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A Study of the Antitumor Activity of Four Dibutyltin(IV)-*N*-arylidene-α-amino Acid Complexes

Nwaka Ogwuru, Lian E. Khoo and George Eng
[1]Department of Chemistry and Physics, University of the District of Columbia, 4200 Connecticut Ave., N.W. Washington, DC 20008, USA
[2]National Institute of Education, Nanyang Technological University, 469 Bukit Timah Road, Singapore 1025, Republic of Singapore

This paper reports the activity of four dibutyltin(IV)–*N*-arylidene-α-amino acid complexes against the National Cancer Institute (NCI) panel of 60 cell lines. The results indicated that three of the organotin complexes ($C_{17}H_{25}NO_3Sn$, $C_{18}H_{27}NO_3Sn$ and $C_{20}H_{31}NO_3Sn$) exhibit their highest cytotoxic effect on the NCI-522 (non-small cell lung cancer) cell line. The fourth complex, $C_{21}H_{27}NO_3Sn$, exhibits its highest cytotoxic activity on the cell line RXF-631L (renal cancer). In general, a low to moderate cellular response was observed for all the organotin complexes, with at least one cell line in each subpanel of cells exhibiting a very low growth inhibition response to all the organotin complexes. The low-responding cell lines included HOP-62 (non-small lung cancer), DLD-1 (colon cancer), SF-539 (CNS cancer), SK-MEL-5 (melanoma), IGROV-1 (ovarian cancer) and RPMI-8226 (leukemia). The results also indicated that the compounds did not exhibit any significant subpanel activity and suggested that the compounds were not active in all the cell lines contained in any subpanel. The low to moderate activity of these compounds across the cell lines was attributed to the presence of nitrogen-bearing ligands which prevented the dissociation of the compound and the subsequent binding to DNA. © **1998 John Wiley & Sons, Ltd.**

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* Correspondence to: Dr. George Eng, Department of Chemistry and Physics, University of the District of Columbia, 4200 Connecticut Ave., N.W. Washington, DC 20008, USA.

INTRODUCTION

Organotin compounds have a wide range of applications, which include their use as therapeutics for the treatment of viral diseases and certain tumors. A number of organotin compounds have been found to be active *in vivo* and *in vitro* against P388 lymphocytic leukemia tumor.[1,2] Antitumor properties have been reported for various octahedral diorganotin dihalide complexes, $R_2SnX_2{\cdot}L_2$, where L_2 is generally a bidentate ligand.[3–5] Some organotin compounds have selective effects on lymphocytes, as observed with the di-[6–8] and trialkyltin compounds.[9,10] The selective effects of these organotin compounds can be used in cancer chemotherapy or to control other pathological effects. To this end, four dibutyltin(IV)–*N*-arylidene-α-amino acid complexes were tested across a panel of 60 cell lines to establish the disease-oriented activity for these compounds. The results are reported herein.

EXPERIMENTAL

The antitumor screenings were carried out on a panel of 60 human tumor cell lines derived from seven cancer types [leukemia, lung, colon, central nervous system (CNS), melanoma, ovarian and renal cancers]. Cell lines derived from a particular type of cancer are classified as belonging to the same subpanel. The cell lines for which results are reported here were:

Leukemia: CCRF-CEM, HL-60 TB, K562, MOLT-4 and RPMI-8226.
Non-small cell lung cancer: A-549, EKVX, HOP-18, HOP-62, HOP-92, NCI-H23, NCI-H322, NCI-H460, NCI-H522 and LXFL-529 L.
Small cell lung cancer: DMS-114 and DMS-273.
Colon cancer: COLO-205, DLD-1, HCC-2998,

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Table 1 The dibutyltin–*N*-arylidene-α-amino acid complexes ($OArCR''=NCHR'CO_2)SnBu_2$ screened against the NCI panel of cell lines[a]

Compound	Formula	Ar	R′	R″
I	$C_{17}H_{25}NO_3Sn$	C_6H_4	H	H
II	$C_{18}H_{27}NO_3Sn$	C_6H_4	H	CH_3
III	$C_{20}H_{31}NO_3Sn$	C_6H_4	i-C_3H_6	H
IV	$C_{21}H_{27}NO_3Sn$	$C_{10}H_8$	H	H

[a] The structure of compound **III** has been determined by X-ray analysis (Ref. 11)

HCT-116, HCT-15, HT-29, KM-12, KM-20L2 and SW-620.
CNS cancer: SF-268, SF-295, SF-539, SNB-19, SNB-75, SNB-78, U251 and XF-498 L.
Melanoma: LOX-IMVI, MALME-3M, M14, ME19-MEL, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257 and UACC-62.
Ovarian cancer: IGROV-1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8 and SK-OV-3.
Renal cancer: 786-O, A498, ACHN, CAKI-1, RXF-393 L, RXF-631 L, SN-12C, TK-10 and UO-31.

The four dibutyltin(IV)–*N*-arylidene-α-amino acid complexes were synthesized according to previously published methods[11] and are listed in Table 1. The complexes have been previously characterized using ^{1}H and ^{13}C NMR and IR spectroscopies.[11] In addition, the structure for compound **III** has been previously determined by X-ray analysis.[11] The compounds were tested by the Division of Cancer Treatment, National Cancer Institute (NCI), Bethesda, MD 20014, USA. The NCI protocol for the 48-h continuous drug exposure method, utilizing a high initial inoculum of tumor cells, was used to assess the antitumor activity of the dibutyltin(IV)–*N*-arylidene-α-amino acid complexes.[12] The cells were inoculated into a series of standard 96-well microtiter plates (20 000 cells/well) and preincubated for 24 h. The test compounds were added after the preincubation period in five 10-fold dilutions (10^{-8} M to 10^{-4} M). The cells were incubated for 48 h, then fixed with trichloroacetic acid and washed several times with deionized water. The dye sulforhodamine B (SRB)[13] was added and the cells were washed and dried. Cell viability was determined by solubilizing the bound dye and determining the concentration spectrophotometrically at 564 nm.

RESPONSE PARAMETERS

Under assay conditions *in vitro*, exposure to a chemical agent may decrease the number of viable cells either by direct killing or by decreasing the rate of cell proliferation and growth. The viability of the cells is measured by the response parameters (LC_{50}, GI_{50} and TGI). The LC_{50} is the concentration at which only 50% of the cells are viable (percentage growth is −50), the GI_{50} value is the concentration that yields 50% growth (percentage growth is +50), and total growth inhibition (TGI) is the concentration at which no growth is observed (percentage growth is 0).

The statistics program COMPARE was used to detect differences and similarities among the mean graph fingerprints of the four compounds[14] and was also used to calculate the differential sensitivities for the compounds.

Mean graph representations

The mean graphs (Figs 1–4) compare the relative cell line sensitivities derived from the LC_{50}, GI_{50} and TGI results for each cell line. The bars projecting to the right represent a logarithmic increase in the sensitivity of the cell line to the test agent in excess of the average sensitivity. Bars projecting to the left represent a logarithmic decrease in cellular sensitivity.

Differential cellular sensitivity

The differential sensitivity (Δ) represents the mean cyctotoxic potency of a compound over the entire panel of 60 cell lines and can be calculated for each response parameter using the equation:

$$\Delta = \log_{10}(1/X) - \text{mean } [\log_{10}(1/X)]$$

where $X = LC_{50}$, GI_{50} or TGI.[15] Values lower than unity for the differential sensitivity response represent a low sensitivity of the compound to the panel of cells, values between 1 and 3 represent moderate sensitivity, and values that are greater than 3 represent high sensitivity of the compound to the panel of cells.

Differential subpanel sensitivity

The differential subpanel sensitivity values (D_{LC50}, D_{GI50} and D_{TGI}) represent the mean subpanel potency exhibited by a compound based on the calculated LC_{50}, GI_{50} and TGI values. A result of

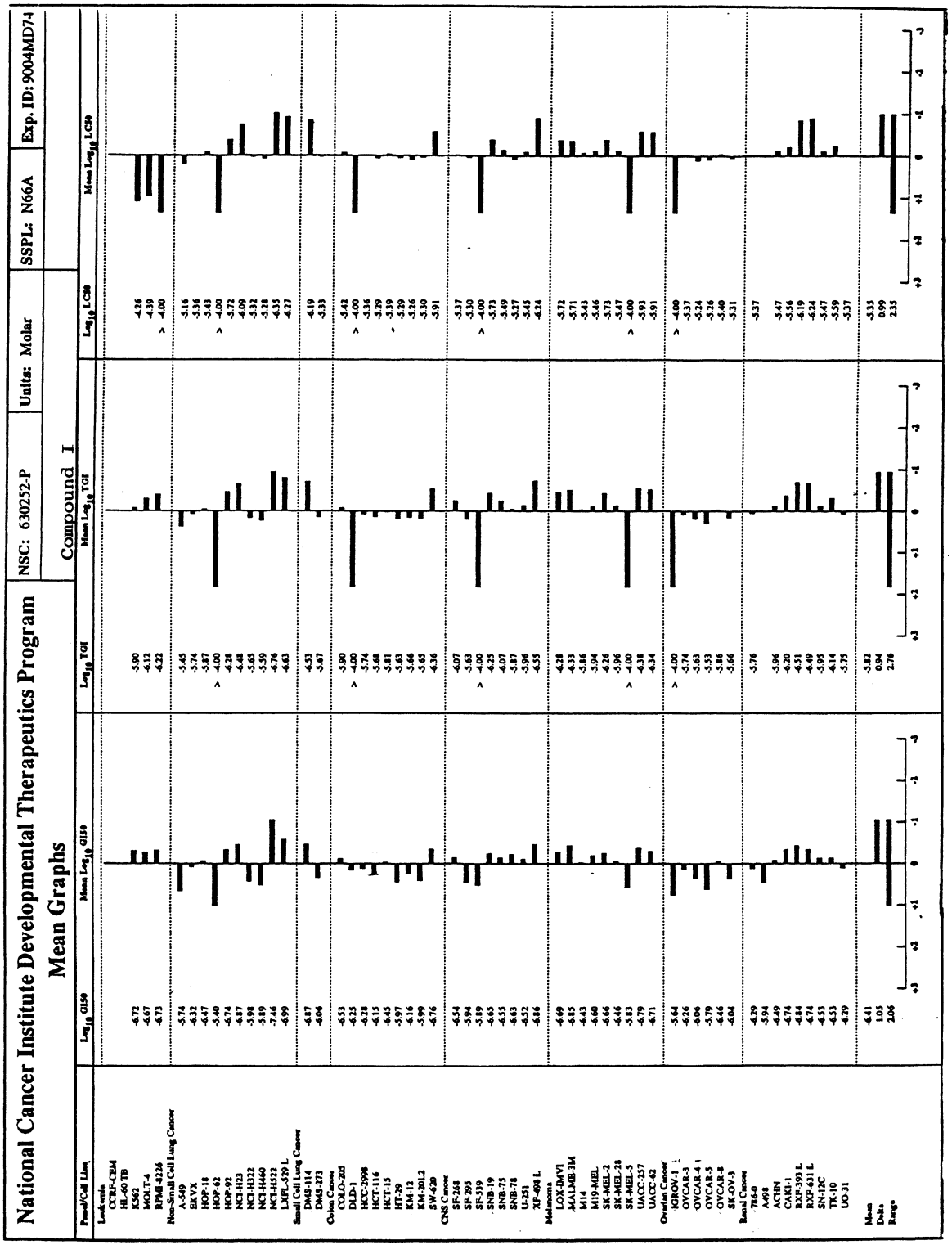

Figure 1 Mean graph presentation of the differential data for compound **I**.

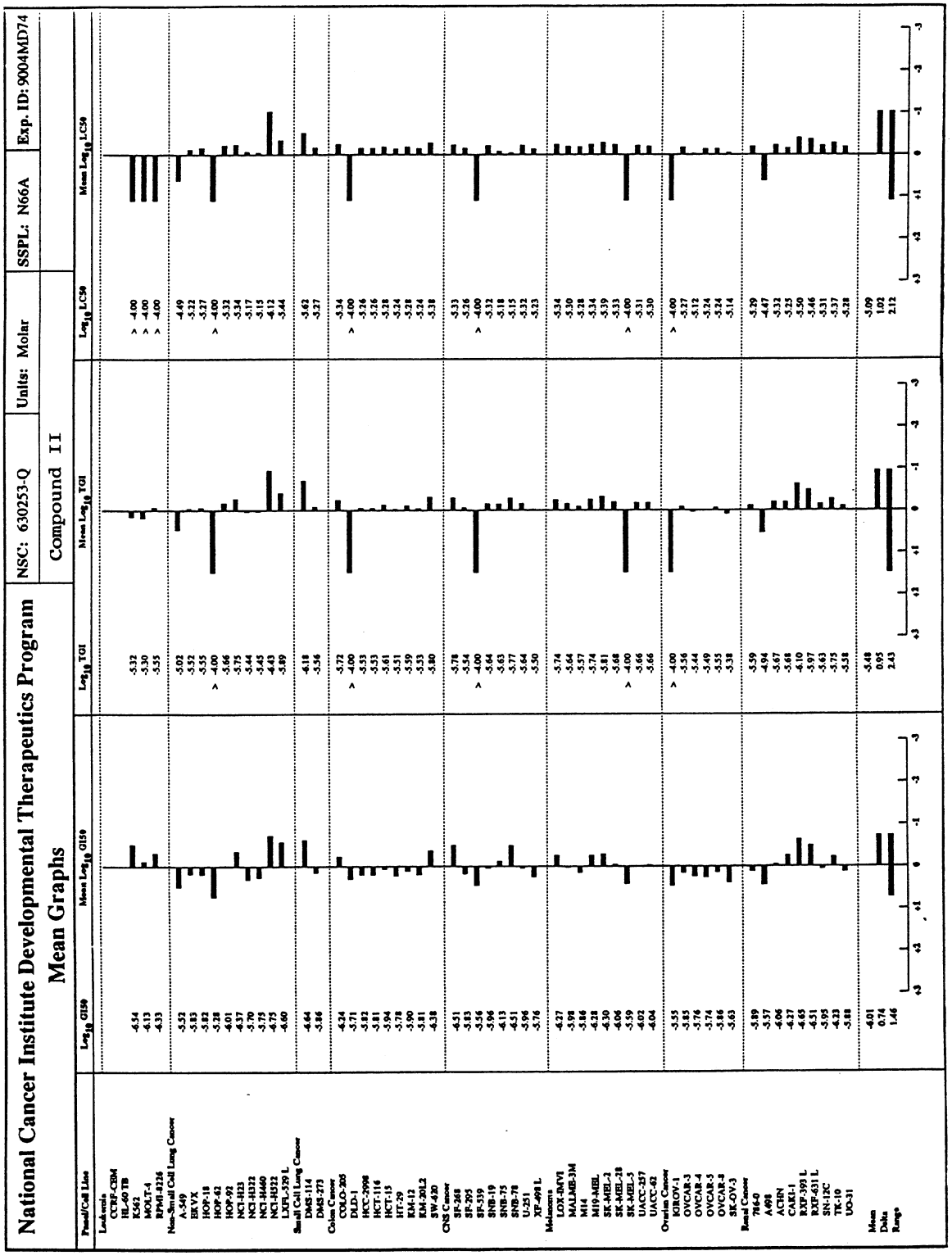

Figure 2 Mean graph presentation of the differential data for compound **II**.

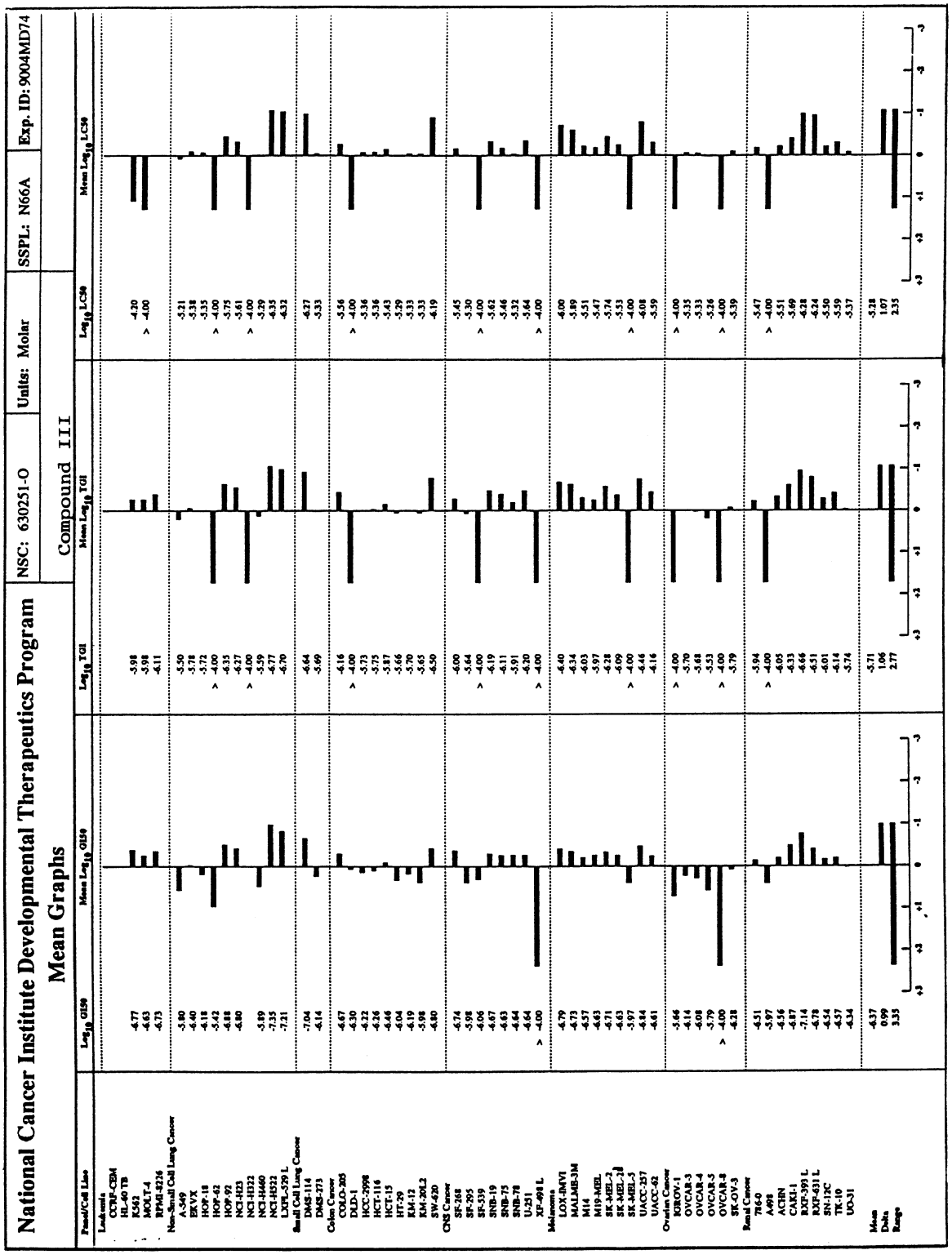

Figure 3 Mean graph presentation of the differential data for compound **III**.

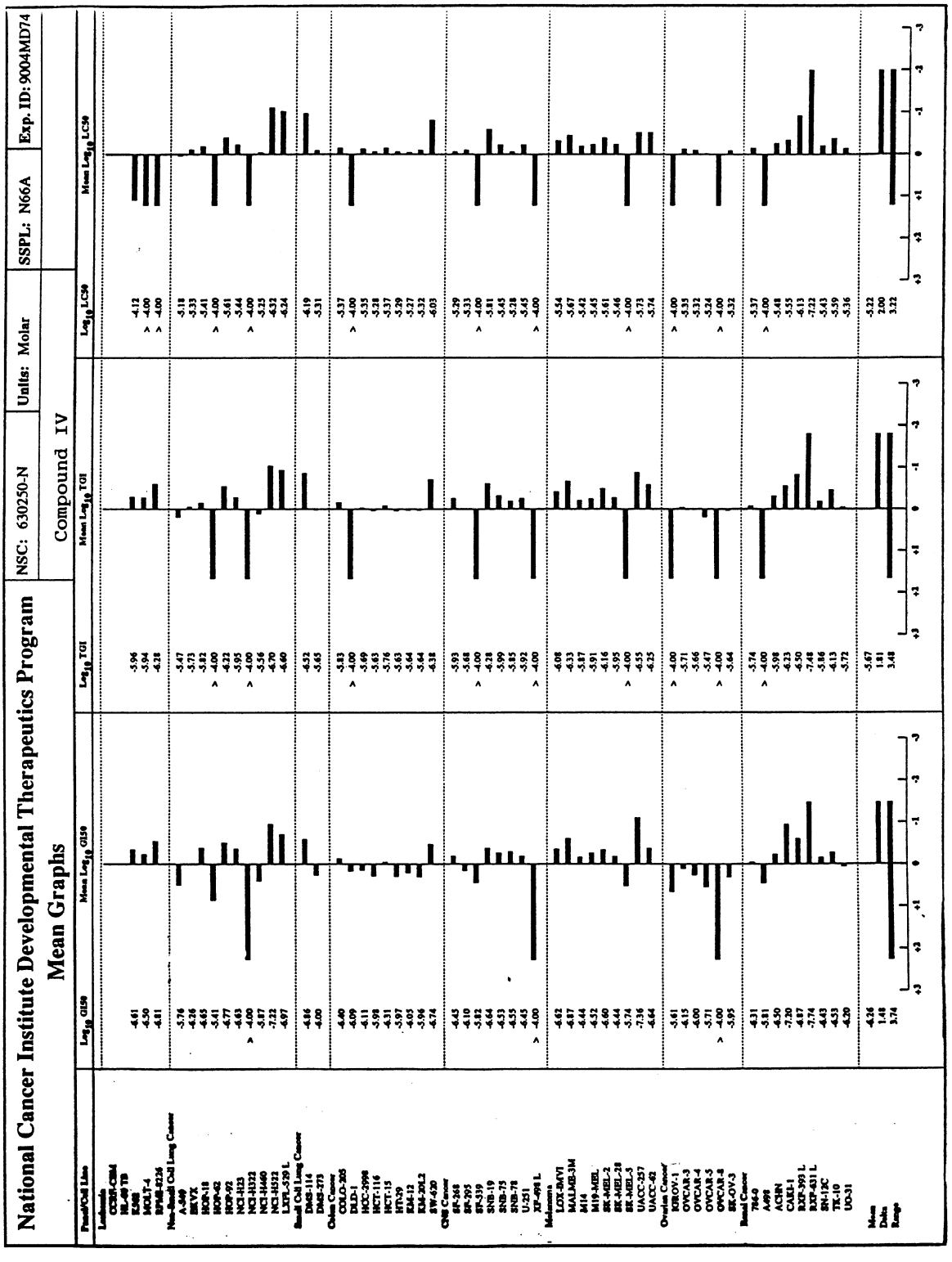

Figure 4 Mean graph presentation of the differential data for compound **IV**.

Table 2 The mean response concentrations (M) for the four dibuyltin(IV)–*N*-arylidene-α-amino acid complexes screened against the panel of cell lines

Mean response parameter	Compound **I**	Compound **II**	Compound **III**	Compound **IV**
GI_{50}	3.98×10^{-7}	1.00×10^{-6}	3.98×10^{-7}	5.01×10^{-7}
TGI	1.58×10^{-6}	3.16×10^{-6}	2.00×10^{-6}	2.00×10^{-6}
LC_{50}	3.98×10^{-6}	7.94×10^{-6}	5.01×10^{-6}	6.31×10^{-6}

Table 3 The differential sensitivities (Δ) for the various dibutyltin(IV)–*N*-arylidene-α-amino acid complexes to the panel of cell lines

Differential cellular sensitivity	Compound **I**	Compound **II**	Compound **III**	Compound **IV**
GI_{50}	1.1	0.7	1.0	1.5
TGI	0.9	1.0	1.1	1.8
LC_{50}	1.0	1.0	1.1	2.0

Table 4 Subpanel specificities for the four dibutyltin(IV)–*N*-arylidene-α-amino acid complexes

Subpanel Specificity	Compound **I**	Compound **II**	Compound **III**	Compound **IV**
D_{GI50}	23.5	6.4	44.4	33.3
D_{TGI}	30.8	46.3	43.3	33.3
D_{LC50}	38.9	18.5	37.0	40.7
D_H	67.3	73.5	66.0	51.9

50 or larger indicates a significant subpanel effect for that compound.

The D_H value confirms the measure of subpanel sensitivity. Values greater than 75 indicate a significant subpanel or selective effect for the compound.

RESULTS

The mean concentrations required to achieve GI_{50}, TGI and LC_{50} levels for the four compounds against the panel of cells tested are given in Table 2. Compound **I** was found to be the most cytotoxic compound, where the GI_{50}, TGI and LC_{50} concentrations were 3.98×10^{-7} M, 1.58×10^{-6} M and 3.98×10^{-6} M, respectively. The least cytotoxic compound was compound **II**, where the GI_{50}, TGI and LC_{50} concentrations were 1.00×10^{-6} M, 3.16×10^{-6} M and 7.94×10^{-6} M, respectively.

The four dibutyltin(IV)–*N*-arylidene-α-amino acid complexes exhibited a low to moderate response to all the differential sensitivity parameters (Table 3), since the values were less than 3. This indicates that the compounds only inhibited cellular growth at a low to moderate rate (from the GI_{50} and TGI parameters), and were at most moderately cytotoxic (from the LC_{50} parameter). From the results in Table 3, compound **IV** was active against more cell lines from different subpanels than the other three compounds, since its differential cellular sensitivity values were higher.

The response of each compound to the subpanels of cells was also evaluated. The results are reported in Table 4 as subpanel specificities using the four parameters D_{GI50}, D_{TGI}, D_{LC50} and D_H. None of the four compounds tested exhibited significant subpanel specificities as indicated by the D_{GI50}, D_{TGI} and D_{LC50} parameters, since their values were less than 50. The D_H values for the compounds confirm these findings, since their values were less than 75.

DISCUSSION

For compound **I**, the differential cellular sensitivity was low to moderate for the cell lines tested (Table 3) and the compound did not exhibit a specific activity towards any particular subpanel of cells, as indicated by Table 4. However, a higher than average response was observed for this compound in the cell line NCI-H522 (non-small cell lung

cancer), where the concentrations required to achieve the GI_{50}, TGI and LC_{50} values were one-tenth of the average concentration for the cell lines (Fig. 1).

A low to moderate differential cellular response was also observed for compound **II** and there was no subpanel selective effect. This compound did not exhibit any cytotoxic effect in the leukemia subpanel of cells at the maximum dose tested (Fig. 2).

The differential cellular sensitivity of the cell lines toward compound **III** was moderate. Even though no subpanel specificity was observed for this compound, some cell lines were particularly sensitive to it. These cell lines included the NCI-H522, LXFL-529L (non-small cell lung cancer) and RXF-393L (renal cancer), where the LC_{50} concentrations were one-tenth of the mean LC_{50} concentration (Fig. 3). In addition, two cell lines exhibited very low sensitivities to this compound: the OVCAR-8 (ovarian cancer) and SF-498L (CNS cancer) cell lines exhibited a growth rate of more than 50% at the maximum dose tested.

For compound **IV**, the differential cellular sensitivity was also moderate. As with the other compounds, no subpanel specificity was observed. However, the highest cytotoxic response was recorded by this compound toward the RXF-631L (renal cancer) cell line (Fig. 4). The higher differential cellular sensitivity may be explained by the increased lipophilicity of this compound when compared with the other compounds tested. This may allow the compound to pass through the cell membranes more effectively. The total growth inhibition was observed at concentrations less than one-hundredth of the mean LC_{50} and TGI concentrations (Fig. 4). The effect of compound **IV** on NCI-H522 was similar to the response observed with compounds **I** and **III** for the same cell line. The lowest cellular response to compound **IV** was observed with NCI-H322 (non-small cell lung cancer), OVCAR-8 and SF-498L cell lines. The growth rate in these cell lines was more than 50% at the highest tested concentration.

SUMMARY

There was no subpanel selective effect observed for any of the four dibutyltin(IV)–-*N*-arylidene-α-amino acid complexes. At least one cell line in each subpanel exhibited a low response (TGI and LC_{50} parameters) to the four compounds tested. These cell lines included HOP-62 (non-small cell lung cancer), DLD-1 (colon cancer), SF-539 (CNS cancer), SK-MEL-5 (melanoma), IGROV-1 (ovarian cancer) and RPMI-8226 (leukemia), where the growth rates were greater than 50% at the highest tested dose. Unlike the activity reported for other organotin compounds which are active against leukemia cell lines,[1] the dibutyltin(IV)–*N*-arylidene-α-amino acid complexes showed very little cytotoxic effect in the leukemia cell lines tested.

The low to moderate activity of the complexes against most of the cell lines may be attributed to the presence of the nitrogen-bearing ligand, which increases the stability of the compounds[16] and is believed to reduce the activity of these compounds because its dissociation, which is necessary for the binding of the organotin to DNA, is hindered.[16] It may also be due to the fact that these compounds are not very hydrophilic, and are prevented from forming a biologically effective intermediate.

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