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Preclinical report

Methyl protogracillin (NSC-698792): the spectrum of cytotoxicity against 60 human cancer cell lines in the National Cancer Institute's anticancer drug screen panel

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Methyl protogracillin (NSC-698792) was a furostanol saponin isolated from the rhizome of *Dioscorea collettii* var. *hypoglauca* (Dioscoreaceae), a Chinese herbal remedy for the treatment of cervical carcinoma, carcinoma of urinary bladder and renal tumor for centuries, in our previous studies. In order to systematically evaluate its potential anticancer activity, methyl protogracillin was tested for its cytotoxicity *in vitro* against 60 human cancer cell lines in the National Cancer Institute (NCI)'s anticancer drug screen. As a result, it was found that methyl protogracillin was cytotoxic against all the tested cell lines from leukemia and solid tumors in the NCI's human cancer panel; it showed particular selectivity against one colon cancer line (KM12), one central nervous system (CNS) cancer line (U251), two melanoma lines (MALME-3M and M14), two renal cancer lines (786-0 and UO-31) and one breast cancer line (MDA-MB-231) with $GI_{50} \leqslant 2.0$ μM. The selectivity between these seven most sensitive lines and the least sensitive line (CCRF-CEM) ranged from 26- to 56-fold. In the same cancer subpanel, selectivity more than 15-fold was observed between MDA-MB-231 and MCF-7, NCI-ADR-RES, BT-549 in breast cancer. From a general view of the mean graph, CNS cancer is the most sensitive subpanel, while ovarian cancer and renal cancer are the least sensitive subpanels. Based on an analysis of the COMPARE computer program with methyl protogracillin as a seed compound, no compounds in the NCI's anticancer drug screen database have similar cytotoxicity patterns (mean graph) to that of methyl protogracillin, indicating a potential novel mechanism of the anticancer action involved. [© 2001 Lippincott Williams & Wilkins.]

Key words: cytotoxicity, *Dioscorea collettii* var. *hypoglauca*, methyl protogracillin, NCI's anticancer drug screen, NSC-698792.

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Introduction

The rhizome of the medicinal plant *Dioscorea collettii* var. *hypoglauca* (Dioscoreaceae) has been a Chinese herbal remedy for the treatment of cervical carcinoma, carcinoma of urinary bladder and renal tumor for centuries. It was included in the 1985, 1990 and 1995 versions of *The Pharmacopoeia of the People's Republic of China*. By bioactivity-guided isolation with *Pyricularia oryzae* (a phytopathogenic fungus responsible for rice blast) assay[1] in our previous studies, three spirostanol saponins,[2] nine furostanol saponins[3,4] and two pregnane glycosides,[5,6] i.e. a total of 14 steroidal saponins, were isolated from the rhizome of *D. collettii* var. *hypoglauca*, and showed antifungal activity by inducing morphological deformation of mycelia and conidia of *P. oryzae*. Among them, 11 steroidal saponins were cytotoxic against human acute myeloid leukemia K562 (AML) cell line *in vitro*.[2,3]

In our continuous investigation of the potential anticancer activity of steroidal saponins from the rhizome of *D. collettii* var. *hypoglauca*, methyl protogracillin (NSC-698792), a furostanol saponin, was tested by the National Cancer Institute (NCI)'s anticancer drug discovery screen, which is an *in vitro* disease-oriented screening system with a panel of 60 human cancer cell lines from leukemia, non-small cell lung cancer (NSCLC), colon cancer, central nervous system (CNS) cancer, prostate cancer, melanoma, ovarian cancer, renal cancer and breast cancer.[7–9] Most cell lines were from solid tumors and a few from leukemia. This paper presents the cytotoxicity spectrum of methyl protogracillin against the NCI's 60 human cancer cell lines *in vitro*.

Materials and methods

Chemicals

Methyl protogracillin was purified from the rhizome of *D. collettii* var. *hypoglauca* as previously described.[3] The stock solutions were prepared in 100% dimethyl sulfoxide (DMSO) and stored at -20°C.

The NCI's anticancer drug screen

A total of 60 human cancer cell lines were used for the NCI's anticancer drug screen, including the subpanels of leukemia, melanoma, and cancers of lung, colon, brain, ovary, renal, breast and prostate. These cell lines were adaptable to a single growth medium, and had reproducible profiles for growth and drug sensitivity.[7,10]

Briefly, each cell line, suspended in the cell culture medium, was inoculated onto 96-well tissue culture plates on day 0 and then incubated for 24 h. The stock solution of methyl protogracillin in DMSO was diluted at five concentrations, i.e. 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M, which were incubated with cells for a further 48 h. At the end of drug incubation, the cells were fixed *in situ*, washed and dried. Sulforhodamine B (SRB), a protein stain binding to basic amino acids of cellular macromolecules, was added, and followed by further washing and drying of the stained adherent cell mass.[11,12] The bound stain was solubilized with Tris buffer and then the optical density (OD) was measured spectrophotometrically on an automatic plate reader interfaced with a microcomputer at a single wavelength of 515 nm.

Percentage growth (PG) was calculated according to one or the other of the following two expressions. If $(\text{Mean OD}_{\text{test}} - \text{Mean OD}_{\text{tzero}}) \geq 0$, then $\text{PG} = 100 \times (\text{Mean OD}_{\text{test}} - \text{Mean OD}_{\text{tzero}})/(\text{Mean OD}_{\text{control}} - \text{Mean OD}_{\text{tzero}})$. If $(\text{Mean OD}_{\text{test}} - \text{Mean OD}_{(\text{tzero})}) < 0$, then $\text{PG} = 100 \times (\text{Mean OD}_{\text{test}} - \text{Mean OD}_{\text{tzero}})/\text{Mean OD}_{\text{tzero}}$. Where, Mean OD_{tzero} is the average of OD just before exposure of cells to the test compound, Mean OD_{test} is the average of OD after 48-h exposure of cells to the test compound and Mean $\text{OD}_{\text{control}}$ is the average of OD after 48 h without exposure of cells to the test compound. Table 1 and Figures 1 and 2 were derived from the PG data.

Results and discussion

Methyl protogracillin was tested at five concentrations, i.e. 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M. The PG was calculated based on the OD of SRB-derived color at each concentration (see Methods). Figure 1 presents the dose–response curve of methyl protogracillin against each cell line in the NCI's *in vitro* anticancer drug discovery screen by SRB assay.[11] These cell lines were from nine human human cancer subpanels, i.e. leukemia, NSCLC, colon cancer, CNS cancer, prostate cancer, melanoma, ovarian cancer, renal cancer and breast cancer. Each subpanel uses several cell lines. The dose–response curve is created by plotting the PG against the $\log_{10}$ of the corresponding drug concentration for each cell line by subpanel group. Three horizontal lines are provided at the PG values of +50, 0 and -50, respectively. Thus the molar drug concentrations corresponding to points where the curves cross these lines represent the interpolated values to cause 50% growth inhibition (GI_{50}), total growth inhibition (TGI) and 50% cell killing (LC_{50}), respectively. However, sometimes these response parameters cannot be obtained by interpolation. In such a case, the value given for each response parameter is the highest concentration tested and is preceded by a '>' sign. For methyl protogracillin, the response parameters above the highest test concentration (100 μM) are TGI against CCRF-CEM (leukemia), MOLT-4 (leukemia), HT29 (colon cancer), OVCAR-5 (ovarian cancer) and SN12C (renal cancer); and LC_{50} against CCRF-CEM, K-562 (leukemia), MOLT-4, PRMT-8226 (leukemia), EKVX, HT29, OVCAR-4 (ovarian cancer), OVCAR-5, SN12C and MCF-7 (breast cancer). The values of GI_{50} and LC_{50} are summarized in Table 1.

As shown in Table 1, methyl protogracillin exhibited cytotoxicity against all the test cell lines from leukemia and solid tumors with $GI_{50} < 100$ μM. Very strong cytotoxic effects are observed against one colon cancer line (KM12), one CNS cancer line (U251), two melanoma lines (MALME-3M and M14), two renal cancer lines (786-0 and UO-31) and one breast cancer line (MDA-MB-231) with $GI_{50} \leq 2.0$ μM, i.e. 1.75, 1.50, 1.75, 1.68, 1.90, 1.95 and 0.89 μM, respectively. Methyl protogracillin exhibited moderate cytotoxicity against two leukemia lines (CCRF-CEM and MOLT-4), four NSCLC lines (EKVX, HOP-62, NCI-H226 and NCH-H23), one CNS cancer line (SF-268), three melanoma lines (SK-MEL-2, SK-MEL-28 and SK-MEL-5), three ovarian cancer lines (OVCAR-3, OVCAR-4 and OVCAR-5), four renal cancer lines (A498, ACHN, CAKI-1 and TK-10) and three breast cancer lines (MCF-7, NCI/ADR-RES and BT-549) with GI_{50} from 10 to 50 μM, i.e. 50.2, 25.6, 16.5, 13.9, 14.7, 15.6, 12.4, 15.7, 11.1, 13.5, 16.2, 13.9, 12.4, 12.9, 14.0, 11.9, 16.5, 17.9, 16.4 and 17.9 μM, respectively. The TGI values are from 3 to 47 μM against the tested cell lines ($GI_{50} < 100$ μM) except CCRF-CEM, MOLT-4, HT-29, OVCAR-5 and SN12C with TGI > 100 μM, which are from 2- to 19-

Table 1. The cytotoxicity of methyl protogracillin against the NCI's 60 human cancer cell lines

Panel/cell line	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)
Leukemia			
CCRF-CEM	50.2	>100	>100
K-562	4.25	39.4	>100
MOLT-4	25.6	>100	>100
RPMT-8226	12.0	78.0	>100
NSCLC			
A549/ATCC	2.68	9.37	32.5
EKVX	16.5	43.0	>100
HOP-62	13.9	28.3	57.3
HOP-92	2.46	12.3	35.4
NCI-H226	14.7	30.6	63.8
NCI-H23	15.6	29.0	53.8
NCI-H322M	2.84	9.16	30.2
NCI-H460	3.40	16.2	48.2
NCI-H522	4.58	14.5	38.1
Colon cancer			
COLO 205	4.09	14.0	38.6
HCC-2998	6.47	21.6	51.9
HCT-116	5.50	18.2	45.1
HCT-15	3.28	11.2	39.3
HT29	9.24	>100	>100
KM12	1.75	3.13	5.60
CNS cancer			
SF-268	12.4	25.5	52.3
SF-295	3.81	13.8	37.2
SF-539	2.12	4.67	10.8
SNB-19	3.65	16.4	44.3
SNB-75	2.76	9.09	30.1
U251	1.50	2.88	5.55
Melanoma			
MALME-3M	1.75	4.41	12.7
M14	1.68	3.04	5.52
SK-MEL-2	15.7	34.4	75.3
SK-MEL-28	11.1	23.2	48.5
SK-MEL-5	13.5	31.7	74.7
UACC-257	9.08	21.9	49.2
UACC-62	2.30	12.8	36.5
Ovarian cancer			
OVCAR-3	16.2	31.9	62.8
OVCAR-4	13.9	37.7	>100
OVCAR-5	12.4	>100	>100
SK-OV-3	4.01	15.0	38.8
Renal cancer			
786-0	1.90	4.80	15.3
A498	12.9	26.9	56.0
ACHN	14.0	30.5	66.4
CAKI-1	11.9	25.0	52.2
SN12C	8.91	>100	>100
TK-10	16.5	36.0	78.6
UO-31	1.95	3.83	7.52
Prostate cancer			
PC-3	7.28	24.6	69.9
DU-145	3.70	11.7	34.2
Breast cancer			
MCF-7	17.9	47.1	>100
NCI/ADR-RES	16.4	31.9	61.9
MDA-MB-231	0.89	16.5	67.1
HS 578T	8.51	22.2	51.9
MDA-MB-435	2.36	6.23	21.8
MDA-N	4.08	14.7	38.4
BT-549	17.9	32.1	57.5
T-47D	4.42	15.7	39.6

fold higher than the corresponding GI_{50} values. A ratio of LC_{50} to GI_{50} greater than 20 is only found in leukemia: CCRF-CEM (>24), while others are from 2- to 16-fold.

The data of response parameters in Table 1 can be plotted by the NCI's mean graph as shown in Figure 2. The mean graph facilitates visual scanning of data for potential patterns of selectivity for particular cell lines or for particular subpanels with respect to a selected response parameter.[13] Differences in apparent selectivity patterns may occur against the same cell lines when different parameters are compared. The mean graph shows the pattern at each of the principal response parameters, i.e. GI_{50}, TGI and LC_{50}. The vertical line of each response parameter group represents the mean value of all test data against cell lines. Therefore, bars extending to the right represent sensitivity of the cell line in excess of the average sensitivity of all cell lines. Bars extending to the left imply sensitivity of the cell line less than the average sensitivity. For those insensitive cell lines whose response parameters are more than the highest concentration 100 μM, it was not possible to determine the desired response parameter (e.g. TGI against CCRF-CEM, MOLT-4, HT29, OVCAR-5 and SN12C; and LC_{50} against CCRF-CEM, K-562, MOLT-4, PRMT-8226, EKVX, HT29, OVCAR-4, OVCAR-5, SN12C and MCF-7) by interpolation, the bar length shown is the highest concentration tested and the listed $\log_{10}$ of the response parameter is preceded by a '>' sign.

In Figure 2, at the GI_{50} level, CNS cancer is the most sensitive subpanel, while colon cancer, ovarian cancer and renal cancer are less sensitive subpanels because at least one-third of the cell lines have bars extending to the left. The bars of those log $GI_{50} \leqslant -5.70$ (i.e. $GI_{50} \leqslant 2.0$ μM in Table 1) extending to the right by approximately 0.5 include KM12, U251, MALME-3M, M14, 786-0, UO-31 and MDA-MB-231 cell lines from five subpanels, suggesting the responses against these cell lines are more selective than other lines. The selectivity between these seven most sensitive lines and the least sensitive line (CCRF-CEM) is from 26- to 56-fold. In the same cancer subpanel, selectivity more than 15-fold is observed

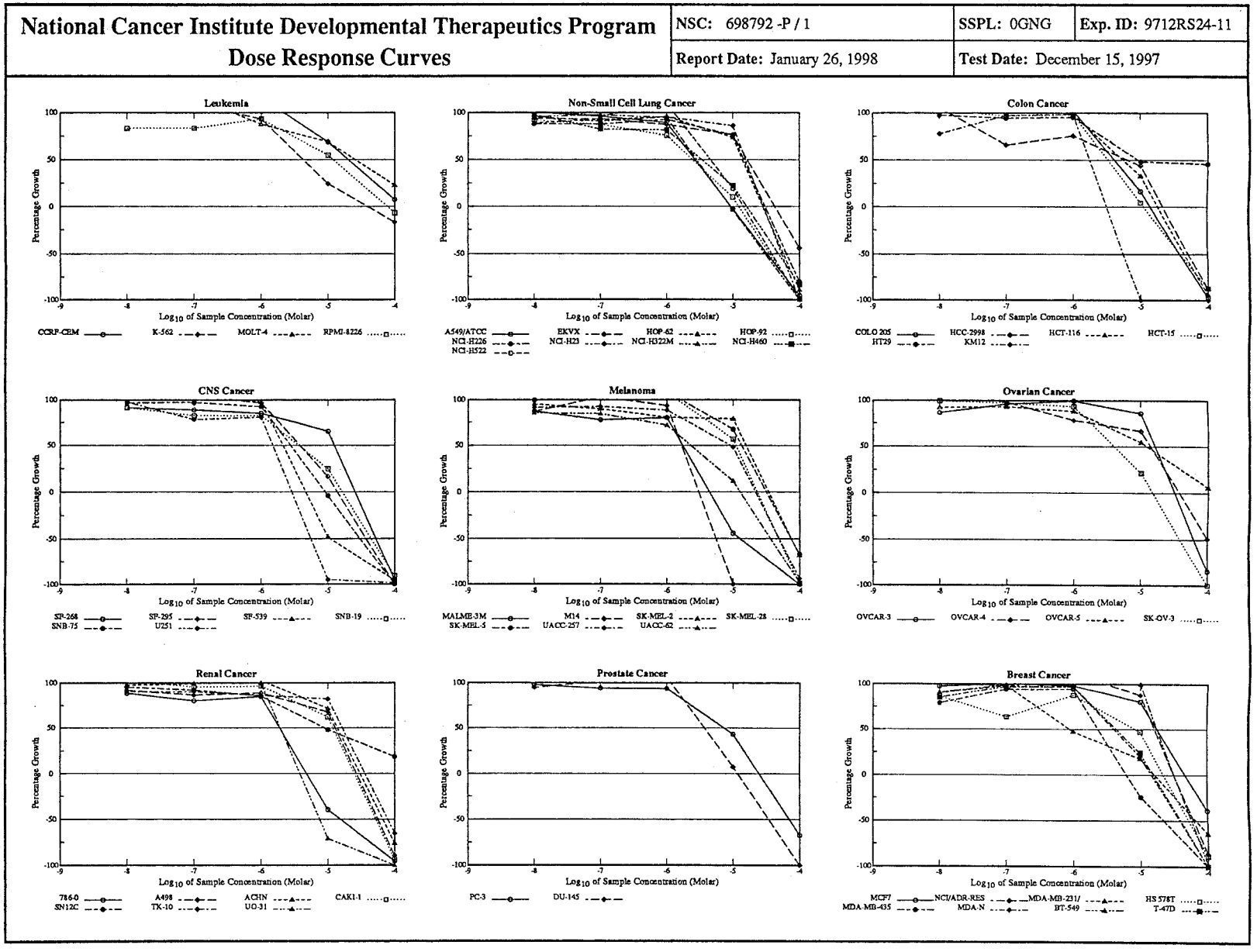

Figure 1. The dose–response curves of methyl protogracillin against the NCI's 60 human cancer cell lines.

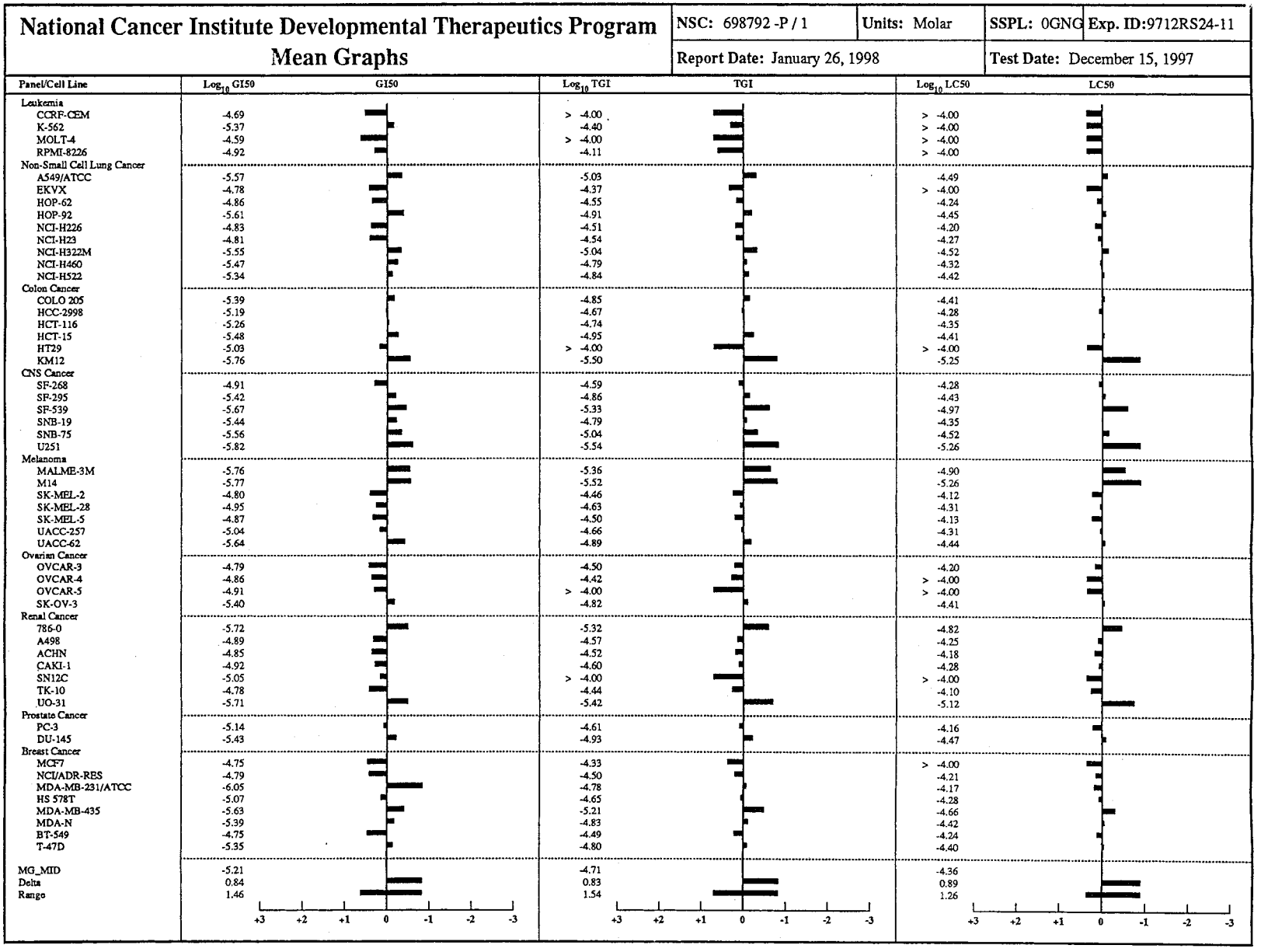

Figure 2. The mean graphs of methyl protogracillin against the NCI's 60 human cancer cell lines.

between MDA-MB-231 and MCF-7, NCI-ADR-RES, BT-459 in breast cancer. Very similar profiles are found in solid tumors rather than leukemia at TGI and LC_{50} levels, respectively.

To further characterize the mechanism of action of methyl protogracillin and to identify compounds with potentially similar mechanism of action, we performed COMPARE analysis using the NCI's anticancer drug screen database with methyl protogracillin as a seed. The COMPARE computer program utilizes cytotoxicity data derived from screening compounds against 60 human cancer cell lines to calculate the Pearson correlation coefficient between the data for seed compounds and those for past agents in the database.[13,14] Such comparison can lead to the identification of similarities in action of the test compound to known reference compounds. The ability of COMPARE to group compounds with similar mechanisms of action, similar molecular targets and similar mechanisms of resistance has been previously demonstrated.[15–18] As a result, the COMPARE analysis of methyl protogracillin based on a comparison on the mean graph was negative, suggesting a potential novel mechanism of anticancer action involved.

Thus, methyl protogracillin was selected for follow-up evaluation by the NCI Biological Evaluation Committee (BEC). After determination of its non-toxicity with minimum tolerated dose >600 mg/kg, methyl protogracillin is in the list for the NCI's *in vivo* Hollow Fiber Assay in nude mice.[19] Twelve cell lines, two from each human cancer subpanel, will be used for this assay, including NSCLC (NCH-H23 and NCI-H522), colon cancer (SW-620 and COLO 205), CNS cancer (SF-295 and U251), melanoma (LOX IMVI and UACC-62), ovarian cancer (OVCAR-3 and OVCAR-5) and breast cancer (MDA-MB-231 and MDA-MB-435).

Conclusion

In summary, methyl protogracillin was cytotoxic against all the test cell lines from leukemia and solid tumors in the NCI's human cancer panel with $GI_{50}<100$ μM; it showed particular selectivity against one colon cancer line (KM12), one CNS cancer line (U251), two melanoma lines (MALME-3M and M14), two renal cancer lines (786-0 and UO-31) and one breast cancer line (MDA-MB-231) with $GI_{50}\leqslant 2.0$ μM. The selectivity between these seven most sensitive lines and the least sensitive line (CCRF-CEM) ranged from 26- to 56-fold. In the same cancer subpanel, selectivity more than 15-fold was observed between MDA-MB-231 and MCF-7, NCI-ADR-RES, BT-549 in breast cancer. From a general view of the mean graph, CNS cancer is the most sensitive subpanel, while ovarian cancer and renal cancer are the least sensitive subpanels. Based on an analysis of the COMPARE computer program with methyl protogracillin as a seed compound, no compounds in the NCI's anticancer drug screen database have similar cytotoxicity patterns (mean graph) to that of methyl protogracillin. The COMPARE data analysis indicated a potential novel mechanism of the anticancer action involved, which will trigger us to investigate the pharmacological mechanism of methyl protogracillin based on molecular and cellular levels in the near future.

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