Preparation and anticancer evaluation of potassium-N-(P-anisole)-α-(O-Xanthetovanillin)–nitrone

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ABSTRACT

The new compound, "Potassium -N-(P-methoxy phenyl)-α-(O-Xanthetovanillin)- nitrone was prepared and characterized by elemental analysis, IR and molar conductance. The anticancer activity of it had been achieved by the assistance of the National Cancer Institute (NCI) USA. Here we show that vanillin (3-methoxy-4-hydroxybenzaldehyde); a naturally occurring food component and an acknowledged antimutagen, anticlastrogen and anticarcinogen-is an inhibitor of the activity of nine types of cancer cells specially the Colon, CNS and Renal cancer.

الخلاصـــة

تم تحضير مركب بوتاسيوم -N- (باراميثوكسي فنيل) – الفا – (زانثيتو فانيلين) – نايترون وثم تشخيصه بواسطة التحليل الدقيق للعناصر. الأشعة تحت الحمراء والموصلية الكهربائية. اما الفعالية ضد سرطانية فقد تمت بمساعدة المعهد الوطني للسرطان (NCI) في الولايات المتحدة الأمريكية. ومن خلال البحث اثبت بان الفانيلين – (٣- ميثوكسي – ٤ - بنزالديهايد) وهو المادة الموجودة في الطبيعة بأنها ذات مفعول ضد سرطاني من خلال اثباطه لفعالية تسعة أنواع من الخلايا السرطانية وخاصة سرطان القولون ، والجهاز العصبي المركزي وسرطان الجهاز البولي.

The nitrone, in the present study, contains two functional groups, the nitrogen oxide and xanthate. It is well known that N→O plays an important roles in the preparation of biologically active compound as anticancer.[1] On the other hand, the xanthate derivatives and their metal complexes found vast application in the biological fields, as insecticides, fungicides herbicides and as antibacterial agents.[2,3] As well vanillin (C8H8O3) is a flavoring agent that in previous studies constitutes an important moiety in compounds act as antimutagen, anticlastogen and anticarcinogen.[4-6] The existence of these promising functional groups encourage us to evaluate the anticancer activity, which to our best knowledge has not been attempted yet.

Experimental

A. Material and Measurements

The content of compound of carbon, hydrogen and nitrogen were estimated by Carlo Erba Elemental Analyser MOD 1106. The sulfur and potassium amounts were estimated by application of standard procedures.[7] The infrared spectra recorded in KBr disc on Perkin-Elmer 557 spectrophotometer (400-4000 cm-1). The UV Spectra was achieved using dichloroethane as a solvent and quartz cell of 1 cm diameter by using Shimadzu UV-visible recording Spectrophotometer, UV-160 (200-600 nm). The molar conductance was found at room temperature by using conductivity apparatus type LF-42 in DMF (10-3 M).

B. Synthesis

B.1. Synthesis of a – (vanillin) – N – (P-methoxyphenyl)

In suitable beaker dissolve 0.108 mole of P-methoxy phenyl nitro benzene and 7.5 g (0.141 mole) of NH_4Cl in 200 cm^3 of 92% propanol, cool the mixture at 10°C and add 27g (0.411 mole) of zinc powder gradually with stirring for 4 hrs. filter the mixture to get a solution of hydroxylamine wash the precipitate with 100 ml of hot propanol take filtrate and add to it 8.4g (0.069 mole) of vanillin and set aside the reaction mixture at dark place with continuous stirring product (precipitate) was filtered, washed with 100 ml of ether to get compound 1.[8]

B.2. Synthesis of Potassium – N – (P-methoxy phenyl)–α– (O-Xanthetovanillin) - nitrone.

In boiling flask, dissolve 0.04 mole of compound 1 in 100 ml of dioxane and add to it 0.05 mole of KOH (dissolved – 20 ml of D.W.). Reflex for 1 hr, filter to get rid un reacted KOH, cool to room temperature,

then add 3 ml of 0.05 mole of CS_2 gradually for 4hr, evaporate the solvent under rotatory evaporator to get oily mass, add to it ether to get compd. 2 which recrystallized by using benzene petroleum ether and dried in vacuum.[9]

C. Anticancer Evaluation

The compound under NCI screening has a no. of NSC:717875 have been evaluated in the 3 cell line, one dose primary anticancer assay and was active using MCF7 (Breast), NCI-H 460 (Lung), SF-268 (CNS), the compound which pass the criteria for activity in this assay will be scheduled automatically for evaluation against the full panel of 60 human tumor cell lines which derived from 9 (nine) main types of cancer Leukemia, Lung Colon, CNS, melanoma, ovarian, renal, prostate and breast cancer. The test repeated by expose the tumor cell lines to the compound under investigation 5 times at different concentration starting from 1×10^{-8} to 1×10^{-4} and for 48 hr.

Result and Discussion

The compound has been characterized by using elemental analysis CHN yield the following values

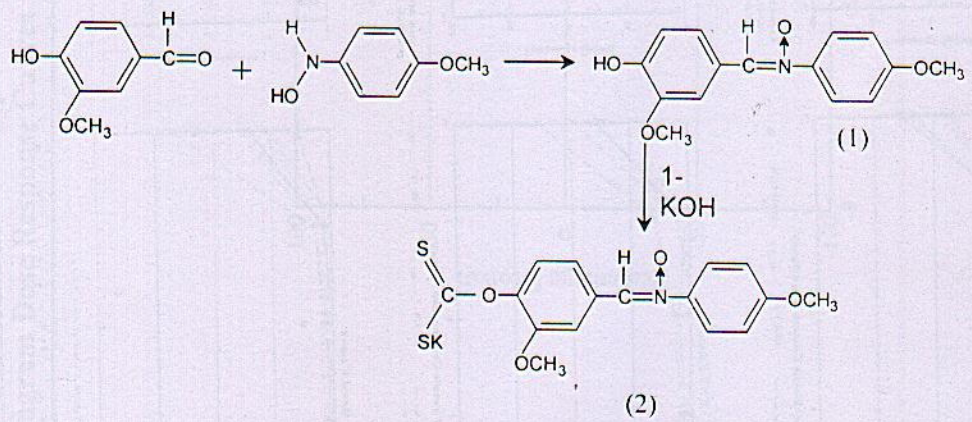
	C %	H %	N %	K %
Calc./ (Found)	49.61 (49.23)	3.62 (3.42)	3.61 (3.33)	10.30 (10.21)

The Molar conductivities for the compound show it behaves as 1:1 electrolyte in DMF of 10^{-3} M solution at room temperature and give value of 75 Ω cm^2 mol^{-1} [10] UV. Vis spectra of the compound showed absorption band 400 nm and considered as the charge transfer transition of the whole molecule. The infrared spectra of the compound showed, the following bands at 1630 cm-1, 1265 cm-1, 1110cm-1, 1070 cm-1, 770 cm-1 for y C = N, y N →O, y C = S, y C – O, y C – S respectively.

Concerning the anticancer screening, three main parameters have been utilized to calculate the inhibition concentrations. Those parameters created from dose response curves which are indicated by the horizontal lines and provided at the percentage growth (PG) values of +50,0 and –50.[11,12] The concentrations corresponding to points where the curves cross these lines are GI50 (growth inhibition 50%) TGI (total growth inhibition) and LC50 (Lethal concentration 50), respectively (Fig. 1,2).

Generally, the forcoming concentrations (diluted) 10^{-8},10^{-7},10^{-6}, and 10^{-5} give no significant changes of the activities parameters (Most of cell line panel show GI50, some with TGI while no one with positive LC50 as indicated in (Table 1,2).

The preparation pathway for the titled compound



(2)

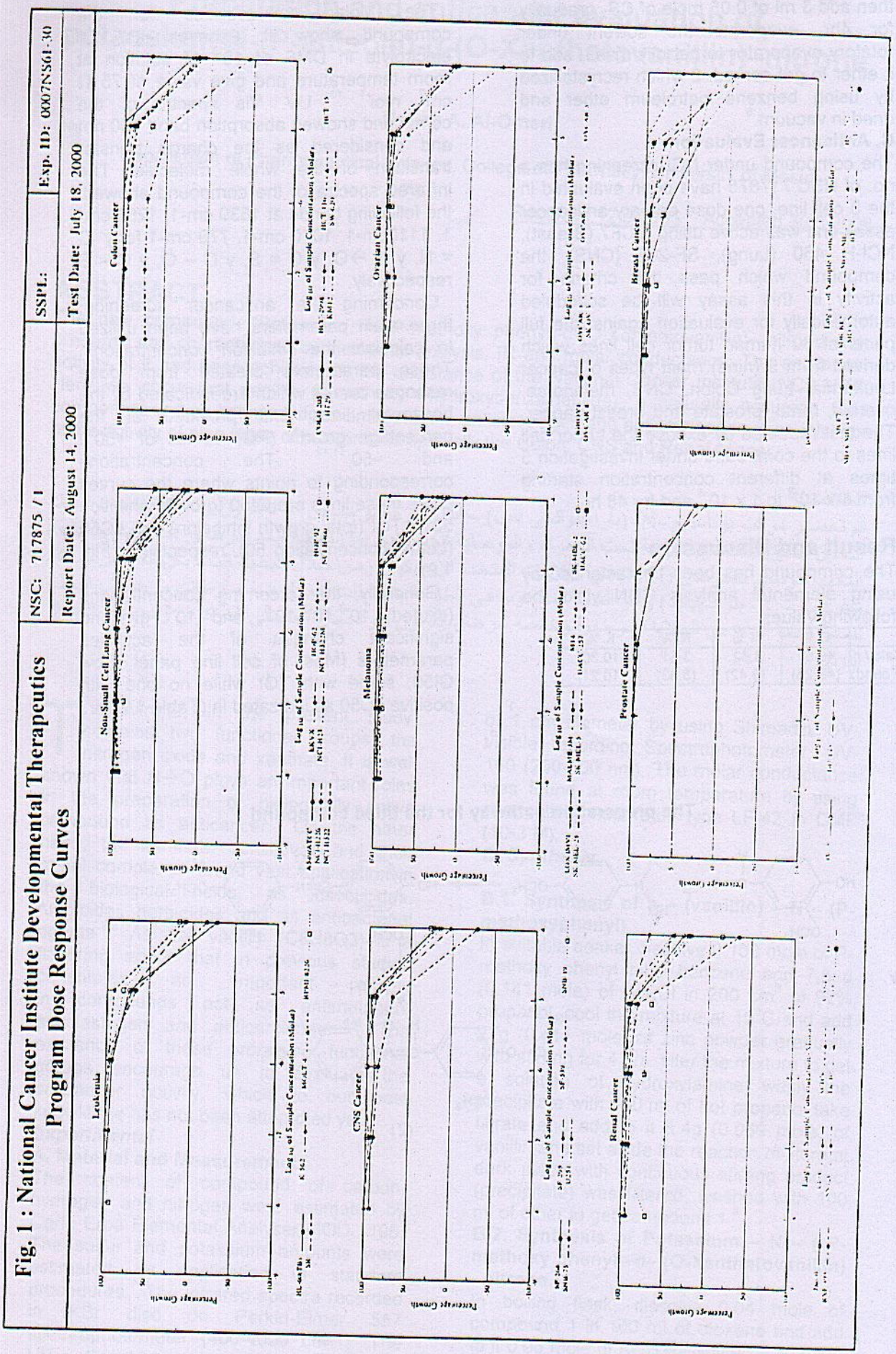


Fig. 2 : National Cancer Institute Developmental Therapeutics Program Dose Response Curves

NSC: 717875 / 1	SSPL:	Exp. ID: 0007NS61-30
Report Date: August 14, 2000	Test Date: July 18, 2000	

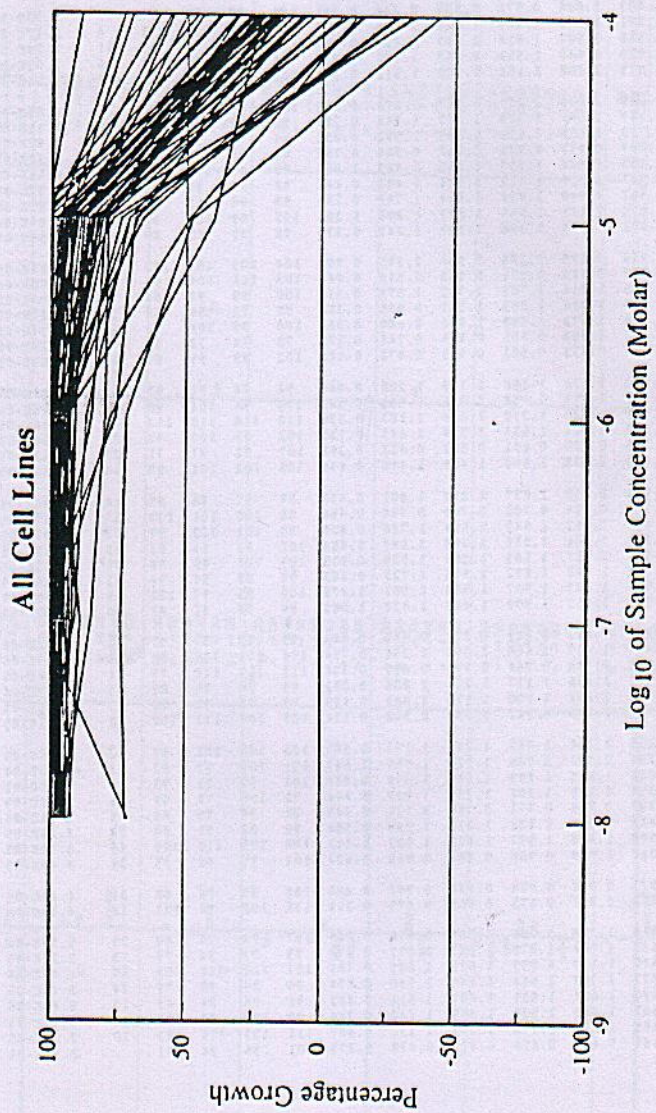


Table 2 : National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC: 717875 / 1	Experiment ID: 0007NS61-30	Test Type: 08	Units: Molar
Report Date: August 14, 2000	Test Date: July 18, 2000	QNS:	MC:
COMI:	Stain Reagent: SRB Dual-Pass	SSPL:	

Panel/Cell Line	Time Zero	Mean Optical Densities: Ctrl	Log10 Concentration: -8.0	-7.0	-6.0	-5.0	-4.0	Percent Growth: -8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
HL-60(TB)	0.389	1.003	1.006	0.978	0.958	0.844	0.281	101	96	93	74	-28	1.73E-05	5.34E-05	>1.00E-04
K-562	0.265	1.892	1.940	1.886	1.826	1.424	0.395	103	100	96	71	8	2.17E-05	>1.00E-04	>1.00E-04
MOLT-4	0.384	1.556	1.586	1.618	1.519	1.466	0.509	102	105	97	92	11	1.29E-05	>1.00E-04	>1.00E-04
RPMI-8226	0.555	1.925	1.840	1.954	1.862	1.670	0.775	94	102	95	81	16	3.03E-05	>1.00E-04	>1.00E-04
SR	0.433	2.073	2.108	2.156	2.100	1.511	0.363	102	105	102	66	-16	1.56E-05	6.35E-05	>1.00E-04
Non-Small Cell Lung Cancer															
A549/ATCC	0.215	1.235	1.235	1.199	1.227	1.173	0.438	100	96	99	94	22	4.06E-05	>1.00E-04	>1.00E-04
EKVX	0.538	1.549	1.506	1.520	1.567	1.364	0.719	96	97	102	82	18	3.14E-05	>1.00E-04	>1.00E-04
HOP-62	0.550	1.722	1.655	1.672	1.685	1.592	0.831	94	96	97	89	24	3.97E-05	>1.00E-04	>1.00E-04
HOP-92	0.766	1.009	0.993	0.983	1.012	0.969	0.702	93	89	101	84	-8	2.32E-05	8.10E-05	>1.00E-04
NCI-H226	0.827	1.453	1.444	1.457	1.465	1.463	1.068	99	101	102	102	38	6.56E-05	>1.00E-04	>1.00E-04
NCI-H23	0.370	1.547	1.529	1.522	1.514	1.421	0.443	98	98	97	89	6	2.97E-05	>1.00E-04	>1.00E-04
NCI-H322M	0.553	1.963	1.940	1.934	1.914	1.749	0.790	98	98	97	85	17	3.25E-05	>1.00E-04	>1.00E-04
NCI-H460	0.335	1.975	1.981	1.956	1.973	1.896	1.261	100	99	100	95	56	>1.00E-04	>1.00E-04	>1.00E-04
NCI-H522	0.289	1.476	1.455	1.468	1.297	1.241	0.370	98	99	85	80	7	2.58E-05	>1.00E-04	>1.00E-04
Colon Cancer															
COLO 205	0.459	2.116	2.179	2.195	2.194	2.195	0.803	104	105	105	105	21	4.48E-05	>1.00E-04	>1.00E-04
HCC-2998	0.235	0.537	0.545	0.571	0.555	0.519	0.446	103	111	106	94	70	>1.00E-04	>1.00E-04	>1.00E-04
HCT-116	0.196	1.813	1.814	1.783	1.722	1.579	0.318	100	98	94	86	8	2.85E-05	>1.00E-04	>1.00E-04
HCT-15	0.217	1.320	1.294	1.295	1.252	0.965	0.396	98	98	94	68	16	2.22E-05	>1.00E-04	>1.00E-04
HT29	0.136	1.074	1.073	1.058	1.080	0.980	0.264	100	98	101	90	14	3.34E-05	>1.00E-04	>1.00E-04
KM12	0.304	1.221	0.968	0.985	0.965	0.746	0.170	72	74	72	48	-44	8.39E-06	3.33E-05	>1.00E-04
SW-620	0.098	0.526	0.533	0.521	0.505	0.472	0.144	102	99	95	88	11	3.09E-05	>1.00E-04	>1.00E-04
CNS Cancer															
SF-268	0.356	1.222	1.174	1.166	1.148	1.200	0.546	94	94	91	97	22	4.24E-05	>1.00E-04	>1.00E-04
SF-295	0.603	2.010	2.014	1.958	2.061	1.865	0.641	100	96	104	90	3	2.86E-05	>1.00E-04	>1.00E-04
SF-539	0.407	1.101	1.229	1.210	1.226	1.183	0.510	118	116	118	112	18	4.54E-05	>1.00E-04	>1.00E-04
SNB-19	0.438	1.543	1.561	1.533	1.539	1.443	0.735	102	99	100	91	27	4.36E-05	>1.00E-04	>1.00E-04
SNB-75	0.521	0.706	0.720	0.691	0.683	0.653	0.368	107	91	87	71	-29	1.63E-05	5.11E-05	>1.00E-04
U251	0.264	1.476	1.533	1.506	1.489	1.416	0.439	105	102	101	95	14	3.62E-05	>1.00E-04	>1.00E-04
Melanoma															
LOX IMVI	0.386	1.875	1.836	1.837	1.813	1.801	0.658	97	97	96	95	18	3.86E-05	>1.00E-04	>1.00E-04
MALME-3M	0.259	0.742	0.724	0.742	0.749	0.756	0.436	96	100	101	103	37	6.27E-05	>1.00E-04	>1.00E-04
M14	0.459	1.825	1.812	1.842	1.824	1.786	0.824	99	101	100	97	27	4.67E-05	>1.00E-04	>1.00E-04
SK-MEL-2	0.460	1.351	1.361	1.279	1.286	1.197	0.668	101	92	93	83	23	3.55E-05	>1.00E-04	>1.00E-04
SK-MEL-28	0.647	1.569	1.617	1.565	1.564	1.550	0.935	105	100	99	98	31	5.23E-05	>1.00E-04	>1.00E-04
SK-MEL-5	0.496	1.964	1.848	1.892	1.876	1.725	0.483	92	95	94	84	-3	2.46E-05	9.32E-05	>1.00E-04
UACC-257	0.677	1.576	1.573	1.537	1.553	1.591	1.476	100	96	97	102	89	>1.00E-04	>1.00E-04	>1.00E-04
UACC-62	0.573	1.552	1.513	1.509	1.468	1.422	1.062	96	96	91	87	50	9.93E-05	>1.00E-04	>1.00E-04
Ovarian Cancer															
IGROV1	0.249	0.892	0.862	0.824	0.872	0.842	0.385	95	89	97	92	21	3.92E-05	>1.00E-04	>1.00E-04
OVCAR-3	0.489	1.396	1.437	1.408	1.396	1.354	0.714	104	101	100	95	25	4.39E-05	>1.00E-04	>1.00E-04
OVCAR-4	0.509	0.738	0.768	0.764	0.776	0.685	0.334	113	111	116	77	-34	1.74E-05	6.90E-05	>1.00E-04
OVCAR-5	0.484	1.167	1.156	1.137	1.125	1.086	0.861	98	96	94	88	55	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-8	0.280	1.121	1.101	1.100	1.110	1.089	0.425	98	98	99	96	17	3.85E-05	>1.00E-04	>1.00E-04
SK-OV-3	0.413	0.920	0.935	0.942	0.988	0.960	0.524	103	104	113	108	22	4.71E-05	>1.00E-04	>1.00E-04
Renal Cancer															
786-0	0.265	1.256	1.256	1.255	1.261	1.183	0.583	100	100	101	93	32	5.06E-05	>1.00E-04	>1.00E-04
A498	0.783	1.986	2.002	2.006	1.921	1.952	1.667	101	102	95	97	73	>1.00E-04	>1.00E-04	>1.00E-04
ACHN	0.408	1.411	1.445	1.399	1.345	1.339	0.848	103	99	93	93	44	7.50E-05	>1.00E-04	>1.00E-04
CAKI-1	0.521	1.354	1.109	1.192	1.155	1.099	0.444	71	105	76	69	-15	1.70E-05	6.67E-05	>1.00E-04
RXF 393	0.440	0.787	0.766	0.775	0.769	0.736	0.285	94	96	95	85	-35	1.96E-05	5.09E-05	>1.00E-04
SN12C	0.352	1.413	1.367	1.326	1.313	1.288	0.584	96	92	91	88	22	3.76E-05	>1.00E-04	>1.00E-04
TK-10	0.746	1.548	1.608	1.587	1.629	1.592	1.113	108	105	110	106	46	8.50E-05	>1.00E-04	>1.00E-04
UO-31	0.219	1.010	1.019	0.968	0.994	0.968	0.424	101	95	98	95	26	4.46E-05	>1.00E-04	>1.00E-04
Prostate Cancer															
PC-3	0.259	0.973	0.986	0.968	0.944	0.843	0.482	102	99	96	82	31	4.24E-05	>1.00E-04	>1.00E-04
DU-145	0.230	0.672	0.832	0.672	0.665	0.675	0.314	136	100	98	101	19	4.16E-05	>1.00E-04	>1.00E-04
Breast Cancer															
MCF7	0.397	1.816	1.769	1.786	1.509	0.971	0.729	97	98	78	40	23	5.59E-06	>1.00E-04	>1.00E-04
NCI/ADR-RES	0.383	1.133	1.094	1.079	1.087	0.977	0.372	95	93	94	79	-3	2.27E-05	9.23E-05	>1.00E-04
MDA-MB-231/ATCC	0.473	0.990	1.130	1.053	1.075	1.005	0.783	127	112	116	103	60	>1.00E-04	>1.00E-04	>1.00E-04
HS 578T	0.432	1.827	1.767	1.614	1.546	1.540	0.774	96	85	80	79	24	3.43E-05	>1.00E-04	>1.00E-04
MDA-MB-435	0.360	1.690	1.614	1.631	1.607	1.011	0.312	94	96	94	49	-13	9.46E-06	6.11E-05	>1.00E-04
MDA-N	0.290	1.503	1.488	1.504	1.459	1.146	0.386	99	100	96	71	8	2.13E-05	>1.00E-04	>1.00E-04
BT-549	0.742	1.165	1.211	1.178	1.226	1.137	0.667	111	103	114	93	-10	2.62E-05	7.98E-05	>1.00E-04
T-47D	0.275	0.487	0.489	0.478	0.475	0.470	0.275	101	96	94	92	.	2.85E-05	9.95E-05	>1.00E-04

Fig. 3 : National Cancer Institute Developmental Therapeutics Program Mean Graphs

NSC: 717875 / 1	Units: Molar	SSPL:	Exp. ID:0007NS61-30
Report Date: August 14, 2000		Test Date: July 18, 2000	

Panel/Cell Line	Log_{10} GI50	GI50	Log_{10} TGI	TGI	Log_{10} LC50	LC50
Leukemia						
HL-60(TB)	-4.76		-4.27		> -4.00	
K-562	-4.66		> -4.00		> -4.00	
MOLT-4	-4.48		> -4.00		> -4.00	
RPMI-8226	-4.52		> -4.00		> -4.00	
SR	-4.81		-4.20		> -4.00	
Non-Small Cell Lung Cancer						
A549/ATCC	-4.39		> -4.00		> -4.00	
EKVX	-4.50		> -4.00		> -4.00	
HOP-62	-4.40		> -4.00		> -4.00	
HOP-92	-4.63		-4.09		> -4.00	
NCI-H226	-4.18		> -4.00		> -4.00	
NCI-H23	-4.53		> -4.00		> -4.00	
NCI-H322M	-4.49		> -4.00		> -4.00	
NCI-H460	> -4.00		> -4.00		> -4.00	
NCI-H522	-4.59		> -4.00		> -4.00	
Colon Cancer						
COLO 205	-4.35		> -4.00		> -4.00	
HCC-2998	> -4.00		> -4.00		> -4.00	
HCT-116	-4.55		> -4.00		> -4.00	
HCT-15	-4.65		> -4.00		> -4.00	
HT29	-4.48		> -4.00		> -4.00	
KM12	-5.08		-4.48		> -4.00	
SW-620	-4.51		> -4.00		> -4.00	
CNS Cancer						
SF-268	-4.37		> -4.00		> -4.00	
SF-295	-4.54		> -4.00		> -4.00	
SF-539	-4.34		> -4.00		> -4.00	
SNB-19	-4.36		> -4.00		> -4.00	
SNB-75	-4.79		-4.29		> -4.00	
U251	-4.44		> -4.00		> -4.00	
Melanoma						
LOX IMVI	-4.41		> -4.00		> -4.00	
MALME-3M	-4.20		> -4.00		> -4.00	
M14	-4.33		> -4.00		> -4.00	
SK-MEL-2	-4.45		> -4.00		> -4.00	
SK-MEL-28	-4.28		> -4.00		> -4.00	
SK-MEL-5	-4.61		-4.03		> -4.00	
UACC-257	> -4.00		> -4.00		> -4.00	
UACC-62	-4.00		> -4.00		> -4.00	
Ovarian Cancer						
IGROV1	-4.41		> -4.00		> -4.00	
OVCAR-3	-4.36		> -4.00		> -4.00	
OVCAR-4	-4.76		-4.31		> -4.00	
OVCAR-5	> -4.00		> -4.00		> -4.00	
OVCAR-8	-4.41		> -4.00		> -4.00	
SK-OV-3	-4.33		> -4.00		> -4.00	
Renal Cancer						
786-0	-4.30		> -4.00		> -4.00	
A498	> -4.00		> -4.00		> -4.00	
ACHN	-4.12		> -4.00		> -4.00	
CAKI-1	-4.77		-4.18		> -4.00	
RXF 393	-4.71		-4.29		> -4.00	
SN12C	-4.42		> -4.00		> -4.00	
TK-10	-4.07		> -4.00		> -4.00	
UO-31	-4.35		> -4.00		> -4.00	
Prostate Cancer						
PC-3	-4.37		> -4.00		> -4.00	
DU-145	-4.38		> -4.00		> -4.00	
Breast Cancer						
MCF7	-5.25		> -4.00		> -4.00	
NCI/ADR-RES	-4.64		-4.03		> -4.00	
MDA-MB-231/ATCC	> -4.00		> -4.00		> -4.00	
HS 578T	-4.46		> -4.00		> -4.00	
MDA-MB-435	-5.02		-4.21		> -4.00	
MDA-N	-4.67		> -4.00		> -4.00	
BT-549	-4.58		-4.10		> -4.00	
T-47D	-4.55		-4.00		> -4.00	
MG_MID	-4.45		-4.04		-4.00	
Delta	0.80		0.44		0.00	
Range	1.25		0.48		0.00	

Table 1. *The activities of the titled nitrone against the a nine types of tumor (different cell lines)*

Type of Tumor	GI50	TGI
Leukemia	All	Only HL-60 (TB) &SR
Non Small Cell Lung Cancer	All except NCI-H 332M	Only HOP – 92
CNS Cancer	All	Only SNB – 75
Melanoma	All except UACC-257	Only SK-MEL – 5
Colon Cancer	All except HCC-2998	Only KM 12
Ovarian Cancer	All except OVCAR – 5	Only OVCAR – 4
Renal Cancer	All except A-498	Only CAKI-1, RXF 393
Prostate Cancer	All	None
Breast Cancer	All except BT-540	Only NCI/ ADR-RES MDA-MB 435, BT-549, & T-47D

A Tabular presentation of the entire series of dose-response curves, plotted for each disease-related sub panel. Also, a mean graph (Fig. 3) displays of the relative cellular responsiveness with respect to the three different parameters GI50, TGI and LC50 may give a comprehensive detailed package of data and, therby modification of the chemical data will provide an opportunity for improved evaluation of structure activity relationships (SAR) comparing with other compounds in the same series and will introduce the potential capability for molecular modeling in the future to facilitate and/or sub panel specific antitumor activity.

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