Bioorganic & Medicinal Chemistry 16 (2008) 8003–8010

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis, and antitumor activity of new bis-aminomethylnaphthalenes

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ARTICLE INFO

Article history:
Received 16 May 2008
Revised 22 July 2008
Accepted 23 July 2008
Available online 29 July 2008

Keywords:
Bis-aminomethylnaphthalenes
Synthesis
Antitumor activity
DNA binding

ABSTRACT

A new series of bis-aminomethylnaphthalenes were synthesized in satisfactory overall yield, through a simple synthetic strategy using reductive amination. The DNA binding properties of these compounds have been examined and compared to those of reference drugs using an UV spectroscopy method. The compounds were evaluated for their in vitro anticancer activity and some of them were studied in vivo. Compound **15** exhibited remarkable antitumor activity and represents a novel template for anticancer chemotherapy and can serve as a new lead compound.

1. Introduction

The majority of the drugs currently used in the treatment of cancer reversibly bind to DNA or induce DNA damage, either directly or via topoisomerase inhibition. DNA and associated proteins remain valid targets for cancer chemotherapy.[1,2] Novel DNA intercalating molecules acridines,[3] indolocarbazoles,[4] naphthalimides,[5] and alkylating agents (benzoacronycines,[6] pyrrolobenzodiazepines,[7] distamicin conjugates[8]) are still being developed. Although it is well established that DNA binding is not sufficient to confer cytotoxic activities, interaction with DNA is often considered as a necessary criterion to maintain a cytotoxic effect, at least for some series of planar intercalating chromophores such as acridines and ellipticines.

Symmetrical bis-1-aminomethylnaphthalenes constitute a new class of molecules (Fig. 1) with cytotoxic activity. Their structure derives from a long development to determine the minimum active molecular structures that result from echinomycine molecule simplification.[9,10] These compounds show a strong interaction with DNA and a potent cytostatic activity.[11,12]

In order to determine whether the activity was due to a non-specific siamese structure or it was influenced by the functional characteristic of the bridge between the two naphthalenes, and the role of aromatic substituents, both the type and positioning, a series of compounds was developed.

All the new compounds were evaluated for their ability to bind DNA and were assayed for antiproliferative properties in vitro and in vivo by the National Cancer Institute (NCI, USA).

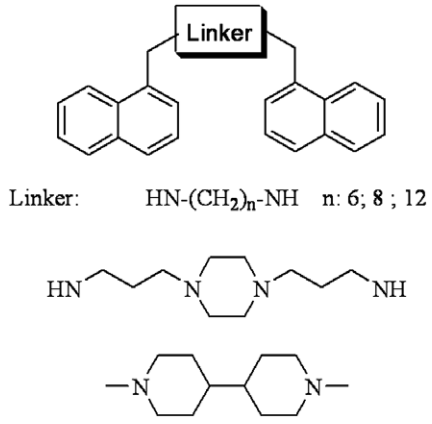

Figure 1. Symmetrical bis-1-aminomethylnaphthalenes derivatives.

2. Results and discussion

2.1. Chemistry

The easier procedure to obtain secondary bis-amines is alkylation of the primary bis-amine with halogenated derivatives. However, yields are not so good and the reaction requires prolonged reaction times. Reductive amination is a good method to obtain these compounds with excellent yields.[13] Several amines condense with aldehydes to form imine products. Imines are sometimes difficult to isolate and purify due to their sensitivity to hydrolysis. In our case, the first step of the synthesis of the compounds was performed by the formation of bis-imines. Derivatives **1**–**7** were ob-

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doi:10.1016/j.bmc.2008.07.069

tained starting from 6-methoxy-2-naphthaldehyde or 2-naphthaldehyde and the corresponding diamines. All the imines were isolated, crystallized, and characterized by their spectroscopic properties (IR, NMR) (Scheme 1).

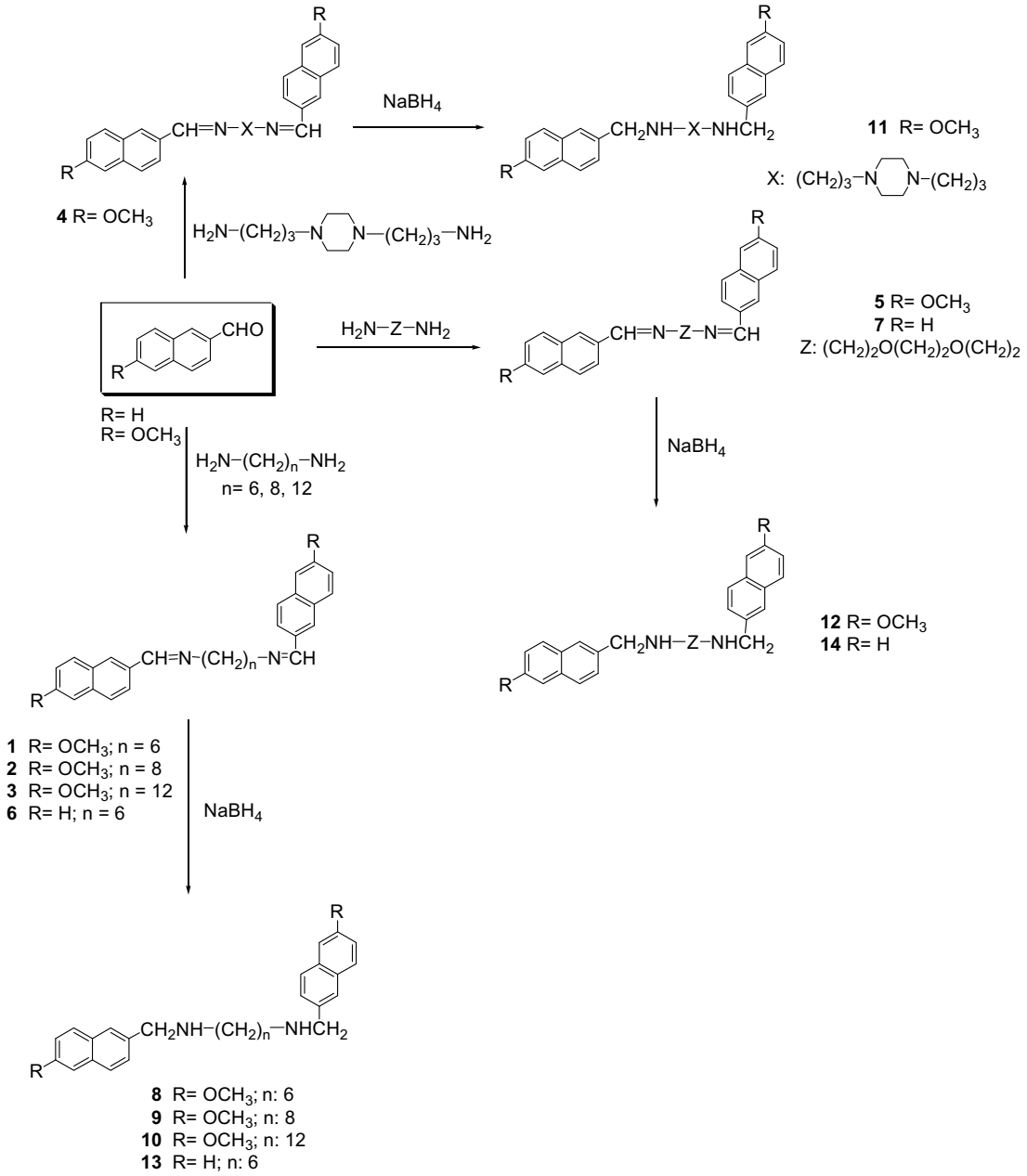
In the second step, we attempted the synthesis of the secondary amine by catalytic hydrogenation over Pd/C (10%) in ethanol at a hydrogen pressure of 0.14 MPa at room temperature, but we recovered the initial imine. However, subsequent reduction of these imines with $NaBH_4$ in methanol[14] gave the desired compounds **8**–**14** in good yields (Scheme 1).

Compounds **15** and **16** were synthesized by alkylation of the corresponding diamine owing to the impossibility to carry out reductive amination. As the desired halogenated compounds were not available, 2-naphthaldehyde and 6-methoxy-2-naphthaldehyde were reduced with $NaBH_4$ in methanol to give the corresponding alcohols. After that, we obtained the halogenated derivatives using thionyl chloride. Finally, alkylation proceeded under reflux with the corresponding diamine in DMF (Scheme 2).

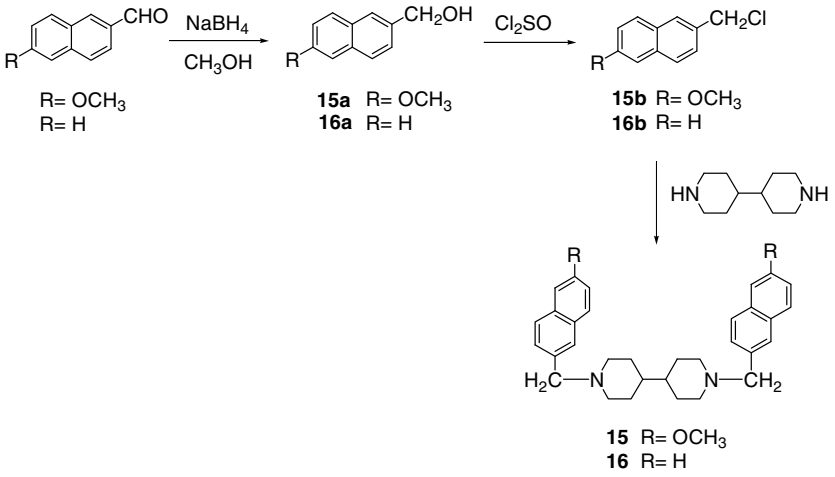
All the bis-amine compounds were characterized by spectral data analysis that confirmed the assigned structures.

2.2. Antineoplastic activity

All the final compounds were evaluated for antiproliferative properties following the NCI in vitro protocols. They were assayed in vitro against a panel of approximately 60 human tumor cell lines, derived from nine cancer types: lung, colon, CNS, melanoma, ovarian, renal, prostate, breast, and leukemia. Compounds were tested at five concentrations at 10-fold dilutions starting from 10^{-4} M. A 48 h continuous drug exposure protocol was used and sulforhodamine B (SRB) protein assay was used to estimate cell growth.[15] The antitumoral activity of tested compounds is given



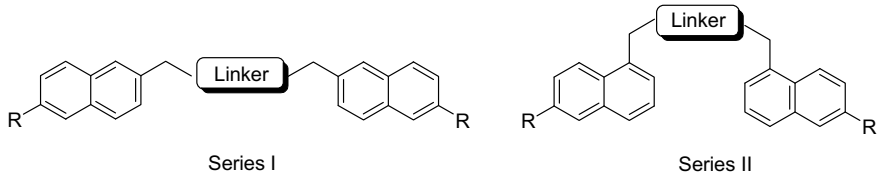
Scheme 1. Synthesis of compounds **1**–**14**.



Scheme 2. Synthesis of compounds **15**–**16**.

by three parameters for each cell line: log GI_{50} value (GI_{50} = molar concentration of the compound that inhibits 50% net cell growth), log TGI value (TGI = molar concentration of the compound leading to the total inhibition), and log LC_{50} value (LC_{50} = molar concentration of the compound leading to 50% net cell death). Furthermore, a mean graph-midpoint (MG-MID) is calculated for each response parameter, which indicates the average sensitivity of all tested cell lines to each tested compound. The results collected in Table 1

Table 1
Average values (MG-MID) for in vitro antitumor activity on 60 human cell lines



Compound (NSC code)	R	Linker	MG-MID[a]			Panel sensitivity
			log GI_{50}[b]	log TGI[c]	log LC_{50}[d]	
Series I						
8 (676426)	O–CH_3	–$NH(CH_2)_6NH$–	−6.22	−5.76	−5.39	Leukemia, colon, breast
9 (676427)	O–CH_3	–$NH(CH_2)_8NH$–	−5.94	−5.62	−5.20	Leukemia, colon, CNS, prostate
10 (676428)	O–CH_3	–$NH(CH_2)_{12}NH$–	−4.81	−4.53	−4.26	Leukemia, colon, CNS
11 (676429)	O–CH_3	—$HN(CH_2)_3$—N⟨⟩N—$(CH_2)_3NH$—	−5.96	−5.61	−5.25	Leukemia, colon
12 (695801)[f]	O–CH_3	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	−6.14	−5.72	−5.28	Leukemia, colon, melanoma, breast, CNS
14 (695800)[g]	H	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	−5.77	−5.43	−5.00	Leukemia, colon
15 (683792)	O–CH_3	—N⟨⟩N—	−5.53	−5.01	−4.39	Leukemia, melanoma, colon, breast
16 (696885)	H	—N⟨⟩N—	−5.54	−5.08	−4.60	Melanoma, colon, leukemia
Series II[11,12]						
629732	H	–$NH(CH_2)_6NH$–	−5.27	−4.91	−4.53	Leukemia, colon, melanoma
629733	H	–$NH(CH_2)_8NH$–	−5.62	−5.28	−4.94	Leukemia, colon, melanoma
629734	H	–$NH(CH_2)_{12}NH$–	−5.66	−5.36	−5.07	Leukemia, colon, melanoma
629735	H	—$HN(CH_2)_3$—N⟨⟩N—$(CH_2)_3NH$—	−5.18	−4.71	−4.36	Leukemia, melanoma, colon
629736	H	—N⟨⟩N—	−5.71	−5.37	−5.02	Leukemia, melanoma, colon, breast, lung
Mitox[e]			−7.32	−6.16	−5.14	Leukemia, lung, CNS, renal
m-AMSA			−6.64	−5.47	−4.68	Leukemia, CNS, renal, lung

[a] MG-MID mean graph-midpoint: arithmetical mean value for all tested cancer cell lines.
[b] The response parameter: log GI_{50} is interpolated value representing the molar concentration at which percentage growth is +50.
[c] The response parameter: log TGI is interpolated value representing the molar concentration at which percentage growth is 0.
[d] The response parameter: log LC_{50} is interpolated value representing the molar concentration at which percentage growth is −50.
[e] Mitox, mitoxantrone.
[f] As hydrochloride.
[g] As hydrochloride.

refer the mean graph-midpoint (MG-MID) values at each of principal response parameters: GI_{50}, TGI, and LC_{50}.

Tested compounds demonstrated a relatively broad spectrum of tumor cell growth inhibition. Compounds displayed selectivity on panel of leukemia, melanoma, colon, breast, and prostate cancers tumor cell lines. In series I, an increase in the number of methylene units between nitrogen atoms resulted in a significant drop in anticancer potency. In series II, antineoplastic activity did not decrease with the enlargement of the linker chain.

Introduction of a methoxy group at 6-position of naphthalene chromophores in compound **14** (NSC 695800) results in a significant increase of antineoplastic activity.

Even though the compound **15** (NCS 696885, series I) has a GI_{50} lower than the compound NCS 629736 (series II), this last one shows an LC_{50} of the same order as GI_{50}. Furthermore, compound **15** is of special interest because there is a substantial difference between the cytostatic and the cytotoxic activities.

Table 2 shows the antitumor activity of compound **15** in most sensitive human cell lines.

Because of this promising in vitro activity, compounds **8** (NSC 676426), **12** (NCS 695801), **15** (NSC 683792), and **16** (NCS 696885) were selected at NCI Biological Testing Branch for preliminary hollow fiber in vivo testing[16] (see Section 3). Among the twelve cell lines forming the standard panel routinely used in such assays, compound **15** showed IP+SC score of 14, an SC score of 6, but demonstrated a net cell kill. Table 3 shows the results of the four compounds tested.

A COMPARE[17] analysis was performed with the more active compound **15** to investigate whether it resembles anticancer drugs of the NCI standard agent database and to probably predict its mechanism of action. The COMPARE algorithm was developed to determine the degree of similarity of mean graph fingerprints obtained from the in vitro anticancer screen with patterns of activity of standard agents. The hypothesis is that if the data pattern of a compound correlates well with the data pattern of compounds belonging to the standard agents database,[18] the compound of interest may have the same mechanism of action as those agents with known mechanism. A correlation coefficient of 0.55–0.6 is considered the lowest correlation that suggests a relationship with another compound.[19] Using GI_{50} values of compound **15** (NSC 683792) as seed, COMPARE analysis showed that compounds in Table 4 had a Pearson's correlation coefficient (PCC) <0.6. The weakly correlated compounds, shows in Table 4, are cytotoxic through diverse mechanisms of action, including DNA alkylation (CCNU), induction of apoptosis (anguidine), and alteration of cell cycle progression involving the MAPK pathway (tetrandine). All in all the COMPARE analysis for the representative compound **15** against the standard agent database showed poor or no correlation indicating that mechanism of action for the novel bis-aminomethylnaphthalenes may differ from that of the standard antitumor drugs. Therefore, antitumoral activity of the novel compound may be caused by a new and unknown mechanism.

Table 2
Antitumor activity of compound **15** in most sensitive human tumor cell lines

Panel/cell line	$\log_{10}$ GI_{50}
Leukemia	
HL-60(tb)	−5.58
K-562	−6.30
MOLT-4	−5.66
RPMI-8226	−5.76
SR	−5.91
Colon cancer	
COLO 205	−5.79
HCT-116	−5.46
HT29	−5.47
KM12	−5.49
SW-620	−5.50
Melanoma	
LOX IMVI	−5.55
MALME-3M	−5.64
M14	−5.61
SK-MEL-2	−5.19
SK-MEL-28	−5.58
SK-MEL-5	−5.83
UACC-257	−5.69
UACC-62	−5.72
Breast cancer	
MCF7	−5.28
MCF7/ADR-RES	−4.88
MDA-MB-231/ATCC	−5.48
HS 578T	−4.97
MDA-MB-435	−5.72
MDA-N	−5.77
T-47D	−5.75

Table 3
The in vivo activity (hollow fiber assay) for compounds **8**, **12**, **15**, **16**

Compound (NSC code)	IP score[a]	SC score[b]	Total score	Cell kill
8 (676426)	4	4	8	N
12 (695801)[c]	6	6	12	N
15 (683792)	8	6	14	Y
16 (696885)	0	4	4	N

% T/C, reduction percentage of the viable cell mass in treated mice relative to a control group.
[a] IP score, % T/C for each of two compounds doses for intraperitoneal samples.
[b] SC score, % T/C for each of two compounds doses for subcutaneous samples.
[c] As hydrochloride.

2.3. DNA binding properties

As the bis-aminomethylnaphthalenes synthesized previously[11] displayed close affinity to calf thymus DNA, we expected the synthesized compounds to conserve this property.

All the final products were tested for their ability to bind DNA. The binding capacity of these compounds was evaluated by measuring the hypochromic and bathochromic effects of their absorbance in the UV spectra.[20] The typical experiment was enhanced by means of a slow rotation of DNA/drug mixture stirring, in a 5:1 ratio for 24 h. The procedure was validated by repeating assays with well-known intercalating agents (*m*-AMSA and mitoxantrone) and a compound which binds closely in the minor groove (bis-benzimide, Hoechst No. 33258). The degree of interaction was expressed by the ratio between the final absorbance area after

Table 4
Compare correlation coefficients (PCC) using GI_{50} values of compound **15** (NSC 683792) as seed

Rank	NSC	PCC	Num common cell lines	Compound
1	68075	0.600	50	Thalicarpine
2	192965	0.595	60	Spirogermanium
3	77037	0.488	60	D-Tetrandrine
4	322921	0.485	59	Hoechst dye
5	45441	0.481	49	CCNU
6	118994	0.465	60	IdA
7	180973	0.464	60	Tamoxifen
8	83265	0.460	47	Tritylthioalanine
9	303861	0.459	60	L-Cysteine
10	141537	0.458	60	Anguidine
11	409962	0.412	60	Carmustine
12	133100	0.408	60	Rifamycin
13	332598	0.408	59	Rhizoxin
14	95441	0.405	60	Semustine
15	349156	0.404	60	Pancratistatin

24 h (a_{24}) and that of the compound at the same concentration (a_0), centered at maximal absorbance. Values of 1 indicate a total lack of affinity and value 0 shows that the whole compound was bound to DNA. The coefficient a_{24}/a_0 values obtained are summarized in Table 5.

Under such experimental conditions, all these compounds exhibited a bathochromic and hypochromic effects. The DNA binding assay showed that compounds tested are excellent DNA ligands with higher affinity than *m*-AMSA and bis-benzimide, except compounds **10** and **16**.

A large number of relatively small organic molecules are known to bind to DNA by various mechanisms such as intercalation and ionic attraction. It is important to characterize such phenomena because the mechanism of action of many carcinogenic, mutagenic, and antitumor substances presumably relates to binding with DNA. Additionally, as the interaction is best described as a dynamic equilibrium, we performed a kinetic study of compound **15** using different drug/DNA ratios (1:2; 1:2.5; 1:3; 1:3.5; 1:4 and 1:5). This assay showed that the rate of the process was proportional to relative concentration of drug/DNA ratio. Moreover, Figure 2 shows that in drug/DNA (1:2) ratio, the rate of occupying the sites of union to DNA is faster than in the others ratios.

Table 5
DNA affinity assay

Compound (NSC code)	a_{24}/a_0[a]
8 (676426)	0.51
9 (676427)	0.34
10 (676428)	0.77
11 (676429)	0.52
12 (695801)[b]	0.47
13 (−)[c]	0.53
14 (695800)[d]	0.47
15 (683792)	0.51
16 (696885)	0.61
m-AMSA (249992)	0.54
Mitoxantrone (301739)	0.00
Bis-benzimide (322921)	0.57

[a] a_{24}, final absorbance area (DNA-compound) after 24 h; a_0, initial absorbance area (compound) at the same concentration.
[b] As hydrochloride.
[c] Not studied by NCI.
[d] As hydrochloride.

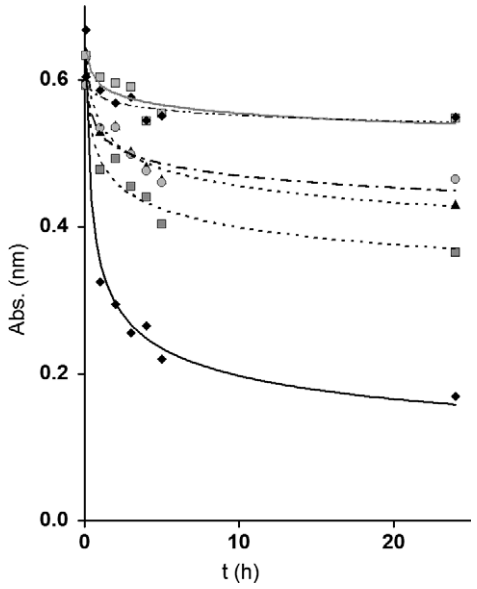


Figure 2. Absorbance (λ_{max} = 232 nm) versus times of drug–DNA (–◆– 1:2; ..■.. 1:2.5; ▲ 1:3; ● 1:3.5; —■— 1:4; -.-◆-.- 1:5).

When plotting absorbance against the different ratios of drug–DNA mixture at 1 and 24 h the observed process is in one step which indicates a single mode of interaction of compound **15** with DNA (Fig. 3).[21]

A medium correlation between degree of affinity and antineoplastic activity was observed (R^2 = 0.62), so a different mechanism of action is suggested, like an inhibition of specific enzymes such as DNA-topoisomerases.[22]

2.4. Cell cycle effects

In the search of a possible mechanism of action responsible for compound **15** antitumor activity we have investigated its effect on the cell cycle by fluorescence activated cell sorting (FACS). TSU-Pr1 cell line derived from prostate carcinoma was used in this assay. Cells were treated with 10 μM of compound **15** and after 48 h fixed and labeled with propidium iodide. The different phases of cell cycle were analyzed by cytometry. TSU-Pr1 cells treated with compound **15** did not show changes of the cell cycle profiles as shown by the histograms depicted in Figure 4. Flow cytometry of propidium labeled cells indicates that treatment with compound **15** does not provoke a significative induction of cell apoptosis.

In conclusion, we have synthesized a series of bis-aminomethylnaphthalenes. The new compounds were evaluated for their ability to bind DNA and were assayed for antiproliferative properties both in vitro and in vivo. Compound **15** exhibited remarkable antitumor activity, being an appropriate ligand (in structure and function) for interaction with DNA, and represents a novel template for anticancer chemotherapy and could serve as a new lead compound.

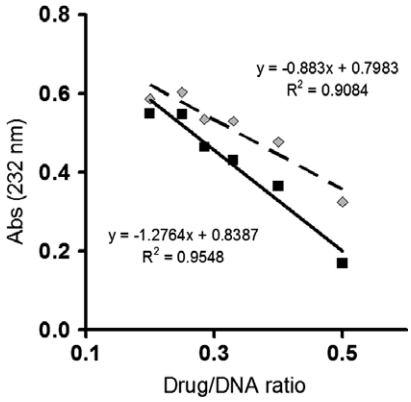


Figure 3. Absorbance (λ_{max} = 232 nm) versus different ratios of drug–DNA at 1 and 24 h.

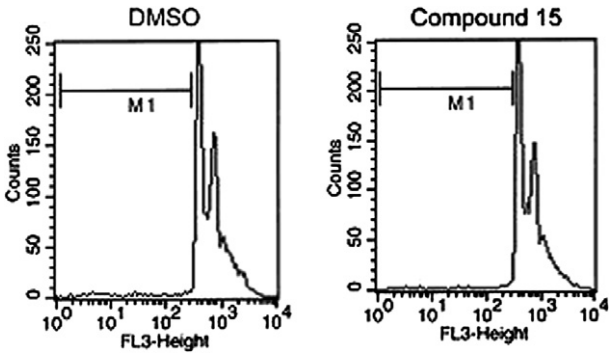


Figure 4. DNA fragmentation analysis for determination of apoptosis by FACS. TSU-Pr1 cells were treated with compound **15** and DNA content in the cells was determined after 48 h. Drug treated cells did not show any change in the histogram indicating that compound **15** is not able to induce apoptosis.

3. Experimental

3.1. Chemistry

Melting points were determined in a capillary with an Electrothermal 9100 SERIES-Digital apparatus and are uncorrected. IR spectra were recorded with a FT Perkin-Elmer Spectrum One from KBr discs. UV spectra were measured with a Jasco V-570 UV/vis/NIR spectrophotometer. ^{1}H NMR (200 MHz) spectra were obtained with a Bruker spectrometer at room temperature with tetramethylsilane as internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. Elemental analysis was carried out in our laboratories with a Coleman Analyzer.

3.1.1. General procedure for the synthesis of compounds 1–4

To a solution of 6-methoxy-2-naphthaldehyde (4.6 g, 24.7 mmol) in 20 mL of dry EtOH, the appropriate diamine (12.6 mmol) in 10 mL of dry EtOH was added dropwise. The reaction mixture was heated at reflux for 30 min; the crude products were filtered off and were crystallized from an appropriate solvent.

3.1.1.1. *N,N′*-Bis((6-methoxynaphthalen-2-yl)methylene)hexane-1,6-diamine (1). Yield: 96%, white solid, mp: 178–180 °C (ethanol). IR (KBr): 2950, 2890, 1590, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.20 (s, 2H, C*H*=N), 7.66–7.73 (m, 6H, β-naphthyl-*H*), 7.31 (dd, J = 8.3, 1.7 Hz, 2H, β-naphthyl-*H*), 7.06 (d, J = 6.7 Hz, 2H, β-naphthyl-*H*), 7.04 (d, J = 6.7 Hz, 2H, β-naphthyl-*H*), 3.57 (s, 6H, O–C*H*$_3$), 2.56 (t, J = 6.9 Hz, 4H, HC=N–C*H*$_2$–), 1.20–1.60 (m, 8H, (–C*H*$_2$)$_4$). Anal. Calcd for $C_{30}H_{32}N_2O_2$: C, 79.61; H, 7.13; N, 6.19. Found: C, 79.95; H, 7.01; N, 6.20.

3.1.1.2. *N,N′*-Bis((6-methoxynaphthalen-2-yl)methylene)octane-1,8-diamine (2). Yield: 97%, white solid, mp: 132–138 °C (ethanol). IR (KBr): 2900, 2890, 1595, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.20 (s, 2H, C*H*=N), 7.62–7.80 (m, 6H, β-naphthyl-*H*), 7.51 (dd, J = 8.1, 1.4 Hz, 2H, β-naphthyl-*H*), 7.10 (d, J = 6.6 Hz, 2H, β-naphthyl-*H*), 7.00 (d, J = 7.0 Hz, 2H, β-naphthyl-*H*), 3.90 (s, 6H, O–C*H*$_3$), 2.64 (t, J = 7.0 Hz, 4H, HC=N–C*H*$_2$–), 1.20–1.49 (m, 12H, (–C*H*$_2$)$_6$). Anal. Calcd for $C_{32}H_{36}N_2O_2$: C, 79.96; H, 7.55; N, 5.83. Found: C, 79.82; H, 7.81; N, 5.63.

3.1.1.3. *N,N′*-Bis((6-methoxynaphthalen-2-yl)methylene)dodecane-1,12-diamine (3). Yield: 76%, white solid, mp: 139–141 °C (ethanol). IR (KBr): 2950, 2850, 1595, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.00 (s, 2H, C*H*=N), 7.60–7.79 (m, 6H, β-naphthyl-*H*), 7.46 (dd, J = 8.3, 1.2 Hz, 2H, β-naphthyl-*H*), 7.10 (d, J = 6.6 Hz, 2H, β-naphthyl-*H*), 6.90 (d, J = 6.9 Hz, 2H, β-naphthyl-*H*), 3.95 (s, 6H, O–C*H*$_3$), 2.62 (t, J = 6.9 Hz, 4H, HC=N–C*H*$_2$–), 1.20–1.52 (m, 20H, (–C*H*$_2$)$_{10}$). Anal. Calcd for $C_{36}H_{44}N_2O_2$: C, 80.56; H, 8.26; N, 5.22. Found: C, 80.43; H, 8.05; N, 5.38.

3.1.1.4. 3,3′-(Piperazine-1,4-diyl)bis(*N*-((6-methoxynaphthalen-2-yl)methylene)propan-1-amine) (4). Yield: 86%, white solid, mp: 165–167 °C (ethanol). IR (KBr): 2900, 2850, 1590, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.10 (s, 2H, C*H*=N), 7.61–7.65 (m, 6H, β-naphthyl-*H*), 7.32 (dd, J = 8.3, 1.7 Hz, 2H, β-naphthyl-*H*), 7.08 (d, J = 6.9 Hz, 2H, β-naphthyl-*H*), 7.06 (d, J = 7.0 Hz, 2H, β-naphthyl-*H*), 3.85 (s, 6H, O–C*H*$_3$), 2.62 (t, J = 6.8 Hz, 4H, HC=N–C*H*$_2$–), 2.27–2.38 (m, 12H, piperazine-*H* and –C*H*$_2$-piperazine) 1.60–1.72 (m, 4H, –C*H*$_2$). Anal. Calcd for $C_{34}H_{40}N_4O_2$: C, 76.09; H, 7.51; N, 10.44. Found: C, 75.88; H, 7.69; N, 10.58.

3.1.1.5. 2,2′-(Ethane-1,2-diylbis(oxy))bis(*N*-((6-methoxynaphthalen-2-yl)methylene)ethanamine) (5). A solution of 2-[2-(2-aminoethoxy)-ethoxy]-ethylamine (0.7 mL, 4.7 mmol) in 10 mL of dry EtOH was added dropwise to a solution of 6-methoxy-2-naphthaldehyde (1.5 g, 8.1 mmol) in dry EtOH (20 mL).

The mixture was stirred at reflux temperature for 3 h. 3/4 parts of solvent were evaporated under reduced pressure; the white crude product was filtered off and recrystallized from EtOH. Yield: 58.9%, mp: 121–123 °C.

IR (KBr): 2900, 2850, 1595, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.30 (s, 2H, C*H*=N), 7.61–7.65 (m, 6H, β-naphthyl-*H*), 7.30 (dd, J = 8.0, 1.2 Hz, 2H, β-naphthyl-*H*), 7.08 (d, J = 6.9 Hz, 2H, β-naphthyl-*H*), 7.06 (d, J = 7.0 Hz, 2H, β-naphthyl-*H*), 3.82 (s, 6H, O–C*H*$_3$), 3.60 (t, J = 4.5 Hz, 8H, O–C*H*$_2$–), 2.82 (t, J = 5.3 Hz, 4H, HC=N–C*H*$_2$–). Anal. Calcd for $C_{30}H_{32}N_2O_4$: C, 74.18; H, 6.23; N, 5.97. Found: C, 73.98; H, 6.40; N, 5.7.

3.1.1.6. *N,N′*-Bis-naphthalen-2-ylmethylene-hexane-1,6-diamine (6). To a solution of 2-naphthaldehyde (4.6 g, 29.7 mmol) in 20 mL of dry EtOH, a solution of 1,6-hexanediamine (1.72 g, 14.6 mmol) in 10 mL of dry EtOH was added dropwise. The reaction mixture was heated at reflux for 15 min. The formed precipitate was filtered off and recrystallized from EtOH. Yield: 95%, mp: 137–139 °C.

IR (KBr): 2900, 1595, 850, 780 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 8.06 (s, 2H, C*H*=N), 7.82–7.90 (m, 8H, β-naphthyl-*H*), 7.50–7.68 (m, 6H, β-naphthyl-*H*), 3.00 (t, J = 6.1 Hz, 4H, HC=N–C*H*$_2$–), 0.90–1.44 (m, 8H, –C*H*$_2$). Anal. Calcd for $C_{28}H_{28}N_2$: C, 85.67; H, 7.19; N, 7.14. Found: C, 85.40; H, 7.40; N, 6.90.

3.1.1.7. 2,2′-(Ethane-1,2-diylbis(oxy))bis(*N*-((naphthalen-2-yl)-methylene)ethanamine) (7). A solution of 2-[2-(2-aminoethoxy)-ethoxy]-ethylamine (0.7 mL, 4.7 mmol) in 10 mL of dry EtOH was added dropwise to a solution of 2-naphthaldehyde (1.5 g, 8.1 mmol) in dry EtOH (20 mL). The mixture was stirred at reflux temperature for 3 h. 3/4 of solvent volume was evaporated under reduced pressure; the product was filtered, washed with EtOH. Yield: 60%, mp: 108–110 °C.

IR (KBr): 2950, 2840, 1595, 1300, 850, 780 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 8.00 (s, 2H, C*H*=N), 7.70–7.90 (m, 8H, β-naphthyl-*H*), 7.50–7.60 (m, 6 H, β-naphthyl-*H*), 3.20–3.24 (m, 4H, O–C*H*$_2$–C*H*$_2$–O), 3.00 (m, 8H, HC=N–C*H*$_2$–C*H*$_2$–O–). Anal. Calcd for $C_{28}H_{28}N_2O_2$: C, 79.22; H, 6.65; N, 6.6. Found: C, 79.12; H, 6.81; N, 6.44.

3.1.2. General procedure for the synthesis of compound 8–12

$NaBH_4$ (1.4 g, 39.6 mmol) was added in small portions over several minutes to a suspension of corresponding compounds **1**–**7** (3.15 mmol) in 10 mL of dry methanol. The mixture was heated at reflux for 3 h and then was allowed to come to room temperature and stirred overnight. The products were filtered, washed with H_2O, and were crystallized from an appropriate solvent.

3.1.2.1. *N,N′*-Bis-(6-methoxy-naphthalen-2-ylmethyl)-hexane-1,6-diamine (8). Compound **8** was recrystallized from cyclohexane/benzene (1:1) (1.03 g, 79% yield), mp: 117–119 °C.

IR (KBr): 3330, 2900, 2850, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.30–7.60 (m, 6H, β-naphthyl-*H*), 7.10 (dd, J = 8.0, 1.6 Hz, 2H, β-naphthyl-*H*), 7.04 (d, J = 6.9 Hz, 2H, β-naphthyl-*H*), 7.00 (d, J = 7.0 Hz, 2H, β-naphthyl-*H*), 3.83 (s, 6H, O–CH$_3$), 3.80 (s, 4H, naphthyl-C*H*$_2$–N), 2.52 (t, J = 6.8 Hz, 4H, N–C*H*$_2$–), 1.25–1.47 (m, 10H, –(C*H*$_2$)$_4$ and NH). Anal. Calcd for $C_{30}H_{36}N_2O_2$: C, 78.91; H, 7.95; N, 6.13. Found: C, 78.73; H, 8.10; N, 6.28.

3.1.2.2. *N,N′*-Bis((6-methoxynaphthalen-2-yl)methyl)octane-1,8-diamine (9). Compound **9** was crystallized from methanol/ciclohexane (1:1) (1.25 g, 82% yield), mp: 93–94 °C.

IR (KBr): 3330, 2900, 2850, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.30–7.60 (m, 6H, β-naphthyl-*H*), 7.18 (dd, J = 8.1, 1.6 Hz, 2H, β-naphthyl-*H*), 7.00 (d, J = 7.0 Hz, 2H, β-naphthyl-*H*), 6.90 (d, J = 7.0 Hz, 2H, β-naphthyl-*H*), 3.98 (s, 6H, O–C*H*$_3$), 3.85 (s, 4H, naphthyl-C*H*$_2$–N), 2.68 (t, J = 6.8 Hz, 4H, N–C*H*$_2$–), 1.25–1.47 (m,

14H, –(CH_2)$_6$ and N*H*). Anal. Calcd for $C_{32}H_{40}N_2O_2$: C, 79.30; H, 8.32; N, 5.78. Found: C, 79.12; H, 8.49; N, 5.89.

3.1.2.3. *N,N′*-Bis((6-methoxynaphthalen-2-yl)methyl)dodecane-1,12-diamine (10). Compound **10** was recrystallized from ethanol (1.4 g, 82% yield), mp: 110–115 °C.

IR (KBr): 3330, 2950, 2890, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.30–7.60 (m, 6H, β-naphthyl-*H*), 7.15 (dd, *J* = 8.1 Hz, *J* = 1.6 Hz, 2H, β-naphthyl-*H*), 7.10 (d, *J* = 6.9 Hz, 2H, β-naphthyl-*H*), 7.00 (d, *J* = 7.0 Hz, 2H, β-naphthyl-*H*), 3.95 (s, 6H, O–C*H*$_3$), 3.90 (s, 4H, naphthyl-C*H*$_2$–N), 2.65 (t, *J* = 6.9 Hz, 4H, N–C*H*$_2$–), 1.25–1.47 (m, 22H, –(CH_2)$_{10}$ and N*H*). Anal. Calcd for $C_{36}H_{48}N_2O_2$: C, 79.96; H, 8.95; N, 5.18. Found: C, 79.78; H, 9.05; N, 5.0.

3.1.2.4. 3,3′-(Piperazine-1,4-diyl)bis(*N*-((6-methoxynaphthalen-2-yl)methyl)propan-1-amine) (11). Compound **11** was recrystallized from ethanol (1.02 g, 60% yield), mp: 75–80 °C.

IR (KBr): 3330, 2950, 2800, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.60–7.66 (m, 6H, β-naphthyl-*H*), 7.31 (dd, *J* = 8.4, 1.7 Hz, 2H, β-naphthyl-*H*), 7.19 (d, *J* = 6.9 Hz, 2H, β-naphthyl-*H*), 7.09 (d, *J* = 6.9 Hz, 2H, β-naphthyl-*H*), 3.98 (s, 6H, O–C*H*$_3$), 3.83 (s, 4H, naphthyl-C*H*$_2$–N), 2.62 (m, 16H, N–C*H*$_2$– and C*H*$_2$-piperazine), 1.90 (s, 2H, N*H*), 1.60–1.72 (m, 4H, –C*H*$_2$). Anal. Calcd for $C_{34}H_{44}N_4O_2$: C, 75.52; H, 8.20; N, 10.36. Found: C, 72.30; H, 8.4; N, 10.46.

3.1.2.5. 2,2′-(Ethane-1,2-diylbis(oxy))bis(*N*-((6-methoxynaphthalen-2-yl)methyl)ethanamine) (12). Compound **12** was crystallized from acetone, mp: 200–202 °C (d). **12** was obtained as hydrochloride using ethanol/HCl to give 1.42 g, 86% yield, mp 222–223 °C. IR (KBr) (as free base): 3320, 2950, 2820, 1260, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) (as free base) δ: 7.80–7.84 (m, 6H, β-naphthyl-*H*), 7.40 (dd, *J* = 7.9, 1.5 Hz, 2H, β-naphthyl-*H*), 7.31 (d, *J* = 6.9 Hz, 2H, β-naphthyl-*H*), 7.20 (d, *J* = 6.9 Hz, 2H, β-naphthyl-*H*), 3.98 (s, 6H, O–C*H*$_3$), 3.90 (s, 4H, naphthyl-C*H*$_2$–N), 3.1–3.15 (m, 8H, C*H*$_2$–O), 2.89–3.00 (m, 4H, N–C*H*$_2$–), 1.90 (s, 2H, N*H*). Anal. Calcd for $C_{30}H_{36}N_2O_4$: C, 73.74; H, 7.43; N, 5.73. Found: C, 73.54; H, 7.60; N, 5.86.

3.1.2.6. *N,N′*-Bis-((naphthalen-2-yl)methyl)hexane-1,6-diamine (13). Compound **13** was washed with H_2O (1.04 g, 84%), mp: 74–76 °C.

IR (KBr): 3330, 2900, 850, 780 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 7.73–7.82 (m, 8H, β-naphthyl-*H*), 7.41–7.48 (m, 6H, β-naphthyl-*H*), 3.93 (s, 4H, naphthyl-C*H*$_2$–N), 2.64 (t, *J* = 6.8 Hz, 4H, N–C*H*$_2$–), 1.31–1.40 (m, 10H, (CH_2)$_4$ and N*H*). Anal. Calcd for $C_{28}H_{32}N_2$: C, 84.80; H, 8.13; N, 7.06. Found: C, 84.65; H, 8.36; N, 7.25.

3.1.2.7. 2,2′-(Ethane-1,2-diylbis(oxy))bis(*N*-(naphthalen-2-ylmethyl)-ethanamine) (14). Compound **14** was recrystallized from ethanol/HCl to give compound **14** as hydrochloride (1.28 g, 88%), mp: 232–234 °C.

IR (KBr) (as free base): 3300, 2900, 2830, 1100, 850, 780 cm^{-1}. ^{1}H NMR (DMSO-d_6) (as free base) δ: 7.73–7.79 (m, 8H, β-naphthyl-*H*), 7.38–7.46 (m, 6H, β-naphthyl-*H*), 3.93 (s, 4H, naphthyl-C*H*$_2$–N), 3.59–3.64 (m, 8H, C*H*$_2$–O), 2.80–2.85 (m, 4H, N–C*H*$_2$–), 1.90 (s, 2H, N*H*). Anal. Calcd for $C_{28}H_{32}N_2O_4$: C, 78.47; H,7.53; N, 6.54. Found: C, 78.33; H, 7.22; N, 6.69.

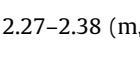
3.1.3. General procedure for the synthesis of compounds 15 and 16

To a solution of the appropriate aldehyde (10 mmol) in 30 mL of dry EtOH was added in a small portion 0.2 g $NaBH_4$ over 30 min at 0 °C. Afterwards the mixture was stirred at 50 °C for 3 h. Then, the mixture was filtered, the alcohol solution was evaporated, and the residue was washed with EtOH to yield the compound **15a**, mp: 121–122 °C, and compound **16a**, mp: 79–80 °C (mp lit.: 79–81 °C).

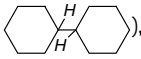
To the appropriate compound **15a** or **16a** (4.5 mmol) in 25 mL of CH_2Cl_2, a solution of thionyl chloride (6.8 mmol) in 5 mL of CH_2Cl_2 was added gradually at 0 °C. Afterwards, the mixture was heated at reflux for 1 h and the solvent was evaporated to dryness under reduced pressure. The residue was washed with cyclohexane and dried to give **15b**, mp: 57–59 °C (mp lit.: 58–59 °C). and **16b**, mp: 48–49 °C (mp lit.: 48–49 °C).

A solution of 4,4′-bipiperidine (1.3 g, 7.7 mmol) in 5 mL of dry EtOH was added dropwise to a solution of the select compound **15b** or **16b** (15 mmol) in 20 mL of dry EtOH and K_2CO_3 (0.7 g, 7.7 mmol). The mixture was heated at reflux for 5 h. The white precipitate formed was filtered to afford the crude product. Purification was effected as noted.

3.1.3.1. 1,1′-Bis-(6-methoxy-naphthalen-2-ylmethyl)-[4,4′]-bipiperidine (15). Compound **15** was recrystallized from acetone (1.05 g, 66%), mp: 212–213 °C.

IR (KBr): 3300, 2950, 2850, 1100, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.61–7.65 (m, 6H, β-naphthyl-*H*), 7.38 (dd, *J* = 8.0, 1.6 Hz, 2H, β-naphthyl-*H*), 7.08 (d, *J* = 6.9 Hz, 2H, β-naphthyl-*H*), 7.06 (d, *J* = 7.0 Hz, 2H, β-naphthyl-*H*), 3.85 (s, 6H, O–C*H*$_3$), 3.80 (s, 4H, C*H*$_2$), 2.27–2.38 (m, 8H, piperazine-*H*) 1.65 (d, 2H, ), 1.10–1.27 (m, 8*H*, piperazine-*H*). Anal Calcd for $C_{34}H_{40}N_2O_2$: C, 80.28; H, 7.28; N, 5.51. Found: C, 80.07; H, 7.44; N, 5.79.

3.1.3.2. 1,1′-Bis-(naphthalen-2-ylmethyl)-[4,4′]-bipiperidine (16). Compound **16** was recrystallized from acetone (1.05 g, 75%), mp: 139–140 °C.

IR (KBr): 3300, 2950, 850, 780 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.61–7.65 (m, 6H, β-naphthyl-*H*), 7.38–7.49 (m, 6H, β-naphthyl-*H*), 3.85 (s, 6H, O–C*H*$_3$), 3.80 (s, 4H, C*H*$_2$), 1.96–2.27 (m, 8H, piperazine-*H*), 1.65 (d, 2H, ), 1.14–1.27 (m, 8*H*, piperazine-*H*). Anal. Calcd for $C_{32}H_{36}N_2$: C, 85.71; H, 8.04; N, 6.25. Found: C, 85.68; H, 8.07; N, 6.24.

3.2. DNA affinity assay

DNA solution: Calf thymus DNA (12.5 mg) was slowly magnetically stirred in 10 mM Tris–HCl buffer, pH 7.4 (5 mL), for 24 h at 4 °C. 0.6 mL was taken from this solution and diluted to 25 mL with the same buffer.

The test compound solution was prepared at a 10^{-4} M concentration using a minimal volume of ethanol and then diluted by adding water to a concentration of 2×10^{-5} M. Three milliliters sample of this solution was mixed with 3 mL of the DNA solution. The mixture was slowly rotated for 24 h and, then, its UV spectra were recorded at 20 °C using a 1 cm cell.

3.3. Antineoplastic activity

3.3.1. Primary disease-oriented in vitro antitumor screen

The evaluation of antiproliferative activity was performed at the National Cancer Institute (NCI, USA), following the well-known in vitro disease-oriented primary antitumor screening against a panel about 60 different cell lines derives from nine clinically isolated human tumors.[15] The tested compounds were assayed in range of doses from 10^{-4}–10^{-8}. Results are reported as GI_{50} (concentration that produces a 50% growth inhibition on each cell line), TGI (concentration that produces a total growth inhibition on each cell line), and LC_{50} (concentration that produces cytotoxic effect in 50% on each cell line) parameters in Table 1.

3.3.2. Preliminary in vivo antitumor test (hollow fiber assay)

Twelve cell lines derived from human tumors are cultivated in polyvinylidene fluoride (PVDP) hollow fibers and a sample of each cell line is implanted into each of two physiologic compartments

(intraperitoneal-IP and subcutaneous-SC) in athymic mice.[16] Each test mouse receives a total of 6 fibers (3 intraperitoneally and 3 subcutaneously) representing 3 distinct cancer cell lines. Three mice are treated with potential antitumor compounds at each of two test doses by intraperitoneal route using a QDx4 treatment schedule. Vehicle controls consist of 6 mice receiving the diluent only. The fiber cultures are collected on the day following the last day of treatment. To assess anticancer effects, viable cell mass is determined for each of the cell lines using a formazan dye (MTT) conversion assay. The data are reported as %T/C for each of the 2 compounds doses against each of the cell lines with separate values calculated for the intraperitoneal and subcutaneous samples. To simplify evaluation, a points system has been adopted which results in a 50% or greater reduction in viable cell mass. Compounds with a combined IP+SC score $\geqslant$20, a SC score $\geqslant$8 or a net cell kill of one or more cell lines are referred for further xenograft testing.

3.4. Cell cycle analysis

For flow cytometry of DNA content, 5×10^5 TSU-Pr1 cells in exponential growth were treated at 10 μM of compound **15** for 48 h. After the incubation period, cells were centrifuged, fixed in ice-cold ethanol (70%), treated with lysis buffer containing RNase, and finally stained with propidium iodide. Samples were analyzed on a Becton Coulter Epics XL-MCL flow cytometer. For cell cycle analysis, DNA histograms were analyzed using MultiCycle® for Windows (Phoenix Flow Systems, San Diego, CA).

Acknowledgment

We thank the Development Therapeutics Program of the National Cancer Institute, Bethesda, MD, for performing biological assays and SECyT-UBA for financial support.

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