Pergamon

Biochemical Pharmacology, Vol. 47, No. 9, pp. 1587–1592, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)E0021-C

CYTOTOXIC ACTIVITY OF N^1- AND N^8-AZIRIDINYL ANALOGS OF SPERMIDINE

ZHI-MIN YUAN,* D. MARC ROSEN,† MERRILL J. EGORIN†‡ and PATRICK S. CALLERY*§

*Department of Pharmaceutical Sciences, School of Pharmacy, and †Division of Developmental Therapeutics, University of Maryland Cancer Center and School of Medicine, University of Maryland, Baltimore, MD 21201; and ‡Baltimore Veterans Administration Hospital, Baltimore, MD 21201, U.S.A.

(*Received* 13 *July* 1993; *accepted* 3 *December* 1993)

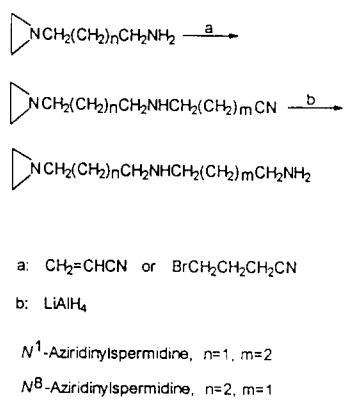
Abstract—Two isomeric aziridine-containing analogs of spermidine, a polyamine, were synthesized and evaluated for cytotoxic activity against cancer cell lines. Replacement of one of the primary amino groups of spermidine with an aziridinyl functionality yielded either N^1-aziridinylspermidine [*N*-(3-aziridinylpropyl)-1,4-diaminobutane] or N^8-aziridinylspermidine [*N*-(4-aziridinylbutyl)-1,3-diaminopropane]. N^1-Aziridinylspermidine was cytotoxic *in vitro* against L1210 murine leukemia cells (IC_{50} 0.15 μM) and HL60 human leukemia cells (IC_{50} 0.11 μM). N^8-Aziridinylspermidine was slightly less potent against L1210 (IC_{50} 0.31 μM) and HL60 (IC_{50} 0.30 μM) cells. When screened by the Developmental Therapeutics Program of the National Cancer Institute, these compounds proved cytotoxic against a wide variety of tumor types. Both compounds inhibited incorporation of radiolabeled thymidine, uridine, and valine into trichloroacetic acid-precipitable material by L1210 cells. Aminoguanidine did not affect the potency of the aziridinylspermidines.

Key words: polyamines; spermidine; aziridines; antineoplastics

Polyamine biochemistry is associated with cell growth and proliferation in part because interference with polyamine biosynthesis and/or function can lead to inhibition of cell growth or cell death [1, 2]. Tissues with a high demand for polyamines, such as tumors, accumulate polyamines effectively [3]. The polyamine homeostatic system is an obvious target for the design of new anticancer agents that can be concentrated in neoplastic cells and tissues by utilizing the polyamine transporter.

The naturally occurring polyamines, which include putrescine, spermidine and spermine, have been chemically modified to yield a number of agents that perturb polyamine biosynthesis, transport, or function [4–13]. For example, replacement of an amino group of putrescine with aziridine (ethylenimine), a ring-strained alkylating functional group that reacts covalently with cellular nucleophiles such as DNA [14, 15], produces the analog *N*-(4-aminobutyl)aziridine [16]. *N*-(4-Aminobutyl)-aziridine has been shown to be cytotoxic against prostatic carcinoma cells [17], as well as a potent reversible inhibitor of the polyamine transporter [18] and an irreversible inhibitor of diamine oxidase [19].

Other polyamines covalently linked with an alkylating functional group, which target tumor cells and DNA, have been reported, including a chlorambucil-spermidine conjugate [12, 13]. Analogues containing an alkylating group attached to the carbon alpha to the secondary amine of spermidine were not substrates of the difluoromethylornithine-inducible polyamine transporter [11]. In another example, cyclic cyclophosphazenes containing combinations of polyamines and aziridine have been found to show antitumor activity [9].



Scheme 1. Synthesis of N^1-aziridinylspermidine and N^8-aziridinylspermidine.

In the present study, two aziridine-containing spermidine analogs, N^1-aziridinylspermidine [*N*-(3-aziridinylpropyl)-1,4-diaminobutane] and N^8-aziridinylspermidine [*N*-(4-aziridinylbutyl)-1,3-diaminopropane] were synthesized (Scheme 1) and assessed for cytotoxic activity.

MATERIALS AND METHODS

NMR and mass spectra of the synthesized

§ Corresponding author: Patrick S. Callery, Ph.D., School of Pharmacy, University of Maryland at Baltimore, 20 North Pine St., Baltimore, MD 21201. Tel. (410) 706-2980; FAX (410) 706-0346.

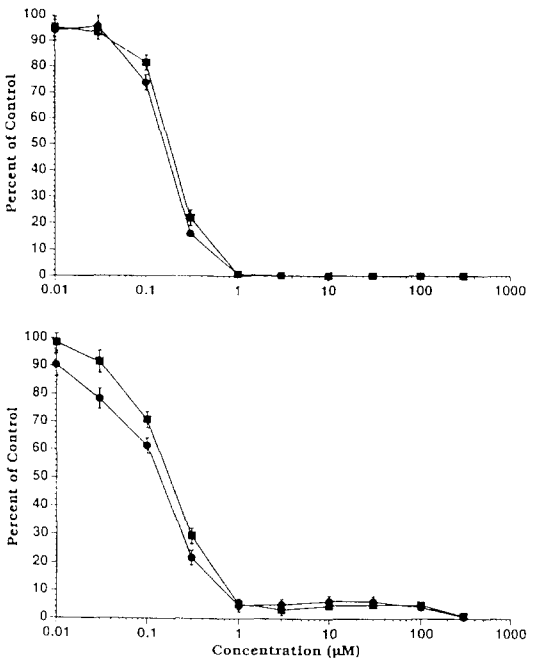


Fig. 1. Concentration–response curve for inhibition of growth by N^1-aziridinylspermidine in the presence (●—●) or absence (■—■) of 1.0 mM aminoguanidine against L1210 cells (upper graph) and HL60 cells (lower graph). Data are derived from representative experiments. Percentages were based on control incubations of 4.7×10^5 L1210 cells and 2.7×10^5 HL60 cells. Values are means ± SD, N = 3.

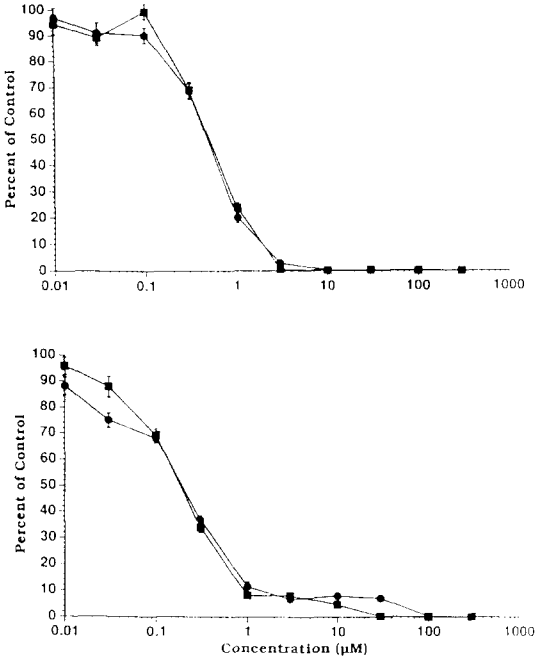


Fig. 2. Concentration–response curve for inhibition of growth by N^8-aziridinylspermidine in the presence (●—●) or absence (■—■) of 1.0 mM aminoguanidine against L1210 cells (upper graph) and HL60 cells (lower graph). Data are derived from representative experiments. Percentages were based on control incubations of 5.6×10^5 L1210 cells and 4.2×10^5 HL60 cells. Values are means ± SD, N = 3.

compounds were consistent with the assigned structures. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and were within 0.4% of the theoretical empirical formulas. Column chromatography separations were performed on silica gel 60 (70–230 mesh). [^{3}H]-Thymidine(2.0 Ci/mmol),[^{3}H]uridine(40 Ci/mmol), and [^{3}H]valine (61 Ci/mmol) were purchased from NEN Research Products (Boston, MA).

N-(3-*Aziridinylpropyl*)-1,4-*diaminobutane*. A solution of 4-bromobutyronitrile (3.0 g, 0.02 mol) in 5 mL of anhydrous tetrahydrofuran was added to a stirred solution of *N*-(3-aminopropyl)aziridine [19] (6.0 g, 0.06 mol) and triethylamine (6.1 g, 0.06 mol) in 40 mL of anhydrous tetrahydrofuran preheated to 40°. One hour later, the mixture was cooled to room temperature and then stirred overnight. After purification by column chromatography [silica gel, CH_2Cl_2:MeOH:$(C_2H_5)_3N$ = 9:1:0.1], the nitrile was reduced to the amine by $LiAlH_4$ [19]. The product was purified by distillation (0.85 g, 25% yield, b.p. 110°/0.15 mm).

N-(4-*Aziridinylbutyl*)-1,3-*diaminopropane*. *N*-(4-Aminobutyl)aziridine [19] (5.7 g, 0.05 mol) was added dropwise to stirred acrylonitrile (3.2 g, 0.06 mol) heated to 35–40°. The temperature was maintained throughout the addition period and for an additional 1 hr. The reaction mixture stood at room temperature until the starting material disappeared (72 hr) as monitored by TLC (silica gel, $CHCl_3$:MeOH:NH_4OH, 9:1:1). The product nitrile was separated and reduced to the amine with $LiAlH_4$ following the method of Conner *et al.* [19]. Purification by distillation yielded 3.1 g of product (36% yield, b.p. 108–110°/0.15 mm).

Cytotoxicity. L1210 murine lymphoblastic leukemic cells [20, 21] or human leukemic cells (HL60) [22] were maintained in logarithmic growth as suspension cultures in RPMI 1640 medium (GIBCO, Grand Island, NY) containing 15% fetal bovine serum (GIBCO) at 37°, 5% CO_2, 95% air and 95% humidity. Cells were treated with the aziridinyl compounds at concentrations ranging from 0.01 to 3000 μM with or without 1.0 mM aminoguanidine. Drug exposures were carried out for 72 hr, an incubation period during which logarithmic growth had been documented for control cells under the conditions of the experiment. Cell number and viability were determined by flow cytometric (FACS IV flow cytometer, Becton Dickinson Immunocytometry Systems, Mountainview, CA) assessment of fluorescein diacetate and propidium iodide fluorescent dyes, following the method of Ross *et al.* [23].

Antitumor screen. Compounds were tested by the Developmental Therapeutics Program at the

Table 1. IC_{50} Values for N^1-aziridinylspermidine, N^8-aziridinylspermidine, spermidine, and thiotepa against L1210 and HL60 cells

	IC_{50} (μM)			
	L1210 cells		HL60 cells	
	With AG	Without AG	With AG	Without AG
N^1-Aziridinylspermidine	0.16	0.15	0.16	0.11
N^8-Aziridinylspermidine	0.39	0.31	0.27	0.30
Spermidine	300.0	15.0	36.0	6.0
Thiotepa	2.4	2.4	2.5	2.5

Compounds were tested in RPMI 1640 medium containing 15% fetal bovine serum with, or without, 1.0 mM aminoguanidine (AG). Data represent mean values from two experiments, each performed in triplicate.

National Institutes of Health at a minimum of five concentrations at 10-fold dilutions using a 48 hr continuous exposure protocol [24]. Briefly, each cell line was inoculated onto microtiter plates. After a preincubation period of 24–48 hr, test agents were added in five 10-fold dilutions, and the culture was incubated for an additional 48 hr. Cell viability or cell growth end-point determinations were performed by *in situ* fixation of cells, followed by staining with a protein-binding dye, sulforhodamine B. The solubilized stain was measured spectrophotometrically to determine relative cell growth or viability.

Tritiated thymidine, uridine, and valine incorporation. L1210 cells were washed twice with phosphate-buffered saline (153 mM NaCl, 1.7 mM KH_2PO_4, 4.9 mM Na_2HPO_4, pH 7.2), resuspended to 1×10^6cells/mL in fresh RPMI 1640 medium containing 15% fetal bovine serum, and added to an equal volume of RPMI 1640 medium that contained 15% fetal bovine serum and the desired concentration of aziridinyl compounds. After incubation for 1, 4, 8 or 24 hr, respectively, the cells were incubated for 1 hr with 2 μCi of [^{3}H]thymidine, 4 μCi of [^{3}H]uridine, or 4 μCi of [^{3}H]valine. Cells were deposited onto a 25 mm glass fiber filter (GFC, Whatman International Ltd., Maidstone, U.K.) that had been pre-wetted with phosphate-buffered saline. Cells were then rinsed twice with 3 mL of ice-cold phosphate-buffered saline, followed by 3 mL of iced 5% trichloroacetic acid solution. After dehydration with 3 mL of iced methanol, the filters were placed into scintillation vials containing 0.5 mL of NCS tissue solubilizer (Amersham, Arlington Heights, IL) and incubated overnight at room temperature. Subsequently, 0.2 mL of glacial acetic acid and 5 mL of Ready Safe scintillation fluid (Beckman, Fullerton, CA) were added to each vial, and the trichloroacetic acid-precipitable radioactivity was counted.

RESULTS AND DISCUSSION

Two novel analogs of spermidine were synthesized, and both were found to be highly cytotoxic. The antiproliferative activities of the N^1- and N^8-aziridinylspermidine isomers were assessed by determining their IC_{50} values against two cancer cell lines, one of which was murine and the other human. N^1-Aziridinylspermidine was more potent than its N^8-isomer (IC_{50} 0.15 μM vs 0.31 μM) against L1210 cells (Figs. 1 and 2 and Table 1). An analogous cytotoxic profile was observed when these compounds were tested against HL60 cells (Figs. 1 and 2 and Table 1).

The flow cytometric method employed in our studies counted cells and identified individual cells as alive or dead, based on differential fluorescent staining. This allowed assignment of the activity of the aziridinyl compounds as being cytotoxic rather than cytostatic.

Spermidine was used as a positive control for cytotoxic activity throughout the experiments. The IC_{50} of spermidine for L1210 cells was approximately 300 μM in the presence of aminoguanidine and approximately 15 μM in the absence of aminoguanidine (Table 1). These results are in excellent agreement with values reported by Porter *et al.* [7]. Aminoguanidine, an inhibitor of amine oxidases [25], was added to incubation mixtures to minimize the potential contribution of the serum amine oxidases in the fetal bovine serum medium component [7, 26]. Whereas spermidine toxicity was reduced greatly by aminoguanidine, in the case of the aziridinyl analogues there was little difference in cytotoxicity in the presence or absence of aminoguanidine. Presumably, either the analogues are not substrates for the plasma amine oxidases that yield toxic end products, or the test compounds themselves are more toxic than their putative oxidative metabolites. Thiotepa is another aziridine-containing cytotoxic agent [27] that also exhibited unchanged cytotoxicity against L1210 and HL60 cells treated with aminoguanidine (Table 1).

Both aziridinylspermidine compounds were subjected to the National Cancer Institute investigational *in vitro* disease-oriented primary antitumor screen against a panel of 56 cell lines [24]. The screen was organized in subpanels representing leukemia, melanoma, and cancers of the lung, colon, kidney, ovary, and central nervous system. The results of the screen are shown in Table 2. Significant cytotoxic

Table 2. Inhibition of cell growth and lethal concentrations of N^1-aziridinylspermidine and N^8-aziridinylspermidine against a panel of fifty-six human tumor cell lines*

Cell lines	N^1-Aziridinylspermidine		N^8-Aziridinylspermidine	
	$Log_{10}GI50$†	$Log_{10}LC50$‡	$Log_{10}GI50$	$Log_{10}LC50$
Leukemia				
CCRP-CEM	−6.49	> −4.00	−6.84	−5.17
HL60(TB)	−6.08	−6.03	−6.91	−6.06
K-562	−6.18	> −4.00	−5.98	> −4.00
MOLT-4	−6.42	> −4.00	−6.44	−4.21
RPMI-8226	−5.64	> −4.00	−5.90	> −4.00
SR	−5.85	> −4.00	−5.79	> −4.00
Non-small cell lung cancer				
A549/ATCC	−5.58	> −4.00	−5.44	> −4.00
EKVX	−6.27	> −4.00	−5.93	−5.01
HOP-62	−6.68	−5.27	−6.00	−4.94
HOP-92	−6.55	> −4.00	−6.13	> −4.00
NCI-H226	−6.38	> −4.00	−5.95	−4.07
NCI-H23	−6.16	> −4.00	−5.72	> −4.00
NCI-H322M	−6.63	> −4.00	−6.04	−4.18
NCI-H460	−6.25	> −4.00	−5.43	> −4.00
NCI-H522	−5.76	> −4.00	−6.29	> −4.00
Colon cancer				
COLO 205	−6.52	−5.29	−6.45	−5.35
HCC-2998	−5.36	> −4.00	−5.61	> −4.00
HCT-15	−5.87	> −4.00	−5.62	> −4.00
HT29	−6.15	> −4.00	−5.67	−4.20
XM12	−5.66	> −4.00	−5.68	−4.51
SW-620	−6.32	> −4.00	−6.47	−5.18
CNS cancer				
SF-268	−6.29	> −4.00	−5.98	> −4.00
SF-295	−6.03	> −4.00	−5.27	> −4.00
SF-539	−6.84	−5.83	−6.89	−5.79
SNB-19	−6.22	> −4.00	−5.81	−4.51
SNB-75	−6.77	−4.64	−6.59	> −4.00
U251	−6.37	> −4.00	−5.97	−4.07
Melanoma				
LOX IMVI	−6.55	> −4.00	−6.20	−4.03
MALME-3M	−6.11	> −4.00	−5.86	−5.03
M14	−6.00	> −4.00	−5.77	> −4.00
SK-MEL-2	−6.66	> −4.00	−6.24	> −4.00
SK-MEL-28	−6.33	> −4.00	−5.90	−4.64
SK-MEL-5	−5.79	> −4.00	−5.77	−5.14
UACC-257	−5.30	> −4.00	−5.86	−4.54
Ovarian cancer				
ICROV1	−6.40	−4.23	−6.22	−5.19
OVCAR-3	−6.21	> −4.00	−5.80	−4.71
OVCAR-4	−6.69	−5.19	−6.59	−4.94
OVCAR-5	−5.67	> −4.00	−6.55	−4.41
OVCAR-8	−6.60	> −4.00	−6.14	−4.67
SK-OV-3	−6.48	> −4.00	−6.06	−4.82
Renal cancer				
786-0	−6.96	−5.21	−6.63	−5.40
A498	−7.72	−6.04	−8.00	−6.07
ACHN	−6.58	> −4.00	−6.44	−4.95
CAKI-1	−7.05	−5.17	−7.31	−5.61
RXF-393	−7.57	−4.77	−6.71	−5.43
SNI2C	−6.59	> −4.00	−6.17	−4.93
TK-10	−6.27	> −4.00	−6.05	> −4.00
Prostate cancer				
PC-3	−5.91	> −4.00	−5.63	> −4.00
DU-145	−6.05	> −4.00	−5.97	−4.79
Breast cancer				
MCF7/ADR-RES	−6.66	> −4.00	−6.07	−4.19
MDA-MB-231/ATCC	−6.61	> −4.00	−5.97	−5.07
HS 578T	−6.68	> −4.00	−6.50	> −4.00
MDA-MB-435	−6.41	> −4.00	−5.93	
MDA-N	−6.26	> −4.00	−5.88	
BT-549	−6.28	> −4.00	−5.88	−4.33
T-47D	−6.89	> −4.00	−7.02	> −4.00

* Compounds were tested by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.

† Mean log_{10} molar concentrations calculated at the point where the test compound achieved 50% inhibition of cell growth.

‡ Mean log_{10} molar concentrations calculated at the point where the test compound achieved 50% cell kill, or 50% lethal concentration.

Table 3. IC_{50} Values for inhibition of incorporation of [^{3}H]thymidine, [^{3}H]uridine, and [^{3}H]valine into trichloroacetic acid-precipitable material by L1210 cells

	Time (hr)	IC_{50} (μM)		
		[^{3}H]Thymidine	[^{3}H]Uridine	[^{3}H]Valine
N^1-Aziridinylspermidine	1	0.78	0.47	13.0
	4	0.45	0.42	2.6
	8	0.35	0.27	
	24	0.40	0.21	0.57
N^8-Aziridinylspermidine	1	3.30	2.40	14.4
	4	3.40	1.54	3.4
	8	2.80	1.30	
	24	2.10	1.00	2.6

Cells were exposed to [^{3}H]thymidine, [^{3}H]uridine, or [^{3}H]valine for 1 hr after incubation with N^1-aziridinylspermidine or N^8-aziridinylspermidine for 1, 4, 8, and 24 hr. Data represent mean values from two experiments, each performed in triplicate.

activity was observed for most of the cell lines. Both compounds are being re-screened, and the results will be evaluated to determine if the compounds have potential as antineoplastic agents that should be tested further.

In experiments with L1210 cells, radioisotopically labeled precursor incorporation data showed that the aziridinylspermidines inhibited both [^{3}H]thymidine and [^{3}H]uridine incorporation into DNA and RNA, respectively. These inhibitory effects were observable within 1 hr after exposure to the aziridinyl compounds (Table 3). The IC_{50} values for incubations of 1–24 hr (Table 3) were somewhat higher than those calculated in the cytoxicity studies where longer incubation periods (72 hr) were used (Table 1). It is not clear from these experiments whether or not DNA and/or RNA may be the target sites of the aziridinylspermidines because the relationship between inhibition of nucleoside incorporation into trichloroacetic acid-precipitable material and cell death is not well established. Inhibition of amino acid incorporation into acid-precipitable material was observed later than the inhibition of incorporation of thymidine or uridine. Between 4 and 24 hr, inhibition of [^{3}H]valine incorporation into cellular proteins was also observed (Table 3). Cell cycle specificity studies with the aziridinylspermidines have not been conducted.

N^1- and N^8-Aziridinylspermidine represent potent cytotoxic polyamine analogs consisting of an alkylating functionality connected to a polycationic chain. These compounds have the potential for reacting with DNA after accumulation in cells by a polyamine transporter. Work is underway to search for additional relationships of these compounds with polyamine biochemistry and DNA reactivity.

Acknowledgements—This work was supported by a Special Research Initiative Support award from the UMAB Designated Research Initiative Fund and by the Office of Research Development, Department of Veterans Affairs. Data from antitumor screens by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, are gratefully acknowledged.

REFERENCES

1. Tabor CW and Tabor H, Polyamines. *Annu Rev Biochem* **53**: 749–790, 1984.
2. Pegg AE, Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res* **48**: 759–774, 1988.
3. Seppanen P, Some properties of the polyamine deprivation-inducible uptake system for methylglyoxal-bis(guanylhydrazone) in tumor cells. *Acta Chem Scand B* **35**: 731–736, 1981.
4. Porter CW, Bergeron RJ and Stolowich NJ, Biological properties of N^4-spermidine derivatives and their potential in anticancer activity. *Cancer Res* **42**: 4072–4078, 1982.
5. Porter CW, Miller J and Bergeron RJ, Aliphatic chain length specificity of the polyamine transport system in ascites L1210 leukemia cells. *Cancer Res* **44**: 120–128, 1984.
6. Nagarajan S and Ganem B, Chemistry of naturally occurring polyamines. 10. Nonmetabolizable derivatives of spermine and spermidine. *J Org Chem* **51**: 4856–4861, 1986.
7. Porter CW, McManis J, Casero RA and Bergeron RJ, Relative abilities of bis(ethyl)derivatives of putrescine, spermidine, and spermine to regulate polyamine biosynthesis and inhibit L1210 leukemia cell growth. *Cancer Res* **47**: 2821–2825, 1987.
8. Heston WDW, Watanabe KA, Pankiewicz KW and Covey DF, Cytotoxic and non-cytotoxic *N*-alkyl derivatives of putrescine: Effect on polyamine uptake and growth of prostatic cancer cells *in vitro*. *Biochem Pharmacol* **36**: 1849–1852, 1987.
9. Darcel F, Chatel M, Gautris P and Labarre JF, The antitumoral action of polyamine linked cyclophosphazenes on human malignant glioma heterografts in nu/nu mice. *Anticancer Res* **10**: 1563–1570, 1990.
10. Lakanen JR, Coward JK and Pegg AE, α-Methyl polyamines: Metabolically stable spermidine and spermine mimics capable of supporting growth in cells depleted of polyamines. *J Med Chem* **35**: 724–734, 1992.
11. Stark PA, Thrall BD, Meadows GG and Abdel-Monem MM, Synthesis and evaluation of novel spermidine derivatives as targeted cancer chemotherapeutic agents. *J Med Chem* **35**: 4264–4269, 1992.
12. Cohen GM, Cullis PM, Hartley JA, Mather A, Symons MCR and Wheelhouse RT, Targeting of cytotoxic

agents by polyamines: Synthesis of a chlorambucil-spermidine conjugate. *J Chem Soc Chem Commun* 298–300, 1992.
13. Holley JL, Mather A, Wheelhouse RT, Cullis PM, Hartley JA, Bingham JP and Cohen GM, Targeting of tumor cells and DNA by a chlorambucil-spermidine conjugate. *Cancer Res* **52**: 4190–4195, 1992.
14. Hemminki K, Kallama S and Falck K, Correlations of the alkylating activity and mutagenicity in bacteria of cytotoxic drugs. *Acta Pharmacol Toxicol* **53**: 421–428, 1983.
15. Musser SM, Pan S-S, Egorin MJ, Kyle DJ and Callery PS, Alkylation of DNA with aziridine produced during the hydrolysis of *N,N',N''*-triethylenethiophosphoramide. *Chem Res Toxicol* **5**: 95–99, 1992.
16. Piper JR, Stringfellow CR, Elliott RD and Johnston TP, *S*-2-(ω-Aminoalkylamino)ethyl dihydrogen phosphorothioates and related compounds as potential antiradiation agents. *J Med Chem* **12**: 236–243, 1969.
17. Heston WDW, Yang C-R, Pliner L, Russo P and Covey DF, Cytotoxic activity of a polyamine analogue, monoaziridinylputrescine, against the PC-3333 human prostatic carcinoma cell line. *Cancer Res* **47**: 3627–3631, 1987.
18. O'Sullivan MC, Golding BT, Smith LL and Wyatt I, Molecular features necessary for the uptake of diamines and related compounds by the polyamine receptor of rat lung slices. *Biochem Pharmacol* **41**: 1839–1848, 1991.
19. Conner JW, Yuan Z-M and Callery PS, Active-site directed irreversible inhibition of diamine oxidase by a homologous series of aziridinylalkylamines. *Biochem Pharmacol* **44**: 1229–1233, 1992.
20. Geran RJ, Greenberg NH, MacDonald MM, Schumacher AM and Abbott BJ, Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother Rep* **3** (Part III): 7, 1972.
21. Egorin MJ, Odders RN, Sanel FT, Felsted RL and Bachur NR, Inhibitory effects of phytohemagglutinin isolectins L_4 and E_4 on L1210 cells. *Cancer Res* **38**: 1677–1687, 1978.
22. Collins SJ, Gallo RC and Gallagher RE, Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. *Nature* **270**: 347–349, 1978.
23. Ross DD, Joneckis CC, Ordóñez JV, Sisk AM, Wu RK, Hamburger AW and Nora RE, Estimation of cell survival by flow cytometric quantification of fluorescein diacetate/propidium iodide viable cell number. *Cancer Res* **49**: 3776–3782, 1989.
24. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J and Boyd M, Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer Inst* **83**: 757–766, 1991.
25. Tamura H, Horiike K, Fukuda H and Watanabe T, Kinetic studies on the inhibition mechanism of diamine oxidase from porcine kidney by aminoguanidine. *J Biochem (Tokyo)* **105**: 299–306, 1989.
26. Averill-Bates DA, Agostinelli E, Przybytkowski E, Mateescu MA and Mondovi B, Cytotoxicity and kinetic analysis of purified bovine serum amine oxidase in the presence of spermine in Chinese hamster ovary cells. *Arch Biochem Biophys* **300**: 75–79, 1993.
27. Miller B, Tenenholz T, Egorin MJ, Sosnovsky G, Rao NUM and Gutierrez PL, Cellular pharmacology of *N,N',N''*-triethylene thiophosphoramide. *Cancer Lett* **41**: 157–168, 1988.