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Synthesis and anti-cancer activity of new thiosemicarbazones of 1-(5-chloro-1*H*-benzimidazol-2-yl) ethanone

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ABSTRACT

Some new Thiosemicarbazones containing Benzimidazole moiety were synthesized by combination of conventional as well as green chemistry methods, which had potential Anti – tumor activity against various cancerous cells. All the compounds were submitted to National Cancer Institute, USA for in vitro anti-cancer screening against 60 human cancer cell lines. Our one compound S_2 (NSC 92491) was selected for five dosage screening and showed remarkable activity.

Keywords: Thiosemicarbazones, Benzimidazole, Anti-cancer activity.

INTRODUCTION

Cancer is the uncontrolled growth and spread of cells. It can affect any part of the body. The growths often invade surrounding tissue and can metastasize to distant sites. Cancer can be caused 90-95% by factors such as tobacco, obesity, infections, radiation and 5-10% due to heredity [1]. Most cancer can be treated by radiology, chemotherapy and surgery. According to WHO, in 2007 cancer caused about 13% of all human deaths worldwide (7.9 million) and it is projected to be 12 million deaths per year by 2030.Thus, invention of newer anti-cancer agents has now become a key aim worldwide[2].

Thiosemicarbazones belong to a class of compounds that occupy a wide range of biological activities and have been studied for their activity against tuberculosis [3], virus and most important against various cancerous cells [4-5]. SAR studies showed that a large number of Thiosemicarbazones of an N- heterocyclic compounds have low π-electron density at the side chain part and the ring N-atom should be reasonably a good electron pair donor to transition metals to form co-ordination compounds [6] (figure-1). Thiosemicarbazones in their neutral or deprotonated form, behave as an N N S thiodentate chelate towards metal ions essential for life. Important finding was that an N N S ligand system was a common feature of all compounds with carcinogenic potency [7]. Also there is a strong correlation between tumor growth rate and the enzyme Ribonucleoside Diphosphate Reductase (RDR) [8]. So, it has been suggested that an inhibitor to RDR would be a good agent for the treatment of cancer and metal complexes formed by Thiosemicarbazones are this type of compounds. The reason for their anti-cancer activity is due to co-ordination of iron by Thiosemicarbazones through N N S thiodentate ligand system, either by preformed iron complex binding to the enzyme or by the free ligand complexing with the iron-charged enzyme [9-11].

The synthesis of transition metal complexes with thiosemicarbazone ligands has been receiving considerable attention due to the pharmacological properties of both ligands and complexes [12-13]. The deprotonated thiosemicarbazone ligands usually coordinate to platinum, palladium, copper, ruthenium, and osmium through oxygen, nitrogen, and sulphur donor atoms in their (N, S) bidentate form or (N, N, S or O, N, S) tridentate form, to form metallic complexes of different molecular geometry [14-16] and all these complexes are active against different cancer cells in their different geometries [17]. The square planar platinum(II) and palladium(II) complexes of M(HL)2Cl and M(L)Cl type with thiosemicarbazone ligands derived from phenylacetaldehyde and 2-

formylpyridine showed high cytotoxicity in vitro against HL60 leukaemia and P388 mouse leukaemia cell lines [18], while platinum(II) and palladium(II) binuclear complexes with p-isopropylbenzaldehyde thiosemicarbazone ligands exhibit strong cytotoxic activities on mouse tumor cell growth inhibition [19-20].

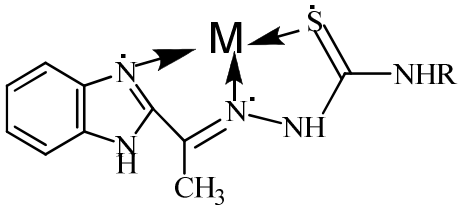
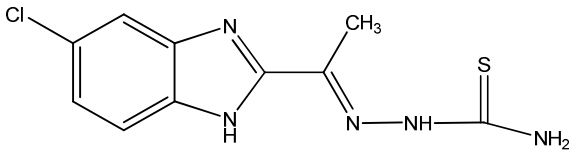


Figure 1: **Complex formation with metal ions**

Some synthetic analogues of Thiosemicarbazone already exist in market like, Triapine, Marboran, etc. [7] Triapine is a potent ribonucleotide reductase inhibitor and used in cancer treatment. Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) is a tridentate chelator that ligates Fe via a sulphur and two nitrogen atoms [21-22]. Triapine is one of the most comprehensively assessed iron chelators with anti-tumor activity. A recent study reported that the Triapine-Fe (II) complex was significantly more active at inhibiting ribonucleotide reductase than free Triapine [23]. Marboran is a good Anti-viral agent. It has activity against pox viruses, Maloney leukaemia viruses and recently against HIV [24]. Generally these complexes are insoluble in water but our new derivatives contain one more heterocyclic N atom which gives the advantage that they form H-bonds with water resulting in more hydrophilicity and somewhat better activity [6]. So, we are encouraged to explore the synthesis of newer derivatives by modification of compound (A).



(A)

Figure 2: Proposed structure of Thiosemicarbazone derivative

MATERIALS AND METHODS

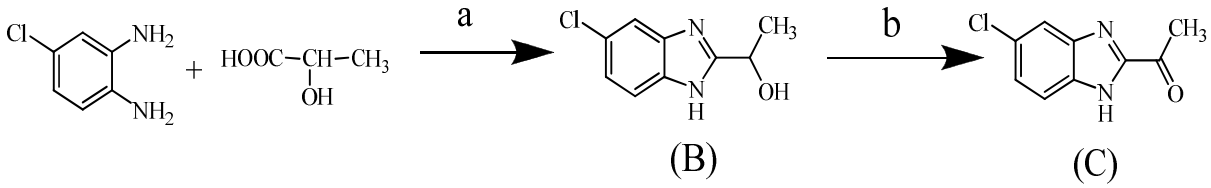
Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 841 using samples in potassium bromide discs. The ^{1}H- NMR spectra were recorded on a Varian 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were recorded on TSQ- Quantum Access Mass spectrometer by ESI (electron spray ionization) method. TLC was performed on Merck DC Alufolien with Kieselgel 60F-254 from Merck Co.,Germany. All the raw-materials were purchased from Sigma-Aldrich Co, Germany.

Novel thiosemicarbazone derivatives were synthesized in accordance with the procedures illustrated in Schemes 1, 2 and 3. The detailed procedures for the intermediates (B), (C) and (D) as well as their characterization data are described here.

Chemistry

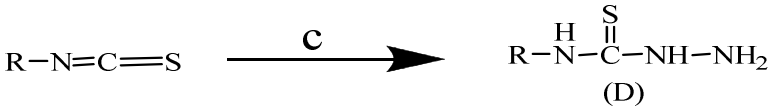
The 1-(5-chloro-1H-benzimidazol-2-yl) ethanone (C) was prepared as outlined in scheme - 1. The condensation reaction between commercially available 4- Chloro benzene - 1, 2 - diamine with lactic acid afforded 1-(5-chloro-1H-benzimidazol-yl) ethanol (B) in quantitative yield. Further oxidation of the *sec*-alcohol gave corresponding ketone (C). The N^4- monosubstituted thiosemicarbazides (D) were synthesized as outlined in scheme - 2. They were formed from hydrazine and various isothiocyanates which were commercially available in the market. Final proposed compound was synthesized as described in scheme - 3. A series of compounds were synthesized by simple condensation reaction between compound (C) and different thiosemicarbazides (D) to form the target structure (A).

Scheme 1:



**Reagents and conditions: (a) Ethylene glycol, MW irradiation, 300 watts, 35 - 45 minutes; (b) CrO_3, CH_3COOH, heat, 87° - 90°C.*

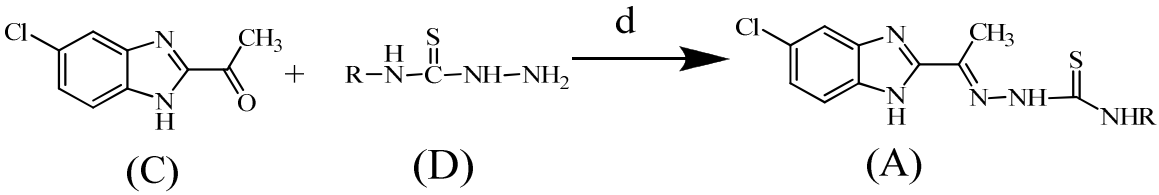
Scheme 2:



Where, R = various alkyl, phenyl, cyclohexyl

**Reagents and conditions: (c) NH_2NH_2 .H_2O, CH_3OH, Room-temperature, 20 – 30 minutes.*

Scheme 3:



Where, R = various alkyl, phenyl, cyclohexyl

**Reagents and conditions: (d) CH_3OH, CH_3COOH, reflux, 65° - 80°C, 2 – 4 hours.*

Synthesis of 1-(5-chloro-1H-benzimidazol-2-yl) ethanol (B)

4-Chlorobenzene 1, 2-diamine 5 g (0.03 mol) and D, L – lactic acid (88% approx.) (8 ml) were mixed. To this mixture, Ethylene glycol (5 ml) was added. The reaction mass were heated in CEM microwave at 300 watts for 35 - 45 minutes. On cooling, the reaction mixture was treated with a saturated solution of $NaHCO_3$ (sodium hydrogen carbonate). (pH = 8) After the neutralisation, the residue salts obtained was dissolved in methanol and filter it. A pinch of charcoal powder was added to eliminate the unwanted colour in that filtrate. The filtrate was concentrated to dryness. The residue then was collected by filtration as yellow solid form. The yield was 50% and product was further used after the crystallization from alcohol. M.P. was taken at 168°- 170°C. Reaction progress was checked by TLC plates using Ethyl acetate: Toluene (4:1) as the mobile phase; IR(cm^{-1}, KBr): NH 3360 (vs), CH(benzene) 3077 (vs), C=C & C=N 1599, C-O 1113; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.49 (d, 3H), 4.68 (q, 1H), 5.77 (d, 1H), 7.14 (d, 1H), 8.40 (dd, 1H).

Synthesis of 1-(5-chloro-1H-benzimidazol-2-yl) ethanone (C)

The 1-(5-chloro-1H-benzimidazol-2-yl) ethanol 2 g (0.01) mol was dissolved in glacial acetic acid (3.75 ml). Chromium Trioxide (CrO_3) 0.38 g (0.003 mol) in H_2O (1.25 ml) was added dropwise in solution of 1-(5-Chloro-1H-benzimidazol -2-yl) ethanol at temperature 87°-92°C. The reaction mixture was heated for 5 minutes on water bath and then added in 50 ml of water in the 250 ml beaker. The yellow precipitates were collected by filtration and dried further. The yield was 70%. The product obtained was not having definite M.P. and it started decomposing at 235°-236°C. The reaction progress was checked by TLC taking Ethyl acetate and Toluene (4:1) as solvents; IR (cm^{-1}, KBr): NH 3368 (m), CH (benzene) 3060 (s), C=O 1699; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.70 (s, 3H), 7.16 (d, 1H), 8.44 (dd, 1H).

General procedure for the synthesis of N^4- alkyl and aryl thiosemicarbazides[6] (D)

The alkyl or aryl isothiocyanate (2-3 ml) was added dropwise in a stirred solution of hydrazine (2-3 ml) in methanol (6 ml) at 65° - 80°C. The stirring was continued for 20 minutes. The solvent was removed by evaporation *in vaccuo* and the crude product was dissolved in chloroform (5 ml) and this solution was added into multifold volume of petroleum ether (40° - 60°C) at constant stirring. The precipitates obtained were used directly without purification or some times after crystallization from the appropriate solvent.

By this method other compounds were prepared: 4- phenyl-, 4- ethyl-, 4-n- butyl- and 4-cyclohexyl-thiosemicarbazides.

General procedure for the synthesis of thiosemicarbazones of the 1-(5-chloro-1H-benzimidazol-2-yl) ethanone (A)
A typical procedure is described here for the synthesis 2-[1-(5-chloro-1H-benzimidazol-2-yl) ethylidene] N-phenylhydrazine carbothioamide. **(S_2)**

The Phenyl thiosemicarbazide 2 g (0.01 mol) and 10 drops of glacial acetic acid were added to a warm solution of 1-(5-chloro-1H- benzimidazol-2 –yl) ethanone in methanol (6 ml). The reaction mass was heated at reflux temperature for 2 - 4 hours. The product was obtained upon cooling, and then dried. The reaction progress was checked by TLC taking Ethyl acetate and Toluene (4:1) as mobile phase. The product was brown colour solid with 50% yield and m.p. at 161°-162°C; IR (cm^{-1}, KBr): NH 3205 (s), C=S 1189 (s), C=N 1514 (s), C=C 1596 (s); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.46-2.48 (s, 3H), 7.24-7.26 (d, 1H), 7.40-7.44 (t, 1H), 7.49-7.51 (d, 1H), 7.59-7.64 (d, 1H), 8.35-8.36 (s, 1H), 10.40 (s, 1H), 11.07 (s, 1H), 13.02 (s, 1H); Mass (m/e, ESI): M^+ 342.9 (found) 343.83(actual), [M +2] 344.9 .

In the same manner, other thiosemicarbazone derivatives were synthesized and characterized.

2-[1-(5-chloro-1H-benzimidazol-2-yl)ethylidene] N-hydrazine carbothioamide. (S_1)
Light brown solid; 55% yield; m.p. 214-215°C; IR (cm^{-1}, KBr): NH 3454 (s), C=S 1213 (s), C=N 1531 (s), C=C 1584 (s); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H), 7.14-7.16(d, 1H), 7.48-7.49 (d,1H), 7.69 (s,1H), 8.57 (s, 2H), 10.73 (s, 1H), 12.86-12.88 (s,1H); Mass (m/e, ESI): M^+ 266.9 (found), 267.73(actual), [M +2] 268.9 .

2-[1-(5-chloro-1H-benzimidazol-2-yl) ethylidene] N-ethylhydrazine carbothioamide. (S_3) Brown solid; 58% yield; m.p. 223-224°C; IR (cm^{-1}, KBr): NH 3435 (s), C=S 1199 (s), C=N 1492 (s), C=C 1549 (s); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.42 (s, 3H), 1.19-1.23 (t, 3H), 3.73 (t, 2H), 7.24-7.26 (d, 1H), 7.54-7.56 (d, 1H), 8.35-8.36 (s, 1H), 9.20 (s, 1H), 10.65 (s,1H), 12.92 (s,1H); Mass (m/e, ESI): M^+ 295.55 (found), 295.8 (actual), [M +2] 297.5 .

2-[1-(5-chloro-1H-benzimidazol-2-yl) ethylidene] N-(n-butyl) hydrazine carbothioamide. (S_4)
Brown solid; 65% yield; m.p. 203-204°C; IR (cm^{-1}, KBr): NH 3448 (s), C=S 1216 (s), C=N 1419 (s), C=C 1541 (s); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.90 (t, 3H), 1.32-1.39 (s, 2H), 1.52-1.57 (q, 2H), 2.42 (s, 1H), 3.64-3.67 (q, 2H), 7.24-7.27 (d, 1H), 7.55-7.57 (d, 1H), 8.34-8.36 (s, 1H), 9.15 (s, 1H), 10.68 (s, 1H), 12.98 (s, 1H); Mass (m/e, ESI): M^+ 323.20(found), 323.8 (actual), [M +2] 325.20 .

2-[1-(5-chloro-1H-benzimidazol-2-yl)ethylidene]N-cyclohexylhydrazine carbothioamide. (S_5)
Brown solid; 70% yield; m.p. 215-216°C; IR (cm^{-1}, KBr): NH 3420 (s), C=S 1206 (s), C=N 1519 (s), C=C 1594 (s); ^{1}H-NMR (400 MHz, DMSO-d_6):δ 1.12-1.75 (m, 11H), 2.43 (s, 3H), 7.24-7.25 (d, 1H), 7.52-7.55 (d, 1H), 8.36-8.38 (s, 1H), 9.02 (s, 1H), 10.55 (s, 1H), 13.24 (s, 1H); Mass (m/e, ESI): M^+ 349.20 (found), 349.44 (actual), [M +2] 351.20 .

In vitro anti-cancer activity [25]
The synthesized thiosemicarbazone derivatives were screened for their anticancer activity by the protocol established at National Cancer Institute, Bethesda, MD, USA. The cell line of interest was seeded at a cell density of 5000 – 40,000 cells/well in a 96 well plate with 100µl RPMI 1640 as the basal media supplemented with 5% fetal bovine serum and 2mM L-glutamine. The cells were incubated for a period of 24 hours at 37°C, 5% carbon dioxide, 95% air, and 37% relative humidity for proper attachment. Upon attachment the 0% cell growth control groups were fixed with TCA, while the appropriate serial dilution of the drug (drug diluted in DMSO) was added to the feeding media in the treatment groups. The cells were then incubated for a period of 48 hours at the afore mentioned conditions. Subsequently, the cells were fixed with 50µl of 50% cold TCA (final concentration 10% TCA) solution for 60 minutes at 4°C. The media was then aspirated, cells washed with tap water for 5 times, and air dried. Finally, 100 µl Sulforhodamine B (SRB) at 0.4% (w/v concentration) in 1% acetic acid was used for quantification. SRB is a colorimetric assay used to determine the cell density based on the cellular protein content. The unbound dye was then removed by washing the cells with 1% acetic acid for 5 times to avoid non specific and background interaction/absorbance and the cells were then air dried. The bound stain was then solubilised with 10mM trizma base, and the absorbance was measured at 515nm. The protocol for suspension cells was the same with the only exception being the concentration of TCA set at 80% (final concentration 16%) for fixing the cells.

RESULTS AND DISCUSSION

The synthesized compounds were screened for their anti-cancer activity. We had submitted five compounds. They had given separate codes to each. They are S_1 (NSC- 92075), S_2 (NSC-92491), S_3 (NSC-95191), S_4 (NSC-99793) and S_5 (NSC-95192). Out of the five compounds four were selected by NCI and screened for their in vitro anti-tumor activity against 60 human cell lines derived from nine clinically isolated cancer types (e.g. Leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast) according to a standard protocol established at the National Cancer Institute, Bethesda, MD, USA.

Four compounds they had screened for one dosage. Out of four compounds, in one compound **S_2** (NSC 92491) was selected for further five dosage screening because it had shown excellent activity in one dosage screening. Compound was tested for five different concentrations starting from $\log_{10}$ (-8.3) M to $\log_{10}$ (-4.3) M. The effectiveness of the drug was judged by the percentage of the cell arrest. In the given data table, negative values indicated growth inhibition. More the negative value more would be the effectiveness of the drug at that concentration. At low concentrations of $\log_{10}$ (-8.3) the cell survival or cell growth is 100% relative to the control group and the cell density at time zero, however with an increase in concentration to $\log_{10}$ (-7) the overall cell growth had been found to be ~40% relative to the control group. Further increase in concentration to $\log_{10}$ (-6) yields 0% cell growth over a period of 2 days, i.e. the average number of cells at time zero was equal to the average number of cells treated with $\log_{10}$ (-6) molar concentration of drug after 2 day incubation. This suggested cell arrest, i.e. the cells failed to grow when treated with this concentration of drug. Finally, at ~$\log_{10}$ (-5) and ~$\log_{10}$ (-4.3) concentrations, ~90 and 100% cell death was observed relative to the control and time zero groups after 2 days. This meant that at this concentration, cellular growth arrest was followed by cell death killing majority or all of the cells initially seeded in the well. In the data-sheet given in table mean optical densities were measured. It was measured by three different ways; at first on time zero which was considered to be 0 % in graphs given in table; secondly in control group i.e. cells allowed to grow for 3 days without any drug which was considered to be 100 % if full growth; and last at different concentrations with drug which was considered to be -100% if total cell arrest otherwise any in between value.

From the compounds that were exposed to the test, compound **S_1** (NSC 92075) exhibited low anticancer activity. This compound had shown activity against cell lines of leukemia, lung, colon, ovarian, renal and breast cancer. However, the sensitivity of these cell lines was not enough above the acceptable values. The similar activity was observed for compound **S_3** (NSC 95191) with the difference being that the cell lines NCI-H460 (lung cancer) and MCF7 (breast cancer) was less sensitive for this compound. The activity of the compound **S_5** (NSC 95192) was at the same level of the above compounds but this compound showed average activity against CCRF-CEM cell line of leukemia. One dosage mean graphs of compounds S_1, S_3 and S_5 are given in table no. 1, 3 and 4 respectively.

The compound S_2 (NSC 92491), a phenyl substituted agent, showed remarkable activity against most of all the cancer cell lines as shown in table no. 2. At the concentration $\log_{10}$ (-4.3) M cell arrest was observed for various cell lines. Variations were observed in percentage growth inhibition / cell arrest as the cancer type was changed. In-vitro testing results for five different dosages and dose response curves against various cancer cell lines are given in table 5 and 6 respectively.

In leukemia, maximum 49% growth inhibition was observed for HL-60(TB) cell line. While this compound showed moderate inhibition for cell lines MOLT-4 (39%), RPMI-8226 (34%) and K-562 (20%).

In non small lung cancer, 100% inhibition was shown for cell line NCI-H460. While moderate inhibition was observed for cell lines HOP-62(95%), EKVX (71%), NCI-H322M (87%), HOP- 92(61%) and NCI-H522 (68%).

In colon cancer, compound S_2 showed 100% inhibition for cell line SW- 620. Moderate activities were showed for KM12 (79%), HCC-2998 (55%) and COLO 205 (45%) cell lines.

In CNS cancer, moderate inhibition observed for SF-268 (71%), SNB-75 (70%), SF-539 (40%) and SF-295 (37%) cell lines.

In melanoma, 100% inhibition observed for SK-MEL-5 cell line. Moderate inhibition was exhibited for UACC-257 (88%), SK-MEL-28 (84%), SK-MEL-2 (80%) and UACC-62 (71%) cell lines.

In ovarian cancer, maximum 96% inhibition indicated for OVCAR-4 cell line. Other cell lines OVCAR-3 (65%), OVCAR-5 (38%) and IGROV 1(41%) were showed medium inhibition.

Table 1: One Dose mean graph for S_1

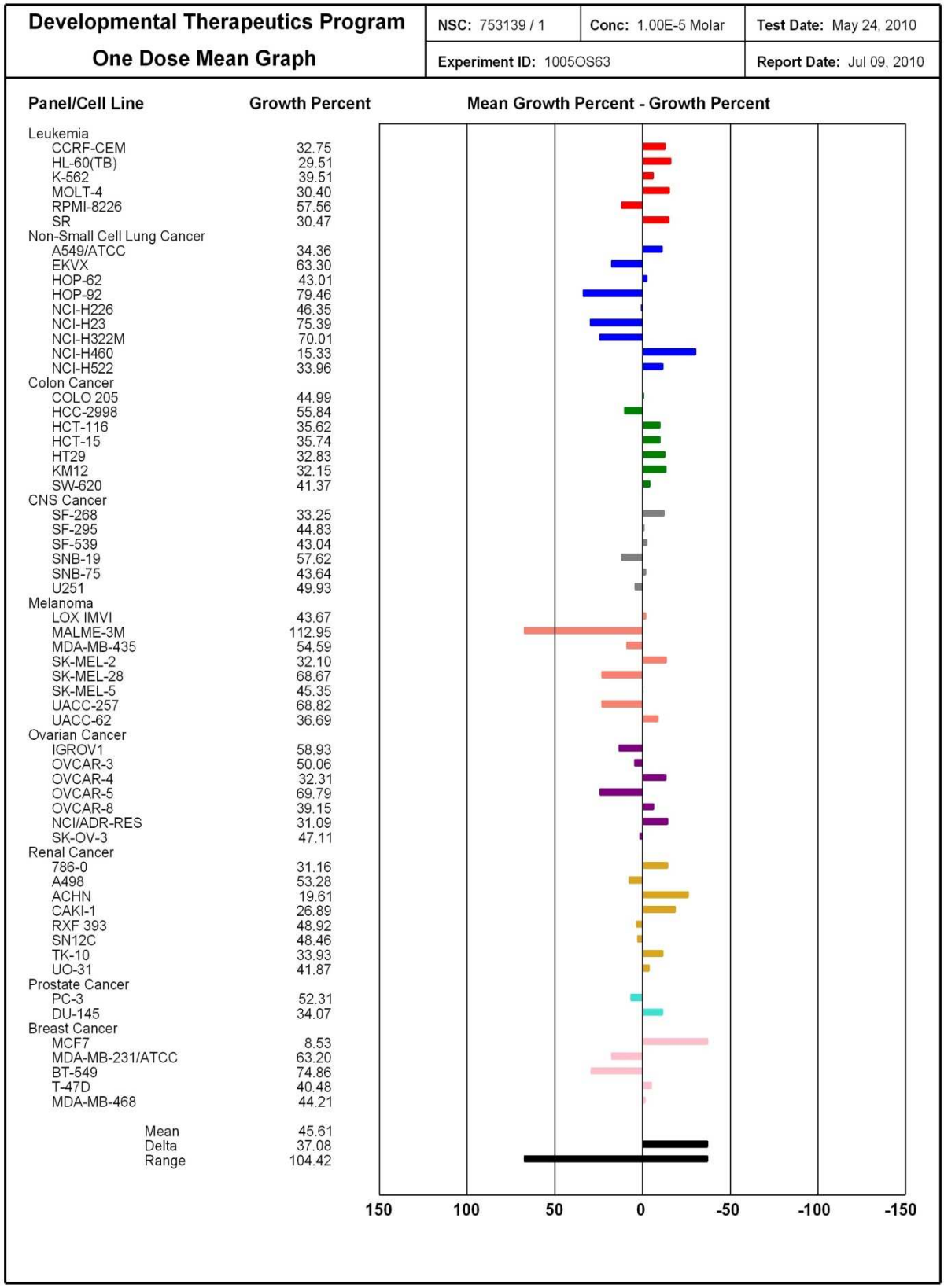


Table 2: One Dose mean graph of S_2

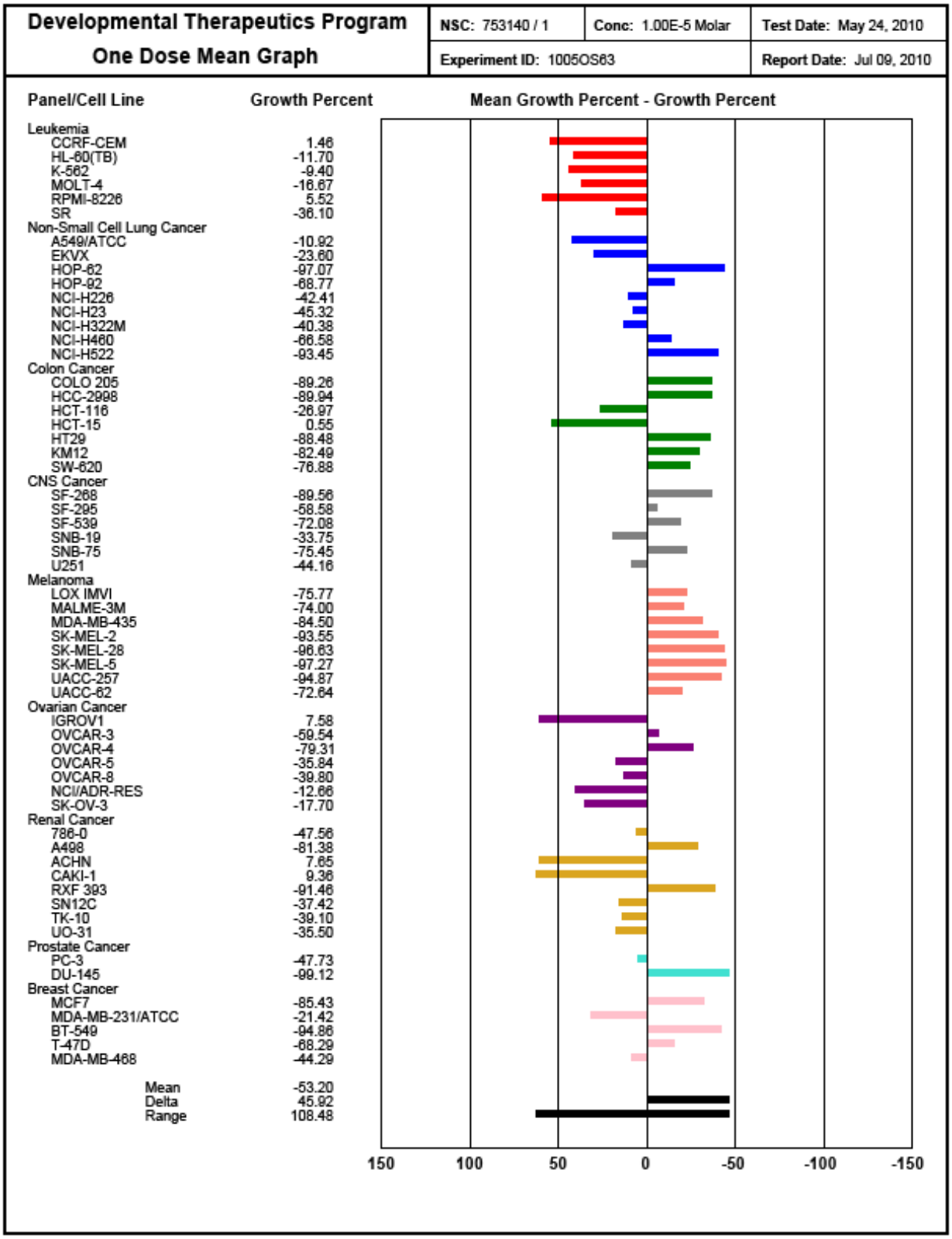


Table 3: One Dose mean graph for S_3

Developmental Therapeutics Program	NSC: 754928 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 06, 2010
One Dose Mean Graph	Experiment ID: 1012OS69		Report Date: Jan 07, 2011

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	68.97
K-562	64.10
MOLT-4	41.25
RPMI-8226	61.56
SR	51.41
Non-Small Cell Lung Cancer	
A549/ATCC	64.58
EKVX	51.14
HOP-62	51.78
HOP-92	58.46
NCI-H226	84.54
NCI-H23	81.02
NCI-H322M	52.19
NCI-H460	36.39
NCI-H522	78.35
Colon Cancer	
COLO 205	52.92
HCC-2998	62.57
HCT-116	73.78
HCT-15	42.64
KM12	65.94
SW-620	70.05
CNS Cancer	
SF-268	54.06
SF-295	45.38
SF-539	76.64
SNB-19	70.16
SNB-75	92.36
U251	53.30
Melanoma	
LOX IMVI	43.09
MALME-3M	102.53
M14	78.74
MDA-MB-435	70.13
SK-MEL-2	104.21
SK-MEL-28	79.72
SK-MEL-5	71.53
UACC-257	91.73
UACC-62	66.38
Ovarian Cancer	
IGROV1	64.66
OVCAR-3	56.96
OVCAR-4	35.97
OVCAR-5	70.76
OVCAR-8	75.44
NCI/ADR-RES	62.66
SK-OV-3	76.25
Renal Cancer	
786-0	75.74
A498	76.50
ACHN	43.36
CAKI-1	29.01
RXF 393	96.32
SN12C	68.66
TK-10	67.04
UO-31	34.23
Prostate Cancer	
PC-3	63.43
DU-145	67.07
Breast Cancer	
MCF7	58.51
MDA-MB-231/ATCC	49.93
HS 578T	78.09
BT-549	78.55
T-47D	74.89
MDA-MB-468	58.70
Mean	65.11
Delta	36.10
Range	75.20

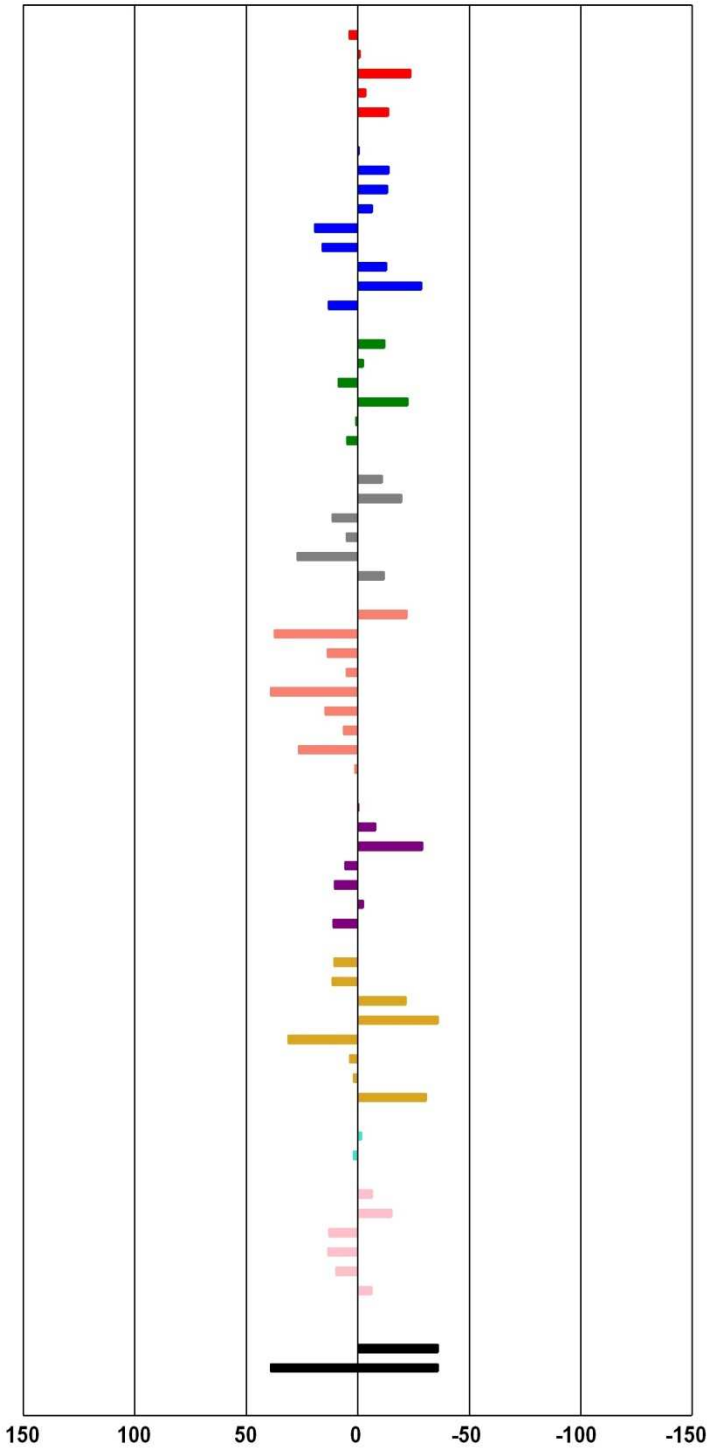


Table 4: One Dose mean graph for S_5

Developmental Therapeutics Program

One Dose Mean Graph

NSC: 754929 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 06, 2010
Experiment ID: 1012OS69		Report Date: Jan 07, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	20.39
HL-60(TB)	95.25
K-562	85.27
MOLT-4	64.18
RPMI-8226	69.61
SR	63.00
Non-Small Cell Lung Cancer	
A549/ATCC	92.81
EKVX	65.25
HOP-62	80.09
HOP-92	76.70
NCI-H226	99.38
NCI-H23	87.49
NCI-H322M	85.25
NCI-H460	81.25
NCI-H522	85.24
Colon Cancer	
COLO 205	96.95
HCC-2998	84.43
HCT-116	84.65
HCT-15	62.18
KM12	78.82
SW-620	92.83
CNS Cancer	
SF-268	76.85
SF-295	69.40
SF-539	81.47
SNB-19	84.91
SNB-75	82.44
U251	81.81
Melanoma	
LOX IMVI	62.51
MALME-3M	121.26
M14	90.71
MDA-MB-435	90.53
SK-MEL-2	96.67
SK-MEL-28	90.99
SK-MEL-5	81.92
UACC-257	99.07
UACC-62	84.79
Ovarian Cancer	
IGROV1	84.64
OVCAR-3	76.10
OVCAR-4	57.39
OVCAR-5	93.74
OVCAR-8	87.35
NCI/ADR-RES	79.08
SK-OV-3	80.20
Renal Cancer	
786-0	94.00
A498	84.92
ACHN	75.11
CAKI-1	58.17
RXF 393	91.28
SN12C	76.57
TK-10	90.97
UO-31	64.86
Prostate Cancer	
PC-3	77.74
DU-145	92.36
Breast Cancer	
MCF7	66.84
MDA-MB-231/ATCC	62.40
HS 578T	90.60
BT-549	84.57
T-47D	93.47
MDA-MB-468	97.12
Mean	81.45
Delta	61.06
Range	100.87

Mean Growth Percent - Growth Percent

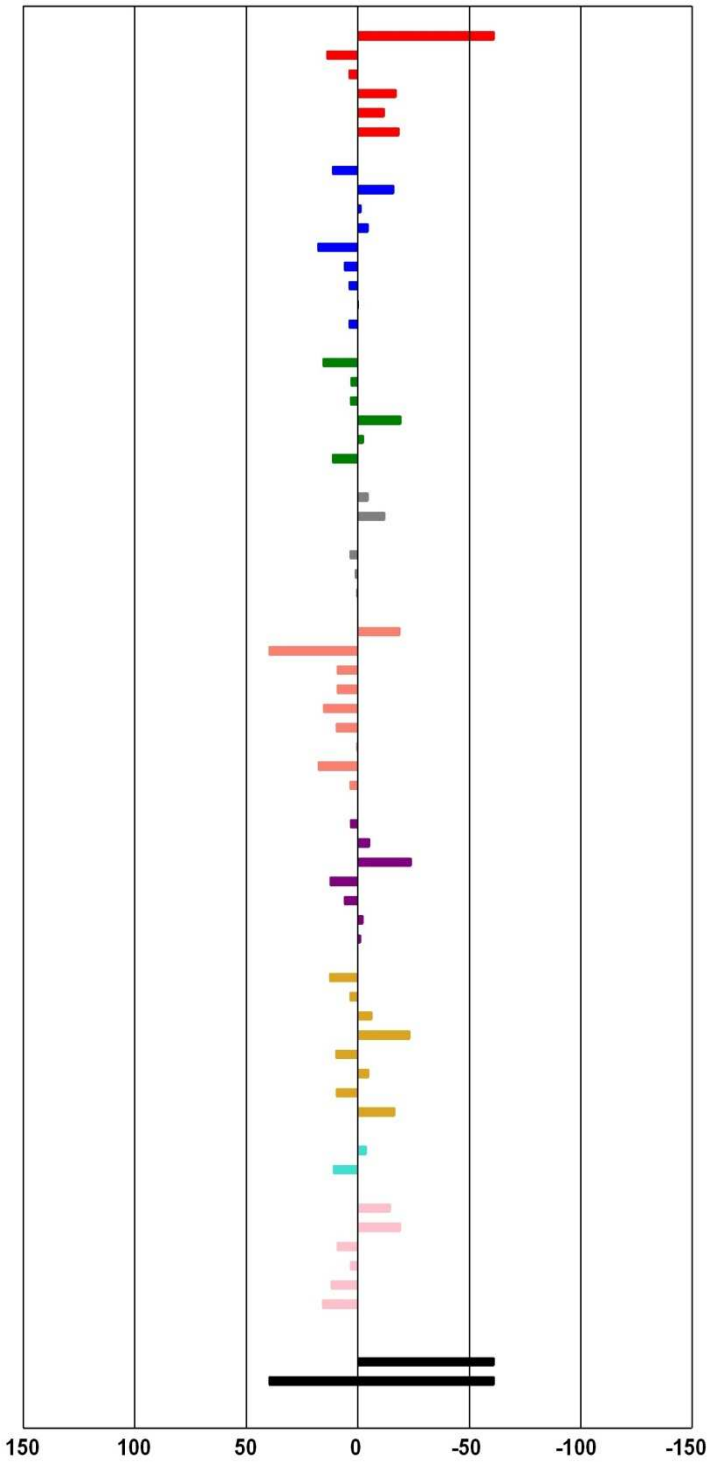


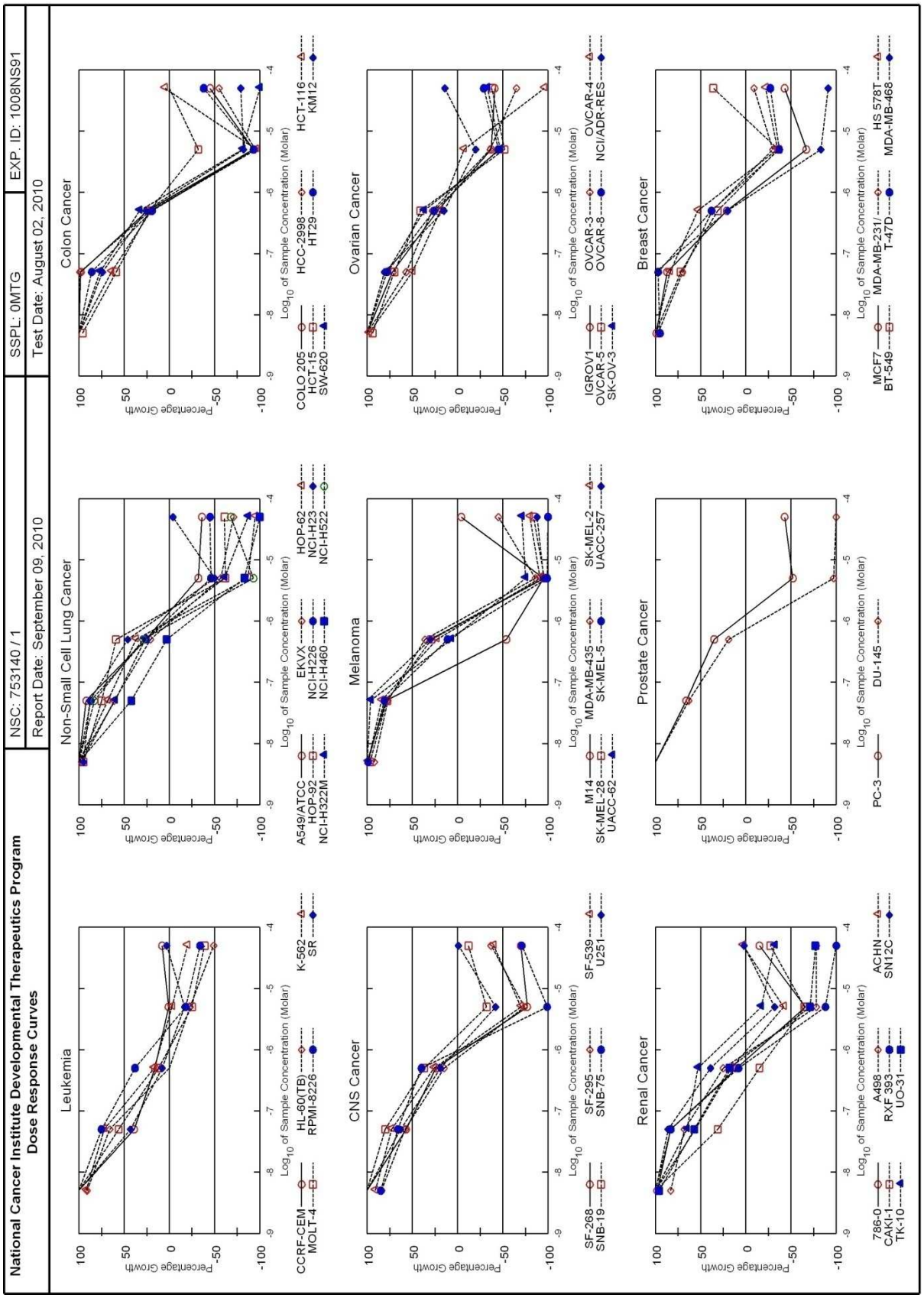
Table 5: *in vitro* testing results for five dosages for S_2

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 753140 / 1	Experiment ID : 1008NS91	Test Type : 08	Units : Molar
Report Date : September 09, 2010	Test Date : August 02, 2010	QNS :	MC :
COMI : S2 (92491)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0MTG	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.385	1.623	1.640	0.864	0.586	0.400	0.483	101	39	16	1	8	3.30E-8	> 5.00E-5	> 5.00E-5
HL-60(TB)	0.671	2.158	2.027	1.659	0.677	0.517	0.344	91	66	.	-23	-49	8.87E-8	5.20E-7	> 5.00E-5
K-562	0.287	2.264	2.102	1.702	0.621	0.279	0.231	92	72	17	-3	-20	1.24E-7	3.61E-6	> 5.00E-5
MOLT-4	0.617	2.412	2.446	1.624	0.837	0.463	0.379	102	56	12	-25	-39	6.88E-8	1.07E-6	> 5.00E-5
RPMI-8226	0.719	1.929	1.991	1.633	1.177	0.587	0.472	105	75	38	-18	-34	2.37E-7	2.36E-6	> 5.00E-5
SR	0.262	1.151	1.223	0.642	0.338	0.219	0.290	108	43	8	-17	3	3.88E-8	.	> 5.00E-5
Non-Small Cell Lung Cancer															
A549/ATCC	0.320	1.551	1.680	1.453	0.642	0.219	0.205	110	92	26	-32	-36	2.17E-7	1.41E-6	> 5.00E-5
EKVX	0.595	1.489	1.462	1.142	0.783	0.257	0.176	97	61	21	-57	-71	9.50E-8	9.31E-7	4.09E-6
HOP-62	0.372	0.952	0.976	0.765	0.587	0.049	0.020	104	68	37	-87	-95	1.90E-7	9.96E-7	2.52E-6
HOP-92	1.012	1.652	1.621	1.492	1.388	0.386	0.396	95	75	59	-62	-61	5.91E-7	1.53E-6	3.98E-6
NCI-H226	0.988	2.119	2.148	1.967	1.277	0.536	0.547	103	87	26	-46	-45	1.99E-7	1.14E-6	> 5.00E-5
NCI-H23	0.532	1.930	1.864	1.763	1.178	0.269	0.509	95	88	46	-50	-4	4.06E-7	1.52E-6	> 5.00E-5
NCI-H322M	0.606	1.454	1.491	1.111	0.834	0.237	0.076	104	60	27	-61	-87	9.80E-8	1.01E-6	3.75E-6
NCI-H460	0.218	1.728	1.784	0.856	0.258	0.037	-0.011	104	42	3	-83	-100	3.74E-8	5.37E-7	2.06E-6
NCI-H522	0.571	1.414	1.414	1.270	0.815	0.040	0.185	100	83	29	-93	-68	2.03E-7	8.64E-7	2.22E-6
Colon Cancer															
COLO 205	0.276	0.936	0.996	0.922	0.436	0.020	0.151	109	98	24	-93	-45	2.23E-7	8.04E-7	.
HCC-2998	1.359	3.331	3.327	3.308	1.781	0.067	0.607	100	99	21	-95	-55	2.14E-7	7.63E-7	2.05E-6
HCT-116	0.170	1.194	1.219	0.824	0.375	0.007	0.219	102	64	20	-96	5	1.04E-7	.	.
HCT-15	0.343	2.501	2.409	1.611	0.821	0.234	0.343	96	59	22	-32	.	8.66E-8	5.00E-5	> 5.00E-5
HT29	0.196	1.255	1.301	1.109	0.394	0.014	0.122	104	86	19	-93	-38	1.72E-7	7.35E-7	.
KM12	0.391	1.631	1.699	1.310	0.710	0.072	0.081	105	74	26	-82	-79	1.57E-7	8.68E-7	2.53E-6
SW-620	0.201	1.059	1.055	0.860	0.488	0.038	-0.014	100	77	33	-81	-100	2.07E-7	9.77E-7	2.67E-6
CNS Cancer															
SF-268	0.448	1.396	1.398	0.984	0.676	0.102	0.136	100	57	24	-77	-70	7.93E-8	8.63E-7	2.69E-6
SF-295	0.476	1.668	1.483	1.167	0.649	0.122	0.300	84	58	15	-74	-37	7.62E-8	7.28E-7	.
SF-539	0.642	2.179	2.048	1.759	1.040	0.192	0.387	91	73	26	-70	-40	1.53E-7	9.30E-7	.
SNB-19	0.546	1.793	1.830	1.539	1.010	0.369	0.482	103	80	37	-32	-12	2.50E-7	1.71E-6	> 5.00E-5
SNB-75	0.371	0.815	0.749	0.664	0.550	0.004	0.107	85	66	40	-99	-71	2.10E-7	9.74E-7	2.23E-6
U251	0.257	1.252	1.258	0.888	0.444	0.150	0.255	101	63	19	-42	-1	9.96E-8	1.02E-6	> 5.00E-5
Melanoma															
M14	0.349	1.151	1.161	0.985	0.159	0.016	0.334	101	79	-54	-95	-4	8.28E-8	1.96E-7	.
MDA-MB-435	0.405	1.489	1.407	1.245	0.793	0.056	0.223	92	77	36	-86	-45	2.28E-7	9.81E-7	.
SK-MEL-2	0.802	2.577	2.561	2.293	1.213	0.076	0.162	99	84	23	-91	-80	1.81E-7	7.99E-7	2.20E-6
SK-MEL-28	0.524	1.509	1.482	1.297	0.810	0.021	0.086	97	78	29	-96	-84	1.88E-7	8.53E-7	2.14E-6
SK-MEL-5	0.626	2.413	2.403	2.076	0.821	0.009	-0.076	99	81	11	-99	-100	1.39E-7	6.29E-7	1.80E-6
UACC-257	0.613	1.394	1.482	1.404	0.845	0.028	0.074	111	101	30	-95	-88	2.60E-7	8.63E-7	2.17E-6
UACC-62	0.763	2.578	2.630	2.498	0.899	0.193	0.219	103	96	7	-75	-71	1.65E-7	6.17E-7	2.50E-6
Ovarian Cancer															
IGROV1	0.398	1.225	1.355	0.997	0.583	0.251	0.233	116	72	22	-37	-41	1.40E-7	1.19E-6	> 5.00E-5
OVCAR-3	0.472	1.217	1.292	0.899	0.679	0.304	0.164	110	57	28	-36	-65	8.86E-8	1.37E-6	1.52E-5
OVCAR-4	0.531	1.347	1.329	0.936	0.689	0.494	0.022	98	50	19	-7	-96	4.91E-8	2.70E-6	1.52E-5
OVCAR-5	0.532	1.562	1.500	1.252	0.958	0.254	0.330	94	70	41	-52	-38	2.49E-7	1.38E-6	.
OVCAR-8	0.375	1.626	1.682	1.350	0.707	0.208	0.267	104	78	26	-45	-29	1.74E-7	1.18E-6	> 5.00E-5
NCI/ADR-RES	0.434	1.584	1.603	1.368	0.609	0.346	0.592	102	81	15	-20	14	1.48E-7	.	> 5.00E-5
SK-OV-3	0.522	1.152	1.195	1.016	0.758	0.279	0.352	107	78	37	-47	-33	2.46E-7	1.39E-6	> 5.00E-5
Renal Cancer															
786-0	0.588	2.029	1.996	1.779	0.731	0.200	0.502	98	83	10	-66	-15	1.41E-7	6.75E-7	.
A498	1.267	1.814	1.720	1.641	1.401	0.284	0.285	83	68	25	-78	-78	1.31E-7	8.69E-7	2.68E-6
ACHN	0.321	1.573	1.600	1.024	0.527	0.187	0.363	102	56	16	-42	3	7.14E-8	.	> 5.00E-5
CAKI-1	0.555	1.760	1.775	0.928	0.472	0.196	0.406	101	31	-15	-65	-27	2.68E-8	2.36E-7	.
RXF 393	0.621	1.143	1.157	1.055	0.665	0.073	-0.024	103	83	8	-88	-100	1.38E-7	6.10E-7	2.01E-6
SN12C	0.531	2.064	2.100	1.848	1.134	0.364	0.558	102	86	39	-32	2	2.95E-7	.	> 5.00E-5
TK-10	0.640	1.426	1.429	1.151	1.060	0.534	0.436	100	65	53	-17	-32	5.60E-7	2.90E-6	> 5.00E-5
UO-31	0.433	1.228	1.195	0.883	0.576	0.125	0.100	96	57	18	-71	-77	7.38E-8	7.95E-7	2.90E-6
Prostate Cancer															
PC-3	0.413	1.513	1.521	1.142	0.800	0.198	0.234	101	66	35	-52	-43	1.67E-7	1.27E-6	.
DU-145	0.330	1.113	1.173	0.826	0.482	0.009	-0.039	108	63	19	-97	-100	1.00E-7	7.33E-7	1.97E-6
Breast Cancer															
MCF7	0.276	1.578	1.568	1.413	0.556	0.092	0.157	99	87	21	-67	-43	1.84E-7	8.76E-7	.
MDA-MB-231/ATCC	0.542	1.485	1.454	1.201	0.895	0.379	0.493	97	70	37	-30	-9	2.05E-7	1.79E-6	> 5.00E-5
HS 578T	0.877	1.758	1.757	1.619	1.346	0.567	0.683	100	84	53	-35	-22	5.44E-7	1.99E-6	> 5.00E-5
BT-549	0.677	1.378	1.375	1.179	0.897	0.432	0.930	100	72	31	-36	36	1.72E-7	.	> 5.00E-5
T-47D	0.529	1.270	1.230	1.244	0.808	0.333	0.388	95	97	38	-37	-27	3.09E-7	1.59E-6	> 5.00E-5
MDA-MB-468	0.523	1.149	1.181	1.165	0.649	0.089	0.046	105	102	20	-83	-91	2.16E-7	7.83E-7	2.39E-6

Table 6: Dose response curves against different cancer cell lines for S_2



In renal cancer, RXF-393 showed 100% growth inhibition. While this compound expressed moderate activity for A498 (78%), UO-31 (77%) and TK-10 (32%) cell lines.

In prostate cancer, 100% inhibition observed for DU-145. While, 40% inhibition was observed for PC-3 cell line.

In breast cancer, 91% growth inhibition was shown for MDA-MB-468 cell line. Other cell lines MCF7 (43%) and T-47D (27%) were moderately inhibited by the above compound.

CONCLUSION

In this Research article, we report convenient and practical procedure for the preparation of some thiosemicarbazones of the 1-(5-chloro-1H-benzimidazol-2-yl) ethanone by using green reaction conditions, simple workup with good yield. As well as their anti-cancer activity in which **S_2** show very good activity, remaining all derivatives are moderately active.

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