Cátedra de Farmacognosia[1], IQUIMEFA-CONICET, Centro de Síntesis y Estudio de Nuevos Compuestos Antineoplásicos[2], Departamento de Química Orgánica, Cátedra de Microbiología Industrial y Biotecnología[3], Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Argentina

Human DNA topoisomerase I poisoning activity of bis-1-aminomethylnaphtalenes. A correlation with their cytotoxic activity

A. Romano[1], E. Mongelli[1], A. M. Bruno[2], C. Gaozza[2], J. Coussio[1] and G. Ciccia[3]

Symmetrical bis-1-aminmoethylnaphtalenes, a group of compounds that demonstrated cytotoxicity towards human tumor cell lines, showed human topoisomerase I poisoning activity. The compounds tested were: *N,N′*-bis-1-naphthylmethyl-1,6-hexanediamine (**1a**), *N,N′*-bis-1-naphthylmethyl-1,8-octanediamine (**1b**), *N,N′*-bis-1-naphthylmethyl-1,12-dodecanediamine (**1c**), *N,N′*-bis-1-naphthylmethyl-4,4-bipiperidine (**2**) and *N*-(1-naphthylmethyl)-*N′*-dimethyl-1,3-diamine propane dichlorhydrate (**3**). All showed human topoisomerase I inhibition by producing protein-linked DNA breaks. The most active were **1a**, **1b**, **1c** with a percentage stimulation of DNA cleavage of 75, 84 and 70% at 100 μg/ml, respectively. Compounds **2** and **3** were moderately active as poisons of topoisomerase I activity, the former showing 58% stimulation of DNA cleavage at 100 μg/ml and the latter a 24% stimulation. The correlation observed between topoisomerase I poisoning and *in vitro* cytotoxic activity suggests that this could be a possible mechanism for the cytotoxicity observed in tumor cell lines.

1. Introduction

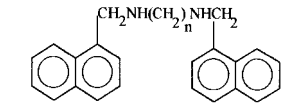
Symmetrical bis-1-aminomethylnaphtalenes constitute a new class of molecules with cytotoxic activity. Their structure derives from a long development to determine the minimum active molecular structures that result from echinomicine molecule simplification [1, 2].

These compounds were found to be cytotoxic when assayed against 60 human tumour cell lines. As a first approach to determine the mode of action, previous studies suggested an interaction between the compounds and DNA [3]. Since many cytotoxic substances that interact with DNA show topoisomerase poisoning activity, a topoisomerase I inhibition assay was carried out [4].

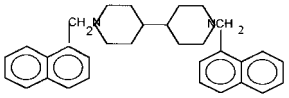
DNA topoisomerases modulate the topological states of DNA during cellular events such as replication, transcription, and recombination. The DNA topoisomerases are classified in two groups: topoisomerase I enzymes relax negatively supercoiled plasmid DNA in the absence of ATP. They introduce a transient breakage in one strand at a time to allow the passage of another strand through the break. During the process the enzyme is found to be covalently linked to the 3′phosphoryl end of the broken DNA strand via a tyrosyl phosphate bond. Type II enzymes work in a similar manner, but they create breaks on both strands of DNA in the presence of ATP [5]. Topoisomerase poisons are distinct from catalytic inhibitors of topoisomerases. Catalytic inhibitors act, for example, by interfering with the binding of topoisomerase to DNA, by interfering with the formation of the active intermediate or by trapping the topoisomerase on the DNA once the reaction is complete. In contrast, a topoisomerase poison stabilizes a ternary complex with the enzyme and DNA [6]. *In vitro*, a poison leads to enhanced recovery of the covalent topoisomerase-DNA complex after the addition of SDS. In a growing cell, the collision of the transcription or replication machinery with the drug-stabilized cleavage complex leads to irreparable DNA damage and cell death.

A substance that can stabilize DNA topoisomerase cleavage complexes to stop the progression of DNA processes may be useful in cancer chemotherapy [4].

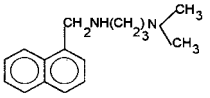
We have studied the effects of the symmetrical bis-1-aminomethylnaphthalenes **1a**, **1b**, **1c** and **2** and a related compound, the assymmetric mononaphthalene **3**, on mammalian topoisomerase I, and report evidence for a drug induced DNA cleavage consistent with topoisomerase I poisoning activity.



n = 6 compound 1a
n = 8 compound 1b
n =12 compound 1c



compound 2



compound 3

2. Investigations, results and discussion

2.1. Cytotoxicity

All the compounds were previously evaluated for *in vitro* antitumor activity at the National Cancer Institute, USA according to the method described [7, 8]. The antitumor activity of the compounds was evaluated using 60 human cell lines derived from seven different cancer types (lung, colon, melanoma, leukemia, renal, ovarian and central nervous system). The compounds were tested in a broad concentration range (10^{-1}M−10^{-8}M) against every cell line of the panel. Data deficient for any individual cell line(s) were deleted from the screening report. The response parameters GI_{50} (concentration giving 50% inhibition), TGI (concentation giving total growth inhibition) and LC_{50} (concentration giving 50% lethal effect) were calculated from dose-response curves. Mean panel $\log_{10}$ values (MG-MID) of the three response parameters were obtained by averaging the individual values for each cell line. The mean GI_{50}, LC_{50} and TGI are presented in Table 1. The symmetrical bis-1-aminomethylnaphtalenes (**1a**, **1b**, **1c** and **2**) showed cytostatic activity values from 5.18 to 5.66. Cytostatic activity increases with methylene chain length in compounds **1a**, **1b** and **1c**. The assymmetric naphtalene

Table 1: Antineoplastic activity of compounds 1a, 1b, 1c, 2 and 3[a]

Compd.	$-\log_{10}GI_{50}$	$-\log_{10}TGI$	$-\log_{10}LC_{50}$
1a	5.27	4.91	4.53
1b	5.62	5.28	4.94
1c	5.66	5.36	5.07
2	5.18	4.71	4.36
3	4.02	4.00	4.06

[a] $\log_{10}$ values of the calculated mean molar GL_{50}, TGI and LC_{50} concentration

3 displayed little cytostatic activity ($-\log GI_{50} = 4.02$). The data presented in Table 2 demonstrate that compounds **1a**, **1b**, **1c** were selective in the leukemia, colon cancer and melanoma panels. Compounds **1a**, **1b**, **1c** and **2** were more active against lines HL-60 (TB), K-562 and SR from the leukemia subpanel. The cells of the line Colo-205 from the colon cancer subpanel were more sensitive to compounds **1a**, **1b**, and **2**. All the other cell lines of this subpanel and melanoma subpanel were sensitive to **1a**, **1b**, **1c** and **2** in high or low concentration. Compound **2** was found to be less active than the others, with specificity in leukemia, colon cancer, melanoma and lung cancer. Compound **3** showed low activity toward all cell lines.

2.2 Topoisomerase I inhibition assay

In order to determine a possible mode of action of the compounds, the DNA-topoisomerase I cleavage complex formation assay was carried out. The reaction products were evaluated by agarose gel electrophoresis. All compounds tested were able to stabilize the topoisomerase cleavage complex and suggesting them to be topoisomerase I poisons. Compound **1b** was the most active, showing an intense band of open circular DNA at 100 μg/ml, that corresponds to the topoisomerase-DNA cleavage complex (Fig. 1). The poisoning activities of compounds **1a**, **1b**, **1c**, **2** and **3** are shown in Figs. 2 and 3.
Topoisomerase I inhibitory activities were quantified as the percentage of DNA cleavage stimulation, as described under Experimental. The comparative percentages obtained in the presence of 1, 10 and 100 μg/ml of each compound are presented in Table 3. Compunds **1b**, **1a**, **1c** and **2** were the most active (84%, 75%, 70% and 58% of

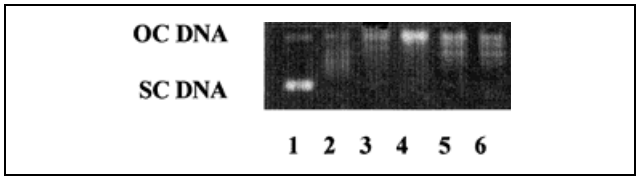


Fig. 1: Poisoning effect of **compound 1b** (agarose gel) Lane 1: Supercoiled DNA Lane 2: Supercoiled DNA plus topoisomerase I Lane 3: Supercoiled DNA plus topoisomerase I plus 0.1 mM camptothecin (control) Lanes 4–6: Supercoiled DNA plus topoisomerase I plus 100, 10 and 1 μg/ml of **compound 1b**

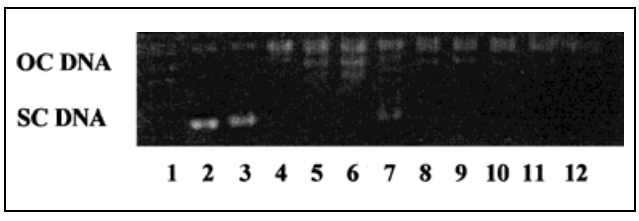


Fig. 2: Comparative poisoning activities of compounds **1a**, **1b** and **2** (agarose gel) Lane 1: Supercoiled DNA plus topoisomerase I Lane 2: Supercoiled DNA Lane 3: Supercoiled DNA plus 1% DMSO (control) Lane 4–6: Supercoiled DNA plus topoisomerase I plus 100, 10 and 1 μg/ml of compound **1b**Lanes 7–9: Supercoiled DNA plus topoisomerase I plus 100, 10 and 1 μg/ml of compound **1a** Lanes 10–12: Supercoiled DNA plus topoisomerase I plus 100, 10 and 1 μg/ml of **compound 2**

Table 2: Data obtained from *in vitro* sreen of compounds 1–3 of 60 human tumor cell lines

		Compd. $-\log(GI_{50})$				
Panel	Cell line	1a	1b	1c	2	3
Leukemia	CCRF-CEM	5.5	5.7	5.9	4.9	4.0
	Hl-60 (TB)	5.8	5.7	5.9	4.9	4.0
	K-562	5.8	6.2	6.3	5.8	4.0
	MOLT-4	5.5	5.7	5.9	5.2	4.0
	RPMI-8226	5.6	5.8	6.0	5.4	4.0
	SR	5.7	5.9	6.0	5.8	4.6
Non-Small Cell Lung	A549/ATCC	4.7	5.7	5.7	4.4	4.0
	EKVX	4.9	5.2	5.5	4.5	4.0
	HOP-18	4.8	5.3	5.5	4.4	–
	HOP-62	4.8	4.8	5.7	4.7	4.0
	HOP-92	5.2	5.6	5.7	5.8	4.1
	NCI-H226	4.8	4.9	5.6	4.5	4.0
	NCI-H23	4.8	5.5	5.8	4.6	4.0
	NCI-H322M	5.5	5.8	5.7	5.2	4.0
	NCI-H460	5.7	5.8	5.8	4.7	4.0
	NCI-H522	5.6	5.7	5.7	4.9	–
	LXFL 529	5.7	5.7	5.7	5.2	–
Small Cell Lung	DMS 114	5.1	5.7	5.7	5.6	–
	DMS 273	4.9	5.7	4.0	5.2	–
Colon	COLO 205	5.8	5.7	4.0	5.8	4.0
	DLD-1	5.4	5.7	5.8	5.7	–
	HCT-116	5.7	5.8	5.8	5.1	4.0
	HCT-15	5.2	5.8	5.8	5.5	4.0
	HT29	5.7	5.7	5.8	5.5	4.0
	KM12	5.4	5.7	5.8	5.0	4.0
	KM20L2	5.6	5.8	5.7	5.6	–
	SW-620	5.4	5.7	5.7	5.6	–
Central Nervous System	SF-268	4.9	5.1	5.7	5.0	4.0
	SF-295	5.7	5.7	4.7	5.1	4.0
	SF-593	5.6	5.8	5.8	5.4	4.0
	SNB-75	5.0	5.7	5.7	5.6	4.0
	SNB-78	5.0	5.7	5.7	4.6	–
	U251	5.6	5.8	5.8	4.9	4.0
	XF 498	5.7	5.7	5.8	5.3	–
Melanoma	LOX IMVI	5.7	5.9	5.9	5.4	4.0
	MALME-3M	5.7	5.7	5.7	5.6	4.0
	M14	5.4	5.8	5.7	5.7	4.2
	M19-MEL	5.8	5.8	5.7	5.2	–
	SK-MEL-2	5.7	5.8	5.8	5.2	4.0
	SK-MEL-28	5.1	5.7	5.7	5.8	4.0
	SK-MEL-5	5.8	5.7	5.7	5.4	4.0
	UACC-257	5	5.7	5.7	5.7	4.0
	UACC-62	4.8	5.7	5.7	5.2	4.0
Ovarian	IGROVI	4.8	5.3	5.7	4.5	4.0
	OVCAR-3	4.8	5.0	5.7	4.4	4.0
	OVCAR-4	4.8	5.6	5.8	4.9	4.0
	OVCAR-5	4.8	5.7	5.8	4.7	4.0
	OVCAR-8	4.8	5.7	5.8	4.7	4.0
	SK-OV-3	4.8	5.7	5.8	4.7	4.0
Renal	786-0	4.7	5.8	5.7	4.8	4.0
	A498	4.8	4.8	5.2	4.7	4.0
	ACHN	5.1	5.5	5.7	4.4	4.0
	CAKI-1	5.7	5.7	5.7	5.4	–
	RXF393	5.4	5.8	5.9	4.9	4.0
	RXF-631	4.9	5.9	5.7	4.9	–
	SN12C	4.9	5.2	5.5	4.2	4.0
	TK-10	4.9	5.8	5.7	4.8	4.0
	UO-31	4.9	5.7	5.8	5.4	4.0

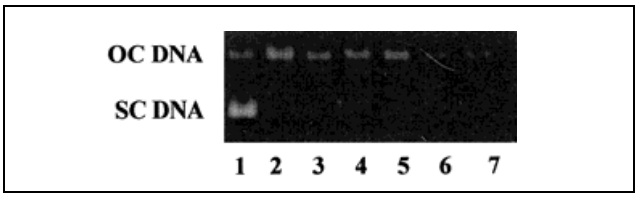


Fig. 3: Poisoning effect of **compounds 1c** and **3**. (agarose gel) Lane 1: Supercoiled DNA Lane 2–4: Supercoiled DNA plus topoisomerase I plus 100, 10 and 1 μg/ml of **compound 1c**. Lane 5–7: Supercoiled DNA plus topoisomerase I plus 100, 10 and 1μg/ml of **compound 3**

Table 3: Relation between antitumor activity of compounds 1–4 and effects on topoisomerase I mediated reactions

Compd.	% stimulation of DNA cleavage[a]			Antitumor activity
	1 μg/ml	10 μg/ml	100 μ/ml	MG MID GI_{50}
1a	49%	62%	75%	5.27
1b	56%	70%	84%	5.62
1c	20%	23%	70%	5.66
2	16%	31%	58%	5.18
3	1%	3%	24%	4.02

[a] Values are the means from the least triplicated experiments, the variance was less than 25% of the mean value

stimulation of DNA cleavage at 100 μg/ml of compound, respectively), while compound **3** was the last potent topoisomerase I inhibitor (24% of stimulation of DNA breaks). The results obtained show that, in general, there is a good correlation between the cytotoxic/cytostatic activities of the compounds and their potency as topoisomerase I poisons. The highest antineoplastic and also the most important poisoning activities were obtained for compounds **1a**, **1b**, and **1c** while the lowest antineoplastic and poisoning activities were obtained with compound **3**, indicating that the presence of two naphthalene groups would increase both cytotoxic/cytostatic and topoisomerase I activities.

In conclusion, this study suggests that the antineoplastic activity of symmetrical bis-1-aminomethylnaphthalenes may be due to their action on topoisomerase I. The possibility, however, is not excluded that these compounds may inhibit other enzymes, such as topoisomerase II.

3. Experimental

3.1 Synthesis of symmetrical bis-1-aminomethylnaphthalenes and asymmetrical mononaphthalene

The preparation of the compounds has been described previously [1].

3.2 Topoisomerase I inhibition assay

Inhibition of topoisomerase I activity was determined using a relaxation assay described by Liu and Miller [9] with modifications. Plasmid pHOT1 (Topogen, USA) was used as a substrate in a final reaction volume of 20 μl containing 10 mM Tris HCL, 1 mM EDTA, 0.15 M ClNa, 0.1% BSA, 0.1 mM spermidine, 5% glycerol and 2 units of purified human topoisomerase I (Topogen, USA). Compounds **1a**, **1b**, **1c**, **2** and **3** were predissolved in DMSO and tested at 100, 10 and 1 μg/ml. The reaction was carried out at 37 °C for 30 min and terminated by addition of 10% SDS and proteinase K (Sigma, USA) (50 μg/ml). After digestion at 37 °C for 30 min, 2 μl of a 0.025% loading dye solution were added to the reaction medium. The sample, previously extracted with 20 μl of a chloroform isoamyl alcohol (CIA) (24:1) solution, was submitted to a 1% agarose gel electrophoresis using TAE buffer during 4 h at 36 V at room temperature. For the quantitative determination of topoisomerase I activity, photographic negatives were scanned in a Hewlett Packard Scanjet scanner (model 11 p), and the bands were analysed with a Jandel SigmaScan image measurement analyzing program. The areas presenting supercoiled DNA, migrating as bands at the buttom of the gel, were determined. Drug-included DNA cleavage was calculated from the formula:

$$\% \text{ of stimulation: } \frac{(N_{ED}/N_E)}{1 - N_E} \times 100$$

where N_{ED} and N_E represents the fraction of nicked DNA measured in the presence of topoisomerase I and drug and N_E in the presence of topoisomerase I alone, respectively.

References

1 Bruno, A. M.; Gaetta, J.; Gaozza, C. H.: A. Quím. **88**, 267 (1992)
2 Bruno, A. M.; Ivanovic, S.; Gaozza, C. H.: A. Quím. **90**, 135 (1994)
3 Bruno, A. M.; Lo Balbo, A.; Asis, S.; Molina, D.; Conti, G,; Gaozza, C. H.: Chem. Farm. Bull. **135**, 374 (1996)
4 Liu, L. F.: Ann. Rev. Bioch. **58**, 351 (1989)
5 Gil, R. R.; Cordell, G. A.: J. Nat. Prod. **59**, 27 (1996)
6 Fostel, J.: Bacteriol. **61**, 168 (1995)
7 Weislow, O. W.; Kieser, R.; Fine, D.; Bader, J.; Shoemaker, R. H.; Boyd, M. R.: J. Natl. Cancer Inst. **81**, 577 (1989)
8 Boyd, M. R.: Princ. Pract. Oncol. **3**, 1 (1980)
9 Liu, L.; Miller, K.: Proc. Natl. Acad. Sci. USA **78**, 3487 (1981)

Received December 13, 1999
Accepted January 25, 2000

Alejandra Romano
Facultad de Farmacia y Bioquímica
Universidad de Buenos Aires
Junín 956 (1113)
Buenos Aires
Argentina
aromano@huemul.ffyb.uba.ar