); 7.36–7.74 (m, 9H).

6.4. General procedures for **10a**,**d–n**

A solution of thionyl chloride (1.77 eq) in dry benzene (3 mL) was added dropwise to a cold (0 °C) solution of **9a**,**d–n** (120 mg) in dry benzene (6 mL).

The reaction mixture was refluxed for 10 min and then cooled at 0 °C. The solvent was removed and the residue was diluted with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*.

6.4.1. 4-(Chloromethyl)-2-(3,4,5-trimethoxyphenyl) pyridine (**10a**)

The residue was crystallized from diethyl ether affording **10a** (99%) as a white powder. ^{1}H NMR ($CDCl_3$): 3.93 (s, 3H); 3.98 (s, 6H); 4.65 (s, 2H); 6.88 (d, 1H, $J = 4.82$ Hz); 6.95 (s, 2H); 7.44 (s, 1H); 8.57 (s, 1H).

6.4.2. 4-(4-Chloromethyl)pyridin-2-ylphenol (**10d**)

The residue was chromatographed on silica gel using light petroleum/EtOAc (6:4) as an eluent yielding **10d** (72%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 4.63 (s, 2H); 6.96 (d, 2H, $J = 8.77$ Hz); 7.24 (d, 1H, $J = 5.04$ Hz); 7.71 (s, 1H); 7.94 (d, 2H, $J = 8.77$ Hz); 8.67 (d, 1H, $J = 5.04$ Hz).

6.4.3. 3-(4-Chloromethyl)pyridin-2-ylphenol (**10e**)

The residue was chromatographed on silica gel using light petroleum/EtOAc (7:3) as an eluent yielding **10e** (70%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 4.61 (s, 2H); 6.91 (dd, 1H, $J = 2.63$–7.89 Hz); 7.3 (d, 1H, $J = 4.82$ Hz); 7.34 (d, 1H, $J = 7.89$ Hz); 7.43 (d, 1H, $J = 7.89$ Hz); 7.55 (t, 1H, $J = 1.97$ Hz); 7.69 (s, 1H); 8.67 (d, 1H, $J = 4.82$ Hz).

6.4.4. 4-(Chloromethyl)-2-p-tolylpyridine (**10f**)

The residue was chromatographed on silica gel using light petroleum/EtOAc (8:2) as an eluent yielding **10f** (80%) as a pale oil. ^{1}H NMR ($CDCl_3$): 2.41 (s, 3H); 4.61 (s, 2H); 7.28–7.32 (m, 2H); 7.75 (s, 1H); 7.91 (d, 2H, $J = 8.77$); 8.68 (d, 1H, $J = 4.82$ Hz).

6.4.5. 4-(Chloromethyl)-2-m-tolylpyridine (**10g**)

The residue was chromatographed on silica gel using light petroleum/EtOAc (75:25) as an eluent yielding **10g** (81%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 2.49 (s, 3H); 4.56 (s, 2H); 7.41–7.44 (m, 2H); 7.79–7.82 (m, 2H); 7.88 (s, 1H); 8.43 (d, 2H, $J = 8.77$); 8.73 (d, 1H, $J = 4.82$ Hz).

6.4.6. N-(4-(4-(Chloromethyl)pyridin-2-yl)phenyl) acetamide (**10h**)

The residue was chromatographed on silica gel using light petroleum/EtOAc (6:4) as an eluent yielding **10h** (73%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 2.44 (s, 3H); 4.48 (s, 2H); 7.25 (d, 1H, $J = 5.26$ Hz); 7.36 (s, 1H); 7.66 (d, 2H, $J = 8.77$ Hz); 7.73 (s, 1H); 8.02 (d, 2H, $J = 8.77$ Hz); 8.67 (d, 1H, $J = 5.26$ Hz).

*6.4.7. N-(3-(4-(Chloromethyl)pyridin-2-yl)phenyl) acetamide (**10i**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (6:4) as an eluent yielding **10i** (75%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 2.44 (s, 3H); 4.44 (s, 2H); 7.43–7.48 (m, 2H); 7.79–7.82 (m, 2H); 7.88 (s, 1H); 8.43 (d, 2H, $J = 8.77$); 8.77 (d, 1H, $J = 4.82$ Hz).

*6.4.8. 4-(Chloromethyl)-2-(4-chlorophenyl)pyridine (**10j**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (7:3) as an eluent yielding **10j** (94%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 4.64 (s, 2H); 7.30 (d, 1H, $J = 5.1$ Hz); 7.47 (d, 2H, $J = 8.6$ Hz); 7.76 (s, 1H); 7.98 (d, 2H, $J = 8.6$ Hz); 8.71 (d, 1H, $J = 5.1$ Hz).

*6.4.9. 4-(Chloromethyl)-2-(4-nitrophenyl)pyridine (**10k**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (6:4) as an eluent yielding **10k** (79%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 4.47 (s, 2H); 7.25 (d, 1H, $J = 5.26$ Hz); 7.36 (s, 1H); 7.66 (d, 2H, $J = 8.77$ Hz); 7.73 (s, 1H); 8.01 (d, 2H, $J = 8.77$ Hz); 8.67 (d, 1H, $J = 5.26$ Hz).

*6.4.10. 4-(Chloromethyl)-2-(3-nitrophenyl)pyridine (**10l**)*

The residue was chromatographed on silica gel using light petroleum/Et_2O (9:1–8:2) as an eluent yielding **10l** (79%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 4.55 (s, 2H); 7.37 (d, 1H, $J = 5.04$ Hz); 7.69 (t, 1H, $J = 7.89$ Hz); 7.85 (s, 1H); 8.32 (d, 1H, $J = 7.89$ Hz); 8.41 (d, 1H, $J = 7.89$ Hz); 8.74 (d, 1H, $J = 5.04$ Hz).

*6.4.11. 4-(Chloromethyl)-2-phenylpyridine (**10m**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (8:2) as an eluent yielding **10m** (71%) as a pale oil. ^{1}H NMR ($CDCl_3$): 4.50 (s, 2H); 7.4–7.6 (m, 4H); 7.78 (s, 1H); 8.04 (d, 2H, $J = 7.23$ Hz); 8.72 (d, 1H, $J = 5.04$ Hz).

*6.4.12. 3-(Chloromethyl)biphenyl (**10n**)*

The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo* to give a residue which was then subjected to flash chromatography. Elution with *n*-hexane/EtOAc (9:1–8:2) afforded **10n** (97%). ^{1}H NMR ($CDCl_3$): 4.69 (s, 2H); 7.35–7.90 (m, 9H).

*6.4.13. 1-(4-Methoxyphenyl)-4-methylpyridine (**12**)*

2-Bromo-4-methyl pyridine (1g, 5.8 mmol) was added to a magnetically stirred solution of $NiCl_2$(dppe) (0.266 g, 0.505 mmol) in dry THF (21 mL).

The reaction mixture was magnetically stirred at room temperature under argon atmosphere for 5 min and then a solution of 4-methoxyphenyl magnesium bromide (1.48 g, 7.0 mmol) in dry THF (10 mL) was added dropwise. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed and the residue was diluted with a concentrated solution of NH_3 (27 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with CH_2Cl_2/Et_2O (95:5) afforded **12** (77%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 2.40 (s, 3H); 3.87 (s, 3H); 6.94–7.04 (m, 3H); 7.49 (br s, 1H); 7.94 (d, 2H, $J = 8$ Hz); 8.51 (d, 1H, $J = 5.2$ Hz).

*6.4.14. 2-(4-Methoxyphenyl)pyridine-4-carboxylic acid (**13**)*

$KMnO_4$ (370 mg, 1.1 eq) was added to a rapidly mechanically stirred solution of **12** (0.425 g, 2.13 mmol) in a mixture of *tert*-butyl alcohol (2.55 mL) and water (1 mL). The reaction mixture was refluxed for 2.5 h. An additional 202.4 mg (0.6 eq) of $KMnO_4$ was added together with 1 mL of a 1:1 mixture of *tert*-butyl alcohol/water. The reaction mixture was refluxed until the permanganate color disappeared (about 2.5 h). An additional 202.4 mg (0.6 eq) of $KMnO_4$ was added together with 2 mL of a 1:1 mixture of *tert*-butyl alcohol/water. After 3.5 h 370 mg of $KMnO_4$ (1.1 eq) was added together with 2 mL of a 1:1 mixture of *tert*-butyl alcohol/water. The reaction mixture was refluxed overnight. Finally, an additional 720 mg of $KMnO_4$ (2 eq) was added and the reaction mixture was refluxed until the permanganate color disappeared. The solution was cooled at room temperature, filtered on Celite and the filtrate was washed more number of times with hot water. The aqueous phase was washed with diethyl ether and then acidified with acetic acid to pH 3.6. The precipitate was collected affording **13** (76%) as a white solid. ^{1}H NMR (CD_3OD): 3.87 (s, 3H); 7.01 (d, 2H, $J = 9$ Hz); 7.79 (d, 1H, $J = 6.0$ Hz); 8.02 (d, 2H, $J = 9$ Hz); 8.23 (s, 1H); 8.73 (d, 1H, $J = 6.0$ Hz).

*6.4.15. (2-(4-Methoxyphenyl)pyridin-4-yl)methanol (**15**)*

Thionyl chloride (1.5 mL, 21 mmol) was added to **15** (0.16 g, 0.7 mmol). The reaction mixture was stirred at 60 °C for 4 h. Thionyl chloride in excess was removed. The residue was cooled at 0 °C and methanol was added dropwise. The reaction mixture was stirred at room temperature for 2 h. The solvent was removed and the residue was diluted with 5% $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give methyl 2-(4-methoxyphenyl)pyridine-4-carboxylate **14** (82%) as a yellow oil. ^{1}H NMR ($CDCl_3$): 9.88 (s, 3H); 3.99 (s, 3H); 7.01 (d, 2H, $J = 8.99$ Hz); 7.71 (d, 1H, $J = 5.04$ Hz); 8.02 (d, 2H, $J = 8.8$ Hz); 8.25 (s, 1H); 8.79 (d, 1H, $J = 5.04$ Hz).

A solution of 1.5 M DIBALH in toluene (0.89 mL, 1.32 mmol) was added dropwise to a magnetically stirred solution of **14** (0.106 g, 0.44 mmol) in toluene (8 mL) and cooled at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with a saturated solution of potassium tartrate (0.297 g). The mixture was filtered and diluted with diethyl ether. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with light petroleum/EtOAc (1:1) afforded **15** (79%) as a pale oil. ^{1}H NMR ($CDCl_3$): 3.89 (s, 3H); 4.81 (s, 2H); 7.02 (d, 2H, $J = 8.99$ Hz); 7.19 (d, 1H,

$J = 5.04$ Hz); 7.70 (s, 1H); 7.98 (d, 2H, $J = 8.99$ Hz); 8.63 (1H, $J = 5.04$ Hz).

*6.4.16. 4-(Chloromethyl)-2-(4-methoxyphenyl)pyridine (**10b**)*

Thionyl chloride (0.473 mL, 6.48 mmol) was added to a solution of **15** (0.08 g, 0.37 mmol) in CH_2Cl_2 (3.5 mL). The reaction mixture was stirred at room temperature for 4 h. The solvent was removed and the residue was diluted with Et_2O and washed with saturated $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated affording **10b** (85%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 3.91 (s, CH_3O); 4.80 (s, CH_2); 7.01 (d, 2H, $J = 8.99$ Hz); 7.20 (d, 1H, $J = 5.04$ Hz); 7.74 (s, 1H); 8.00 (d, 2H, $J = 8.99$ Hz); 8.7 (1H, $J = 5.04$ Hz).

*6.4.17. (2-(3-Methoxyphenyl)pyridin-4-yl)methoxy-tert-butyl-dimethylsilane (**18**)*

Imidazole (0.197 g, 2.9 mmol) and TBDSiCl (0.219 g, 1.93 mmol) were added to a cold (0 °C) solution of (2-bromopyridin-4-yl)methanol (**16**) in dry DMF. The resulting mixture was magnetically stirred at 25 °C for 13 h. The solvent was removed and the residue was diluted with water (13 mL) and the aqueous phase was extracted with Et_2O (3 × 20 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with light petroleum/EtOAc (9:1–1:1) afforded **17** (89%) as colorless oil. ^{1}H NMR ($CDCl_3$): 0.18 (s, 6H); 1.01 (s, 9H); 4.77 (s, 2H); 7.23 (d, 1H; $J = 5.04$ Hz); 7.39 (s, 1H); 8.3 (1H, $J = 5.04$ Hz).

To a solution of $NiCl_2$(dppe) (0.132 g, 0.26 mmol) in dry THF (28 mL), kept under argon atmosphere at room temperature and magnetically stirred, was added **17** (0.88 g, 2.9 mmol). The reaction mixture was stirred for 5 min and then a solution of 3-methoxyphenyl magnesium bromide (0.6 g, 3.49 mmol) in THF (7 mL) was added dropwise. The solution was stirred at room temperature for 18 h. The solvent was removed and the residue was diluted with NH_3 (14 mL) and water (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with hexane/EtOAc (9:1) afforded **18** (65%) as a white solid. ^{1}H NMR ($CDCl_3$): 0.2 (s, 6H); 1.01 (s, 9H); 3.93 (s, 3H); 4.84 (s, 2H); 7.00 (dd, 1H, $J = 8.11$, 2.64 Hz); 7.23 (d, 1H, $J = 4.88$ Hz) 7.41 (t, 1H, $J = 7.89$ Hz); 7.58 (d, 1H, $J = 7.89$ Hz); 7.62 (t, 1H, $J = 2.19$); 7.73 (s, 1H); 8.66 (d, 1H, $J = 4.82$ Hz).

*6.4.18. 4-(Chloromethyl)-2-(3-methoxyphenyl)pyridine (**10c**)*

A solution of TBAF (4.58 mL, 4.58 mmol) in THF was added to a cold (0 °C) solution of **18** (0.58 g, 1.76 mmol) in dry THF (20 mL). The reaction mixture was stirred for 45 min at 0 °C and then for 3 h at 25 °C.

The reaction was diluted with water (40 mL) and stirred for 10 min. The solvent was removed and the residue was diluted with water (10 mL) and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with light petroleum/EtOAc (8:2–1:1) afforded (2-(3-methoxyphenyl)pyridin-4-yl)methanol **19** (86%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 3.93 (s, 3H); 4.85 (s, 2H); 7.01 (d, 1H, $J = 8.11$ Hz); 7.28 (d, 1H, $J = 4.82$ Hz) 7.41 (t, 1H, $J = 8.11$ Hz); 7.59 (d, 1H, $J = 8.11$ Hz); 7.62 (s, 1H); 7.76 (s, 1H); 8.67 (d, 1H, $J = 4.82$ Hz). Thionyl chloride (1.26 mL, 17.2 mmol) was added to a solution of **19** (0.212 g, 0.98 mmol) in CH_2Cl_2 (3.5 mL). The reaction mixture was stirred at room temperature for 4 h.

The solvent was removed and the residue was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography. Elution with light petroleum/EtOAc (7:3) afforded **10c** (96%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): 3.94 (s, 3H); 4.64 (s, 2H); 7.02 (d, 1H, $J = 7.89$ Hz); 7.30 (br, 1H); 7.43 (t, 1H, $J = 7.89$ Hz); 7.58 (d, 1H, $J = 7.89$ Hz); 7.63 (d, 1H, $J = 4.82$ Hz); 7.86 (s, 1H); 8.67 (1H, $J = 4.82$ Hz).

*6.5. General procedures for **2a–r***

DBO (**8**) (1 eq), K_2CO_3 (4 eq), NaI (4 eq), alkyl chlorides (2 eq), DMF (5 mL) were taken in a 6-mL glass tube with a magnetic stir bar.

For derivatives **2o**, **2p** and **2q** the corresponding alkyl halides such as benzyl chloride, 4-phenyl benzyl chloride and 1-(chloromethyl)naphthalene were purchased from Sigma–Aldrich.

For derivative **2r** 1-bromomethylphenanthrene was synthesized as described in the literature [11].

The vessel was sealed with a septum and placed into the microwave cavity. The reaction mixture was then subjected to microwave irradiation under conditions of time, power and temperature as indicated in Table 2.

At the end of irradiation the reaction mixture was cooled to room temperature.

The solvent was removed and the residue was diluted with water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to give a residue which was then subjected to flash chromatography.

*6.5.1. 3,8-Bis((2-(3,4,5-trimethoxyphenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2a**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (2:8) to provide **2a** (70%) as an orange solid. Compound **2a** was converted into its hydrochloric salt. Mp: 195.4 °C; ^{1}H NMR (D_2O): 2.2–2.4 (m, 4H); 2.92–2.98 (m, 4H); 3.83 (s, 3H); 3.84 (s, 3H); 3.91 (s, 6H); 3.93 (s, 2H); 4.00 (s, 2H); 4.17 (s, 2H); 4.6 (s, 2H); 7.16 (s, 2H); 7.23 (s, 2H); 803 (dd, 1H, $J = 5.92$, 1.53 Hz); 8.09 (dd, 1H, $J = 5.92$, 1.53 Hz); 8.23 (s, 1H); 8.49 (s, 1H); 8.64 (d, 1H, $J = 5.92$ Hz); 8.81 (d, 1H, $J = 5.92$ Hz). ^{13}C NMR (D_2O): 22.1; 50.2; 54.3; 55.2; 57.5; 59.1; 60.1; 103.1; 103.2; 123.1;

123.7; 124.1; 125.1; 125.7; 129.1; 141.3; 147.1; 149.8; 151.4; 158.1. MS (ESI) *m/z*: 627.3; 382.0.

*6.5.2. 3,8-Bis((2-(4-methoxyphenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2b**)*

The residue was chromatographed on silica gel using CH_2Cl_2/MeOH (95:5) as an eluent to provide **2b** (82%) as a yellow solid. Compound **2b** was converted into its hydrochloric salt. Mp: 198.5 °C; ^{1}H NMR ($CDCl_3$): 2.4 (s, 4H); 2.86 (d, 2H, J = 12.72 Hz); 2.97 (d, 2H, J = 12.72 Hz); 3.87 (s, 3H); 3.88 (s, 3H); 3.93 (s, 2H); 4.04 (s, 2H); 4.55 (s, 2H); 7.16 (d, 2H, J = 6.58 Hz); 7.19 (d, 2H, J = 7.02 Hz); 7.80 (d, 2H, J = 8.99 Hz); 7.88 (d, 2H, J = 8.77 Hz); 7.90 (d, 1H, 6.14 Hz); 8.00 (d, 1H, J = 6.14 Hz); 8.16 (s, 1H); 8.36 (s, 1H); 8.54 (d, 1H, J = 6.14 Hz); 8.72 (d, 1H, J = 6.14 Hz). ^{13}C NMR ($CDCl_3$): 26.3; 55.3; 56.2; 59.2; 59.9; 60.9; 114.1; 119.6; 121.5; 128.1; 123.1; 132.1; 149.4; 149.5; 157.1; 160.4. MS (ESI) *m/z*: 507.5; 1035.0.

*6.5.3. 3,8-Bis((2-(3-methoxyphenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2c**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (2:8) as an eluent to provide **2c** (62%) as a yellow solid. Compound **2c** was converted into its hydrochloric salt. Mp: 198.7 °C; ^{1}H NMR (D_2O): 2.4 (br, 4H); 2.95 (d, 2H, J = 12.72 Hz); 2.97 (d, 2H, J = 12.72 Hz); 3.89 (s, 3H); 3.90 (s, 3H); 3.99 (s, 2H); 4.07 (s, 2H); 4.6 (s, 2H); 7.25–7.33 (m, 2H); 7.37–7.5 (m, 4H); 7.55–7.64 (m, 2H); 8.02 (dd, 1H, J = 1.53–6.36 Hz); 8.11 (dd, 1H, J = 1.53–6.36 Hz); 8.25 (s, 1H); 8.42 (s, 1H); 8.65 (d, 1H, J = 6.14 Hz); 8.33 (d, 1H). ^{13}C NMR (D_2O): 23.4; 54.1; 55.0; 56.1; 59.3; 64.1; 114.1; 114.7; 117.3; 118.1; 121.7; 122.1; 125.0; 126.1; 126.8; 127.9; 132.1; 133.2; 142.3; 144.1; 149.1; 153.4; 160.1. MS (ESI) *m/z*: 507.2.

*6.5.4. 3,8-Bis((2-(4-hydroxyphenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2d**)*

Elution with hexane/EtOAc (1:1–2:8) afforded **2d** (65%) as a pale oil. Mp: 263.2 °C; ^{1}H NMR (DMSO): 1.99 (m, 4H); 2.38 (d, 2H, J = 9.21 Hz); 2.60 (d, 2H, J = 9.21 Hz); 3.12 (s, 2H); 3.58 (s, 2H); 3.60 (s, 2H); 6.87 (d, 4H, J = 8.11 Hz); 7.21 (d, 1H, J = 5.04 Hz); 7.27 (d, 1H, J = 5.04 Hz); 7.74 (s, 1H); 7.8 (s, 1H); 7.91 (d, 4H, J = 8.11 Hz); 8.51 (t, 2H, J = 4.6 Hz); 9.72 (d, 2H, J = 3.51 Hz). ^{13}C NMR (DMSO): 24.6; 53.8; 57.2; 57.8; 58.5; 108.9; 114.0; 117.1; 119.9; 126.3; 128.2; 147.4; 147.6, 148.4; 154.5; 157. MS (ESI) *m/z*: 478.5.

*6.5.5. 3,8-Bis((2-(3-hydroxyphenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2e**)*

Elution with petroleum ether/EtOAc (1:1–8:2) afforded **2e** (63%) as a white solid. Mp: 93.3 °C; ^{1}H NMR ($CDCl_3$): 1.99 (br, 4H); 2.38 (d, 2H, J = 9.21 Hz); 2.6 (d, 2H, J = 9.21 Hz); 3.12 (s, 2H); 3.58 (s, 2H); 3.60 (s, 2H); 6.83 (d, 2H, J = 7.89 Hz); 7.31 (m, 4H); 7.46 (d, 2H, J = 7.89 Hz) 7.51 (s, 1H); 7.80 (s, 1H); 7.86 (s, 2H); 8.57 (t, 2H, J = 4.37 Hz); 9.56 (d, 2H, J = 4.6 Hz). ^{13}C NMR ($CDCl_3$): 24.6; 53.7; 57.2; 57.9; 58.4; 11.9; 114.5; 115.7; 118.1; 120.8; 128.3; 138.7; 138.7; 147.7; 147.8; 147.9; 148.7; 154.5; 154.6; 156.3. MS (ESI) *m/z*: 479.2.

*6.5.6. 3,8-Bis((2-p-tolylpyridin-4-yl)methyl)-3,8-diaza bicyclo[3.2.1]octane (**2f**)*

Elution with hexane/EtOAc (7:3) afforded **2f** (48%) as a pale oil. Mp: 264 °C; ^{1}H NMR ($CDCl_3$): 1.93–2.04 (m, 4H); 2.44 (s, 6H); 2.49 (d, 2H, J = 10.8 Hz); 2.66 (dd, 2H, J = 10.8–2.85 Hz); 3.18 (br, 2H); 3.58 (s, 2H); 3.61 (s, 2H); 7.22 (d, 1H, J = 5.26 Hz); 7.27–7.35 (m, 5H); 7.73 (s, 1H); 7.80 (s, 1H); 7.93 (d, 4H, J = 7.23); 8.63 (d, 2H, J = 5.26 Hz). ^{13}C NMR ($CDCl_3$): 21.1; 24.1; 55.7; 56.9; 58.7; 63.1; 124.1; 124.9; 125.1; 125.7; 128.9; 132.1; 140.9; 148.3; 154.2. MS (ESI) *m/z*: 475.2.

*6.5.7. 3,8-Bis((2-m-tolylpyridin-4-yl)methyl)-3,8-diaza bicyclo[3.2.1]octane (**2g**)*

Elution with petroleum ether/EtOAc (9:1–8:2) afforded **2g** (75%) as a white solid. Mp (**2g**·HCl): 266.3 °C; ^{1}H NMR ($CDCl_3$): 2.03 (br, 4H); 2.45 (s, 6H); 2.5 (d, 2H, J = 10.8 Hz); 2.6 (d, 2H, J = 10.8 Hz); 3.22 (s, 2H); 3.61 (s, 2H); 3.66 (s, 2H); 7.26–7.28 (m, 4H); 7.41 (t, 2H, J = 7.87 Hz); 7.55 (s, 2H); 7.79 (d, 2H, J = 7.87); 7.86 (s, 2H); 8.69 (d, 2H, J = 5.04 Hz). ^{13}C NMR ($CDCl_3$): 21.1; 24.1; 55.7; 56.9; 58.7; 63.1; 124.1; 124.7; 125.3; 125.7; 128.7; 132.4; 140.8; 148.2; 154.1. MS (ESI) *m/z* : 475.4.

*6.5.8. 3,8-Bis((2-(4-acetamidophenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2h**)*

Elution with $CHCl_3$/MeOH (95:5–9:1) afforded **2j** (77%) as a white solid. Mp (**2h**·HCl): 254.3 °C; ^{1}H NMR ($CDCl_3$): 1.95–2.10 (m, 4H); 2.24 (s, 6H); 2.5 (d, 2H, J = 10.8 Hz); 2.66 (dd, 2H, J = 10.8–2.63 Hz); 3.18 (s, 2H); 3.60 (s, 2H); 3.64 (s, 2H); 4.0 (br, $2NH_2$); 4.48 (s, 4H); 7.25 (d, 2H, J = 5.26 Hz); 7.36 (s, 2H); 7.66 (d, 4H, J = 8.77 Hz); 7.73 (s, 2H); 8.01 (d, 4H, J = 8.77 Hz); 8.67 (d, 2H, J = 5.26 Hz). ^{13}C NMR ($CDCl_3$): 22.7; 23.5; 55.7; 56.9; 58.7; 63.1; 118.7; 120.3; 124.1; 124.7; 130.1; 134.3; 134.4; 141.9; 148.4; 150.1; 155.6. MS (ESI) *m/z*: 561.3.

*6.5.9. 3,8-Bis((2-(3-acetamidophenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2i**)*

Elution with $CHCl_3$/MeOH (95:5–9:1) afforded **2i** (63%) as a pale oil. Mp: 176 °C; ^{1}H NMR ($CDCl_3$): 1.95–2.04 (m, 4H); 2.2 (s, 6H); 2.48 (d, 2H, J = 10.8); 2.63 (d, 2H, J = 10.8); 3.15 (s, 2H); 3.58 (s, 2H); 3.62 (s, 2H); 7.01 (d, 2H, J = 5.26 Hz); 7.57 (s, 2H); 7.73 (d, 4H, J = 8.77 Hz); 7.97 (d, 4H, J = 8.77 Hz); 8.60 (d, 2H, J = 5.26 Hz). ^{13}C NMR ($CDCl_3$): 22.7; 23.5; 55.7; 56.9; 58.7; 63.1; 118.7; 120.3; 124.1; 124.7; 130.1; 134.3; 134.4; 141.9; 148.4; 150.1; 155.6. MS (ESI) *m/z*: 561.3.

*6.5.10. 3,8-Bis((2-(4-chlorophenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2j**)*

Elution with light petroleum/EtOAc (9:1–7:3) afforded **2j** (82%) as a white solid. Mp (**2j**·HCl): 245.3 °C; rt: 17.84 min; ^{1}H NMR ($CDCl_3$): 2.07 (m, 4H); 2.50 (d, 2H, J = 10.8 Hz);

2.65 (d, 2H, $J = 10.8$ Hz); 3.16 (s, 2H); 3.61 (s, 2H); 3.63 (s, 2H); 7.22 (d, 2H, $J = 4.92$ Hz); 7.5 (d, 4H, $J = 8.11$ Hz); 7.65 (s, 1H); 7.80 (s, 1H); 7.96 (d, 4H, $J = 8.11$ Hz); 8.67 (d, 2H, $J = 4.92$ Hz). ^{13}C NMR ($CDCl_3$): 26.3; 30.5; 54.7; 59.1; 60.2; 62.1; 121.1; 122.8; 124.1; 124.7; 130.1; 134.3; 134.4; 141.9; 148.4; 150.1; 155.6. MS (ESI) *m*/*z*: 515.2.

*6.5.11. 3,8-Bis((2-(4-nitrophenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2k**)*

Elution with light petroleum/EtOAc (1:1–2:8) afforded **2k** (77%) as a pale yellow solid. Mp (**2k**·HCl): 198.4 °C; ^{1}H NMR ($CDCl_3$): 2.03 (br, 4H); 2.45 (s, 6H); 2.5 (d, 2H, $J = 10.8$ Hz); 2.6 (d, 2H, $J = 10.8$ Hz); 3.22 (s, 2H); 3.61 (s, 2H); 3.66 (s, 2H); 7.39 (d, 2H, $J = 5.26$ Hz); 7.65 (s, 1H); 7.67 (s, 1H); 8.22 (d, 4H, $J = 8.77$ Hz); 8.36 (d, 4H, $J = 8.77$ Hz); 8.77 (d, 2H, $J = 5.26$ Hz). ^{13}C NMR ($CDCl_3$): 26.3; 30.5; 54.7; 59.1; 60.2; 62.1; 121.1; 122.8; 124.1; 124.7; 130.1; 134.3; 134.4; 141.9; 148.4; 150.1; 155.6. MS (ESI) *m*/*z*: 537.1.

*6.5.12. 3,8-Bis((2-(3-nitrophenyl)pyridin-4-yl)methyl)-3,8-diazabicyclo[3.2.1]octane (**2l**)*

Elution with petroleum ether/EtOAc (1:1–2:8) afforded **2l** (72%) as a yellow solid. Mp (**2l**·HCl): 194.7 °C; ^{1}H NMR ($CDCl_3$): 1.94–2.10 (m, 4H); 2.53 (d, 2H, $J = 10.08$ Hz); 2.68 (d, 2H, $J = 10.52$ Hz); 3.2 (s, 2H); 3.65 (s, 2H); 3.88 (s, 2H); 7.35 (d, 1H, $J = 5.04$ Hz); 7.42 (d, 1H, $J = 5.04$ Hz); 7.69 (t, 2H, $J = 7.89$ Hz); 7.84 (s, 1H); 7.92 (s, 1H); 8.3 (d, 2H, $J = 7.89$); 8.38–8.46 (m, 2H); 8.69 (d, 2H, $J = 5.04$ Hz); 8.90 (s, 2H). ^{13}C NMR ($CDCl_3$): 26.1; 30.1; 55.7; 59.1; 60.2; 62.1; 121.1; 122.3; 124.1; 124.7; 130.1; 134.1; 134.3; 141.9; 148.7; 150.1; 155.3. MS (ESI) *m*/*z*: 537.1.

*6.5.13. 3,8-Bis((2-phenylpyridin-4-yl)methyl)-3,8-diaza bicyclo[3.2.1]octane (**2m**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (7:3) as an eluent to provide **2m** (62%) as a pale yellow solid. Compound **2m** was converted into its hydrochloric salt. Mp: 198.1 °C; ^{1}H NMR (D_2O): 2.3–2.4 (m, 4H); 2.8 (d, 2H, $J = 10.8$ Hz); 3.0 (d, 2H, $J = 10.8$ Hz); 3.9 (s, 2H); 4.2 (s, 2H); 4.5 (s, 2H); 7.51–7.58 (m, 4H); 7.76–7.85 (m, 6H); 7.9 (d, 1H, $J = 2.8$ Hz); 8.1 (d, 1H, $J = 2.8$ Hz); 8.27 (d, 2H, $J = 8.11$ Hz); 8.64 (d, 1H, $J = 5.04$ Hz); 8.77 (d, 1H, $J = 5.04$ Hz). ^{13}C NMR (D_2O): 21.1; 41.3; 52.3; 56.4; 60.3; 123.3; 124.1; 124.7; 125.6; 126.2; 128.1; 130.0; 131.0; 132.1; 140.0; 142.3; 150.1; 154.2; 155.3. MS (ESI) *m*/*z*: 447.1.

*6.5.14. 3,8-Bis(3-phenylbenzyl)-3,8-diaza bicyclo[3.2.1] octane (**2n**)*

Elution with petroleum ether/EtOAc (7:3) afforded **2c** (78%) as a pale oil. ^{1}H NMR ($CDCl_3$): 1.96–2.04 (m, 4H); 2.48 (d, 2H, $J = 10.8$); 2.63 (d, 2H, $J = 10.8$); 3.15 (s, 2H); 3.58 (s, 2H); 3.62 (s, 2H); 7.25–7.5 (m, 12H); 7.6–7.74 (m, 6H). ^{13}C NMR ($CDCl_3$): 21.1; 41.3; 52.3; 56.4; 60.3; 123.3; 124.1; 124.7; 125.6; 126.2; 128.1; 130.0; 131.0; 132.1; 140.0; 142.3; 150.1; 154.2; 155.3. MS (ESI) *m*/*z*: 445.1.

*6.5.15. 3,8-Bis((biphenyl-1-yl)methyl)-3,8-diaza bicyclo [3.2.1]octane (**2o**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (9:1–1:1) as an eluent to provide **2o** (60%) as a white solid. Mp (**2o**·HCl) 254.3 °C dec; ^{1}H NMR ($CDCl_3$): 1.88–2.02 (m, 4H); 2.48 (d, 2H, $J = 10.2$ Hz); 2.63 (d, 2H, $J = 10.2$ Hz); 3.24 (s, 2H); 3.51 (s, 2H); 3.54 (s, 2H); 7.3–7.61 (m, 18H). ^{13}C NMR ($CDCl_3$): 28; 56; 59; 63; 127.5; 127.7; 127.8; 128.3; 129.3; 134.5; 134.7; 140.5; 143.3; 143.9. MS (ESI) *m*/*z*: 445.1.

*6.5.16. 3,8-Bis((phenyl-1-yl)methyl)-3,8-diaza bicyclo [3.2.1]octane (**2p**)*

Elution with $CHCl_3$/MeOH/NH_3 (95:5:0.05) afforded **2p** (74%) as a white solid. ^{1}H NMR ($CDCl_3$): 1.65–1.97 (m, 4H); 2.2 (d, 2H, $J = 12$ Hz); 6.45 (d, 2H, $J = 9.2$ Hz); 3.42 (m, 2H); 3.46 (s, 4H); 7.18–7.38 (m, 10H).

*6.5.17. 3,8-Bis(2-(naphthalen-1-yl)methyl)-3,8-diaza bicyclo[3.2.1]octane (**2q**)*

The residue was chromatographed on silica gel using light petroleum/EtOAc (9:1–1:1) as an eluent to provide **3** (62%) as a white solid. Mp (**2q**·HCl) 278 °C dec; ^{1}H NMR ($CDCl_3$): 1.84–1.94 (m, 4H); 2.58 (d, 2H, $J = 10.08$ Hz); 2.70–2.85 (m, 4H); 3.22–3.42 (m, 4H); 7.37–7.60 (m, 8H); 7.76 (d, 2H, $J = 7.02$ Hz); 7.89 (d, 2H, $J = 4.85$ Hz); 8.12 (t, 2H, $J = 7.67$ Hz). ^{13}C NMR ($CDCl_3$): 30; 33; 45; 56; 59; 124.1; 124.7; 125.2; 125.6; 126.3; 126.8; 127.3; 132.3; 133.4. MS (ESI) *m*/*z*: 421.2.

*6.5.18. 3,8-Bis(phenanthren-3-yl)methyl-3,8-diazabicyclo [3.2.1]octane (**2r**)*

Elution with petroleum ether/EtOAc (7:3) afforded **2r** (69%) as a pale oil. ^{1}H NMR ($CDCl_3$): 1.99–2.04 (m, 4H); 2.54 (d, 2H, $J = 10.8$); 2.69 (d, 2H, $J = 10.8$); 3.25 (s, 2H); 3.78 (s, 2H); 3.82 (s, 2H); 7.5–8.0 (m, 14H); 8.6–8.74 (m, 4H). ^{13}C NMR ($CDCl_3$): 21.1; 41.3; 52.3; 56.4; 60.3; 122.1; 122.4, 126.6; 127.2; 128.3; 130; 131.5; 133.1; 133.4; 134.1. MS (ESI) *m*/*z*: 493.1.

7. Materials and methods

Test compounds were solubilised in DMSO at 100 mM and then diluted into culture medium.

7.1. Cells

Cell lines were purchased from American Type Culture Collection (ATCC). Haematological tumor-derived cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 Units/mL penicillin G and 100 μg/mL streptomycin. Solid tumor-derived cells were grown in their specific media supplemented with 10% FCS and antibiotics. Cell cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

The absence of mycoplasma contamination was checked periodically by the Hoechst staining method.

7.2. Antiproliferative assays

Exponentially growing was re-suspended in growth medium containing serial dilutions of the drugs. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [12,13].

7.3. Cells' culture

MCF-7 cells were cultured in DMEM supplemented with 2 mM L-glutamine, 10% heat-inactivated foetal bovine serum (FBS), 10,000 Uints/mL penicillin and 10 mg/mL streptomycin (all from Cambrex Bioscience, Verviers, Belgium) at 37 °C in an atmosphere of 95% O_2 and 5% CO_2. The cells were plated at a density of 5×10^5 cells in 6-cm cell culture plates the day before treatment. At the end of the incubation time cells were processed for Western blotting and FACS analysis.

7.4. Western blot analysis

Expression of p21CIP1/Waf-1 was detected by SDS-PAGE. Total intracellular protein was extracted from the cells by freezing and thawing in lysis buffer containing protease and phosphatase inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 µg/mL leupeptin, 1 µg/mL pepstatin, 1 µg/mL aprotinin, 1 mM NaF in 50 mM Trizma–HCl) as described [14].

Protein content was estimated by Biorad protein assay (BIO-RAD, Milan, Italy). Samples (30 µg protein) were loaded onto 10% acrylamide gel and separated by SDS-PAGE in denaturating conditions at 100 V. The separated proteins were then transferred electrophoretically (100 mA/blot; Trans Blot Semi-Dry, BIO-RAD) to nitrocellulose paper (Immobilon-NC, Millipore, USA) soaked in transfer buffer [25 mM TRIS, 192 mM glycine and 20% methanol vol/vol (Sigma–Aldrich, Gallarate, Italy)]. Non-specific binding was blocked by incubation of the blot in 5% non-fat dry-milk (BIO-RAD, Milan, Italy) in TBS–Tween [25 mM Tris, 150 mM NaCl, 0.1% Tween vol/vol (all from Sigma–Aldrich, Gallarate, Italy)] for 60 min. The blots were incubated overnight at 4 °C with the primary monoclonal antibody against p21CIP/Waf1 (diluted 1:200) (Santa Cruz Biotechnology, Inc., Germany) and then at room temperature (1 h) with an appropriate secondary mouse antibody (1:10,000) (Sigma–Aldrich, Gallarate, Italy). Immunoreactive protein bands were detected by chemiluminescence reagents (ECL) and exposed to hyperfilm (both from Amersham Biosciences, Milan, Italy). The blots were then scanned and analysed (Gel-Doc 2000 BIO-RAD).

7.5. Analysis of apoptosis

Hypodiploid DNA was analysed using propidium iodide (PI) staining by flow cytometry as described [15].

Briefly, cells were washed in phosphate buffered saline (PBS) and re-suspended in 500 µl of a solution containing 0.1% sodium citrate, 0.1% Triton X-100 and 50 µg/mL PI (Sigma–Aldrich). After incubation at 4 °C for 30 min in the dark, cell nuclei were analysed with Becton Dickinson FACScan flow cytometer using CellQuest program. Cellular debris was excluded from the analysis by raising the forward scatter threshold, and the DNA content of the nuclei was registered on a logarithmic scale. The percentage of cells in the hypodiploid region was calculated.

7.6. Cell-cycle analysis

For analysis of cell cycle distribution, the DNA content of individual cells was measured by flow cytometry. Cells were seeded at a density of 5×10^5 cells/well in six-well plates. After 24 h, cells were stimulated with **2a** (10 µM). After 24 h, cells and supernatants were collected together and incubated with Nicoletti reagent. Core DNA content was measured using linear amplification in the FL2-channel of a FACScan flow cytometer using CellQuest software. Cell cycle was analysed by ModFit LT software.

7.7. Statistical analysis

All results are shown as mean ± SEM of three experiments performed in triplicate. The optical density of the bands of ANXA1 protein expression detected by Western blot was normalized with α-tubulin. Statistical comparison between groups was made using parametric Bonferroni test. P-values < 0.05 were considered significant.

References

[1] W.A. Remers, J.H. Block, J.M. Beale, Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, eleventh ed. (2004) pp.390–453.
[2] J.W. Tracy, L.T. Webster, H.F. Chambers, W.A. Petri, J.E. Bennett, F.G. Hayden, S. Raffanti, D.W. Haas, P. Calabresi, B.A. Chabner, The Pharmacological Basis of Therapeutics, tenth ed. (2001) pp. 1059–1459.
[3] (a) P. Maillet, A. Le-Brun, F. Thompson, G. Tirohoschi, U.S. Pat. Appl. Publ., 2005;
(b) H. Nakao, M. Umetani, M. Suda, T. Nagoya, Eur. Pat. Appl., 1997;
(c) Y.J. Shaw, Y.T. Yang, J.B. Garrison, N. Kyprianou, C.S. Chen, J. Med. Chem. 47 (2004) 4453–4462;
(d) L.J. Lombardo, F.Y. Lee, P. Chen, D. Norris, J.C. Barrish, K. Behnia, J. Med. Chem. 47 (2004) 6658–6661.
[4] (a) PCT Int. Appl. WO 2004 32,933;
(b) U.S. Pat. Appl. Publ., 2005;
(c) Eur. Pat. Appl., 1997.
[5] D. Barlocco, G. Cignarella, P. Vianello, S. Villa, G.A. Pinna, P. Fadda, W. Fratta, Il Farmaco 53 (1998) 557–562.
[6] G. Cignarella, G.G. Nathansohn, J. Org. Chem. 26 (1961) 2747.
[7] G. Caliendo, F. Fiorino, E. Perissutti, B. Severino, S. Gessi, E. Cattabriga, P.A. Borea, V. Santagada, Eur. J. Med. Chem. 36 (2001) 873–886.
[8] N.M. Kernohan, L.S. Cox, J. Pathol. 179 (1996) 1–3.
[9] C.E. Caldon, R.J. Daly, R.L. Sutherland, E.A. Musgrove, J. Cell. Biochem. 97 (2006) 261–274.
[10] C. Bailly, C. Carrasco, A. Joubert, C. Bal, N. Wattez, M.P. Hildebrand, A. Lansiaux, P. Colson, C. Houssier, M. Cacho, A. Ramos, M.F. Brana, Biochemistry 42 (2003) 4136–4150.
[11] A.M. Dance, L. Ralton, Z. Fuller, L. Milne, S. Duthie, C.S. Bestwick, P.K. Lin, Biochem. Pharmacol. 69 (2005) 19–27.
[12] F. Denizot, R. Lang, J. Immunol. Methods 89 (1986) 271–277.
[13] R. Pauwels, J. Balzarini, M. Baba, R. Snoek, D. Schols, P. Herdewijn, J. Desmyter, E. De Clercq, J. Virol. Methods 2 (1988) 309–321.
[14] E. Solito, C. de Coupade, L. Parente, R.J. Flower, F. Russo-Marie, Cell Growth Differ. 9 (1988) 327–336.
[15] I. Nicoletti, G. Migliorati, M.C. Pagliacci, F. Grignani, C. Riccardi, J. Immunol. Methods 139 (1991) 271–279.