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Graphical Abstract

Synthesis, Cytostatic Evaluation and Structure Activity Relationships of Novel Bisindolylmethanes and their Corresponding Tetrahydroindolocarbazoles.

Mardia T. El Sayed[a,b,*], Khadiga M. Ahmed[c], Kazem Mahmoud[a], and Andreas Hilgeroth[a].

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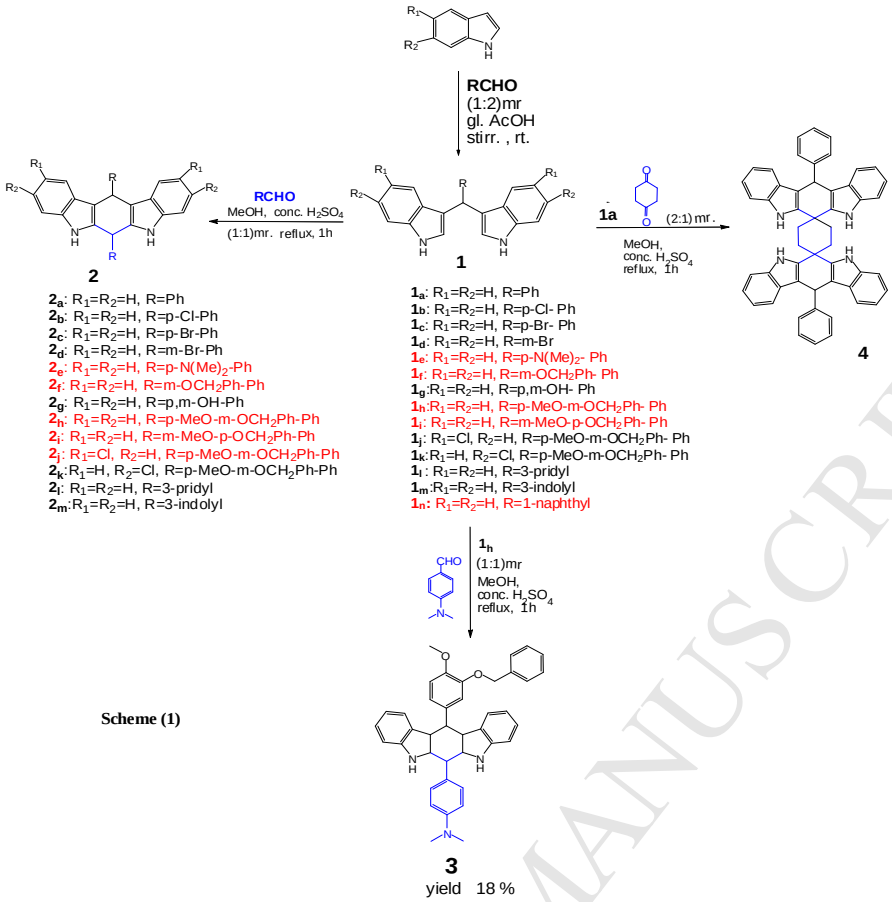
Dear Sir,

BIMs (bis-indolylmethanes) (**1_{a-n}**) were synthesized *via* using glacial acetic acid as a protic acid for promotion of the condensation reaction of indoles with aldehydes in high yields (86-98 %). Corresponding tetrahydroindolo[2,3-*b*]carbazoles (**2_{a-m}**) were synthesized *via* condensation of BIMs with aldehydes. Ten synthesized compounds have been submitted to the national cancer institute in the USA where all the submitted samples have been selected for *one dose screening*. As a result of the *one dose screening* of BIMs (**$1_{e,f,h,i,n}$**) and of the indolocarbazoles (**$2_{e,f,h,i,j}$**) the average highest cytostatic effects was recorded here for the BIM **1_h** and the indolocarbazole (**2_e**) that showed the lowest mean values of "47.39 %" and of "21.63 %" respectively. Both compounds (**1_h** and **2_e**) were further tested in *five dose screening* with the tested substance (**1_h**) being significantly more sensitive for several cancers cell line as corresponding to their GI_{50} values. Furthermore, the basically substituted derivative **2_e** showed the highest antipoliferative activity in a nanomolar scale towards the three selected cancers cell lines Non small lung cell NCI-H460 with GI_{50} "616 nM", Ovarian Cancer cell line OVCAR-4 with GI_{50} "562 nM" and Breast Cancer cell line MCF7 with GI_{50} "930 nM".

The present research proved that, all synthesized BIMs (**$1_{e,f,h,i,n}$**) showed best activities in the same cell lines MOLT-4 in leukaemia cell line and in IGROV1 in an ovarian cancer cell line. Also the basically substituted derivative demonstrates good activity in the renal cancer cell lines CAKI-1 and UO-31. The TGI and LC_{50} values were higher than 100 µM so the compound **1_h** showed noncritical cytotoxic properties. The basically substituted derivative **2_e** gave the highest antipoliferative activity in a nanomolar ranges in selected cell lines with noncritical cytotoxic properties (17 fold higher LC_{50} "34.6 µM" than GI_{50} "2 µM" values). Further SAR modifications in compounds **1_h** and **2_e** that are under investigation in our lab to discover more potent antitumor agents.



2_a: R_1=R_2=H, R=Ph
2_b: R_1=R_2=H, R=p-Cl-Ph
2_c: R_1=R_2=H, R=p-Br-Ph
2_d: R_1=R_2=H, R=m-Br-Ph
2_e: R_1=R_2=H, R=p-N(Me)$_2$-Ph
2_f: R_1=R_2=H, R=m-OCH_2Ph-Ph
2_g: R_1=R_2=H, R=p,m-OH-Ph
2_h: R_1=R_2=H, R=p-MeO-m-OCH_2Ph-Ph
2_i: R_1=R_2=H, R=m-MeO-p-OCH_2Ph-Ph
2_j: R_1=Cl, R_2=H, R=p-MeO-m-OCH_2Ph-Ph
2_k:R_1=H, R_2=Cl, R=p-MeO-m-OCH_2Ph-Ph
2_l: R_1=R_2=H, R=3-pridyl
2_m:R_1=R_2=H, R=3-indolyl

1_a: R_1=R_2=H, R=Ph
1_b: R_1=R_2=H, R=p-Cl- Ph
1_c: R_1=R_2=H, R=p-Br- Ph
1_d: R_1=R_2=H, R=m-Br
1_e: R_1=R_2=H, R=p-N(Me)$_2$- Ph
1_f: R_1=R_2=H, R=m-OCH_2Ph- Ph
1_g:R_1=R_2=H, R=p,m-OH- Ph
1_h:R_1=R_2=H, R=p-MeO-m-OCH_2Ph- Ph
1_i: R_1=R_2=H, R=m-MeO-p-OCH_2Ph- Ph
1_j: R_1=Cl, R_2=H, R=p-MeO-m-OCH_2Ph- Ph
1_k:R_1=H, R_2=Cl, R=p-MeO-m-OCH_2Ph- Ph
1_l : R_1=R_2=H, R=3-pridyl
1_m:R_1=R_2=H, R=3-indolyl
1_n: R_1=R_2=H, R=1-naphthyl

3
yield 18 %

Scheme (1)

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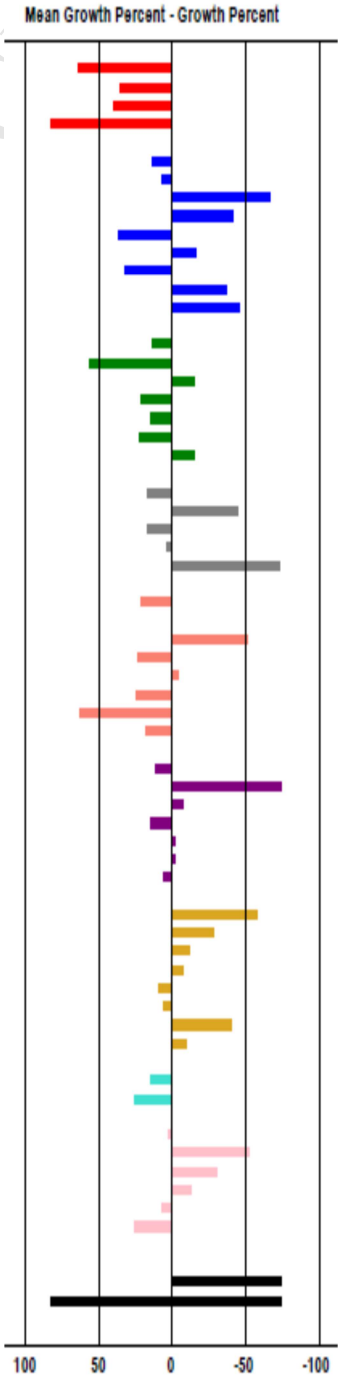

Conflict of interest
The Author(s) declare(s) that they have no conflicts of interest to disclose.

Statement of significance

The present research proved that, all synthesized BIMs (**1**$_{e,f,h,i,n}$) showed best activities in the same cell lines MOLT-4 in leukaemia cell line and in IGROV1 in an ovarian cancer cell line. Also the basically substituted derivative demonstrates good activity in the renal cancer cell lines CAKI-1 and UO-31. The TGI and LC_{50} values were higher than 100 µM so the compound **1**$_h$ showed noncritical cytotoxic properties. The basically substituted derivative **2**$_e$ gave the highest antiproliferative activity in a nanomolar ranges in selected cell lines with noncritical cytotoxic properties (17 fold higher LC_{50} "34.6 µM" than GI_{50} "2 µM" values). Further SAR modifications in compounds **1**$_h$ and **2**$_e$ that are under investigation in our lab wishing to discover more potent antitumor agents.

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Bis-indolylmethanes; tetrahydroindolo[2,3-b]carbazoles; one dose screening; five dose screening; antipoliferative activity; nanomolar scale.

Abstract

BIMs (bis-indolylmethanes) (**1$_{a-n}$**) were synthesized using glacial acetic acid as a protic acid for promotion of the condensation reaction of indoles with aldehydes in high yields (86-98 %). Corresponding tetrahydroindolo[2,3-*b*]carbazoles (**2$_{a-m}$**) were synthesized *via* condensation of BIMs with aldehydes. Ten synthesized compounds have been submitted to the national cancer institute in the USA where all the submitted samples have been selected for *one dose screening*. As a result of the *one dose screening* of BIMs (**1$_{e,f,h,i,n}$**) and of the indolocarbazoles (**2$_{e,f,h,i,j}$**) the average highest cytostatic effects was recorded here for the BIM **1$_h$** and the indolocarbazole (**2$_e$**) that showed the lowest mean values of "47.39 %" and of "21.63 %" respectively. Both compounds (**1$_h$** and **2$_e$**) were further tested in *five dose screening* with the tested substance (**1$_h$**) being significantly more sensitive for several cancers cell line as corresponding to their GI_{50} values. Furthermore, the basically substituted derivative **2$_e$** showed the highest antipoliferative activity in a nanomolar scale towards the three selected cancers cell lines Non small lung cell NCI-H460 with GI_{50} "616 nM", Ovarian Cancer cell line OVCAR-4 with GI_{50} "562 nM" and Breast Cancer cell line MCF7 with GI_{50} "930 nM".

Introduction

In recent years a considerable attention has been paid on the synthetic ways leading to indole derivatives because of their biological activities. Various indole derivatives, such as 3-substituted indoles, are common components of drugs and are generally found to be of pharmaceutical interest in a variety of therapeutic areas [1]. In addition, 3-substituted indole derivatives are also versatile intermediates in organic synthesis [2], due to the feasibility of their 3-position for an electrophilic substitution. The electrophilic substitution reactions of indoles with aromatic aldehydes afford corresponding BIMs. Several catalysts such as protic acids [3-6], Lewis acids [7-10], ionic liquids [11], and others are used to promote these reactions. The 3-position of indole is the preferred site for the electrophilic substitution reactions. A simple and direct method for the synthesis of 3-alkylated indole derivatives involves the condensation of indoles or its substituted derivatives with electrophilies (aldehydes or ketones or imines). Aldehydes either aliphatic or aromatic

are the most important and widely used electrophiles in such reactions. Bisindolylalkane derivatives are found in bioactive metabolites of terrestrial and marine origin. Recently, *Maciejewska* et al. [12] used DNA-based electrochemical biosensors to prove that bis(5-methoxyindol-3-yl)methane [13] considerably reduces the growth of cancer cell lines such as HOP-92 (lung), A498 (renal) and MDAMB-231/1TCC (breast) Their results also indicate that BIMs could potentially be applied as chemotherapeutic agents against tumors [14]. It has been reported that, DIM-C-*p*-PhC_6H_5 substituted in the phenyl ring with a para-*t*-butyl, trifluoromethyl (DIM-C-*p*-$PhCF_3$) substituent and indole ring-substituted analogs are selective PPARγ modulators [15] in several cancer cell lines with high antiproliferative activity [16 - 25]. Other study investigated the antileukaemic activity and molecular mechanisms of action of a newly synthesized ring-substituted diindolylmethane derivative, 1,1-bis[3′-(5-methoxyindolyl)]-1-(*p*-t-butylph- enyl) methane in acute myelogenous leukemia (AML) cells [26]. Inaddition, Indolocarbazoles have been reported as a primary compound for the synthesis of various drugs with important biological, pharmacological, and medicinal activities [27-32]. Indolocarbazoles are associated with anticancer, antimicrobial, and antifungal activities. In most cases biological activity is correlated with indolocarbazoles containing heteroatoms. The biological activity depends on the interaction potential with DNA [33,34]. Furthermore, many experimental studies have indicated that the size, shape and planarity of this structure are important criteria in such DNA interaction [35].

Result and Discussion

All the submitted compounds [BIMs (**$1_{e,f,h,i,n}$**) and the indolocarbazoles **($2_{e,f,h,i,j}$)]** to National Cancer Institute (NCI), USA, have been selected by the NCI for anticancer screening. The tumor growth inhibition properties of the ten compounds **1_e, 1_f, 1_h, 1_i, 1_n, 2_e, 2_f, 2_h, 2_i** and **2_j** with the NCI codes **NSC D-755521/1, D-755518/1, D-755517/1, D-755519/1, D-755520/1, D-758513/1, D-758511/1, D-758510/1, D-758512/1** and **D-758514/1** were evaluated. The selected compounds were screened on human tumor cell lines at 10^{-5} M at the 60-Cell-Line Screenings of the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI, Bethesda, Maryland, USA) under the drug discovery program of the NCI. Among the selected 10 compounds the two compounds **1_h** (**NSC D- 755517/1**) and **2_e** (**D-758513/1**) were further screened for five-log dose molar range as they have shown prominent cell growth inhibition at 10^{-5} M concentration against variety of cancers cell lines. All the one dose mean graphs, the superposition curves and the dose response curves are included in the electronic supplementary file. The 60-cell-line-screening of the NCI includes 60 different tumor cell lines, the nine various organs and tumor types derived (leukaemia, non-small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer).

Chemistry

Many procedures for the synthesis of BIMs have been published by varying the nature of the catalyst used. Generally, BIMs are synthesized by a reaction analogous to the *Ehrlich test*, where indoles react with aliphatic or aromatic aldehydes or ketones in presence of acid catalyst to give *azafulven* [36,37,38], which undergoes further addition with the second indole molecule to afford BIMs. In view of our previous work performed in our lab using glacial acetic acid as a protic acid for efficient promotion of the condensation reaction of indoles with different types of aldehydes, we have synthesized the BIMs *via* using glacial acetic acid with indoles and aromatic aldehydes. In brief, glacial acetic acid

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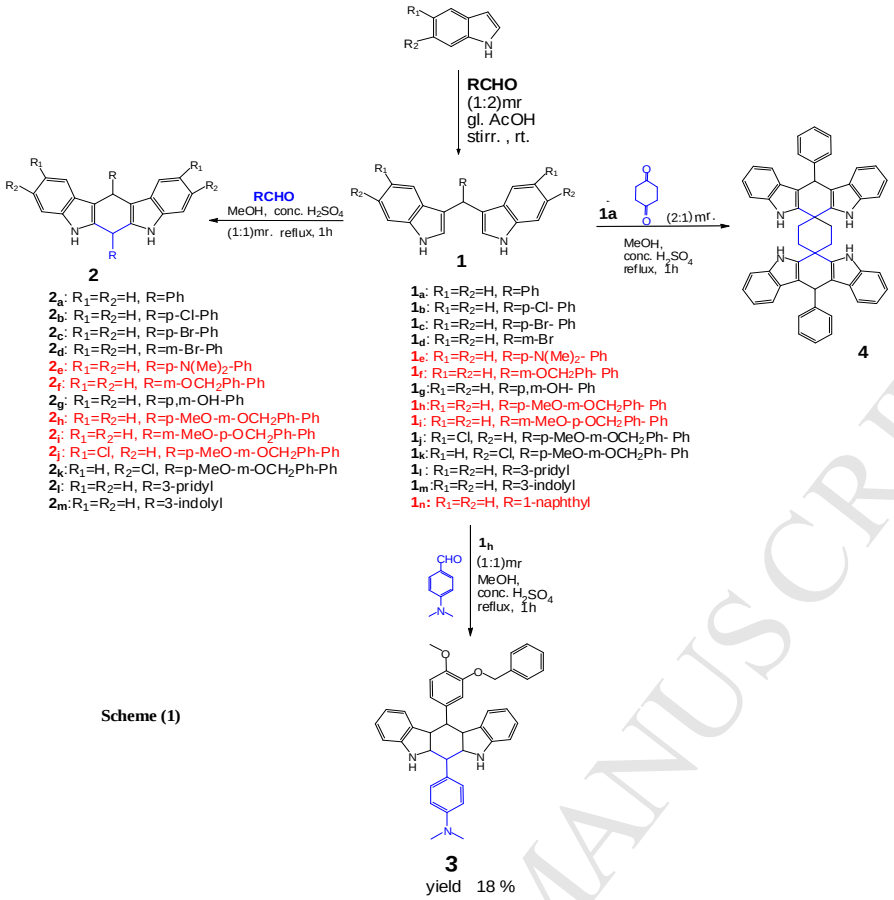

Scheme (1)

without solvent was used to catalyze the reaction of indoles (two equivalent moles) and aryl or heteroaryl aldehydes (one equivalent mole). With our new method *via* glacial acetic acid as a solvent the corresponding BIMs were formed in a high yields (86-98 %) and after a few hours (4-6) hours of stirring at room temperature. Some BIMs (**1**$_{a,b,c,d,e,k,n}$) are known [39-45], their identities were proven by means of MS, NMR, IR spectra, and the other BIMs, (**1**$_{f,g,h,i,j,l}$), are novel and could not be found in the literature. The short reaction time and the simplicity of the reaction procedure makes this method one of the most efficient methods for the synthesis of this class of compounds.

As an extending study of our present lab work, we used the prepared BIMs **1**$_{a-n}$ as a starting materials for the synthesis of biologically active tetrahydroindolo[2,3-*b*]carbazoles of type **2**$_{a-m}$, **3** and the extended spirocyclic biscarbazoles **4**. The reaction has been done according to the few reported cases of condensation of BIMs with aldehydes or ketones [46] in which the BIM and the aromatic aldehyde (the same aldehyde which condensed with indoles in the synthesis of the used BIM) in molar ratios (1:1) were dissolved in methanol and few drops of conc. H_2SO_4 were added dropwisly. The mixture was refluxed under stirring for about 1 h. The product precipitated and was isolated from the reaction mixture while the solution is hot yielding few amounts of the pure tetrahydroindolo[2,3-*b*]carbazoles of type **2**$_{a-m}$. The rest of the compounds **2**$_{a-m}$ could be extracted and purified from the reaction mixture affording the second crop in good to better yields, scheme (1). The formation of the tetrahydroindolo[2,3-*b*]carbazoles (**2**$_{a-m}$) was due to the fact that a cyclizative condensation can occur by an acid catalyzed nucleophilic attack of an indole nucleus at the 2-position, when the 2-position is free. The unsubstituted

2-position of the two indole nucleus in BIMs can react with a carbonyl group of either aldehydes or ketones, affording the corresponding tetrahydroindolo[2,3-*b*]carba--zoles. The ^{1}H-NMR of compounds $\mathbf{2_{a-m}}$ showed the two aliphatic CH protons as a single signal at δ between 5.50 to 5.90 ppm. The acid catalyzed condensation of indoles with aldehydes has been reported as a method for the preparation of substituted isomers of tetrahydroindolo[3,2-*b*]carbazole (*trans* isomer) and tetrahydroindolo[2,3-*b*]carbazoles (*cis*-isomer), figure (1) in the presence of phosphoryl chloride as the acid catalyst [47]. However the products are not stable under this reaction condition where they readily converted *via* oxidation with air to the dihydroindolocarbazoles. The formation of the *trans* isomer has also recently confirmed and published by *Rong Gu* and et al. [47a]. However the reaction was accomplished using indoles with aromatic aldehydes in (1:1) molar ratio in presence of 2 mol % of iodine as a catalyst in acetonitrile under reflux affording 6,12-trans–isomer which was confirmed by x-ray crystallography, figure (1). This behaviour is due to the presence of the free 2- and 3-positions of the indole ring which both of them can undergo a neucleophilic attack at a carbonyl group leading to the expected formation of a mixture of tetrahydroindolo-[2,3-*b*]carbazoles and tetrahydroindolo[3,2-*b*]carbazoles, figure (1). However the reaction of indoles with aromatic aldehydes using iodine as a catalyst is a selective reaction for the preparation of tetrahydroindolo [3,2-*b*]carbazoles and none of the other isomer tetrahydroindolo[2,3-*b*]carbazoles were observed in the reaction mixture. In our reaction using BIMs and aldehydes in methanolic sulfuric acid solution, the cis isomer of tetrahydroindolo[2,3-b]carbazoles ($\mathbf{2_{a-m}}$) were obtained. The products $\mathbf{2_{a-m}}$ which were prepared by this method were found to be more stable than the same products using $POCl_3$ as a catalyst as they were rapidly converted into the oxidized form, figure (1).

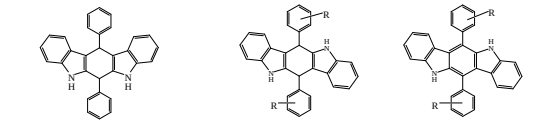

Figure (1): Cis and Trans isomers of indolocarbazoles.

In this context and as a continuation of our work concerning the synthesis of tetrahydroindolo[2,3-*b*]carbazoles, the reaction of BIMs ($\mathbf{1_h}$) (1 mole equivalent) and *p*-dimethylaminobenzaldehyde (1 mole equivalent) has been done by the method of methanol sulphuric solution as a possible route for the synthesis of 4-(8-(3-(benzyloxy)-4-methoxyphenyl)1,1a,2,2a,3,7b,8,8a-octahydro-indolo[2,3-*b*]carbazol-2-yl)-*N*,*N*-dimethylani-lin (**3**). TLC of the reaction mixture showed the formation of four products were identified after purification by column chromatography as BIM ($\mathbf{1_e}$) as a main product with a 30 % yield, compound $\mathbf{2_e}$ with a 15 % yield, compound $\mathbf{1_h}$ with a yield of 10 % and the formation of desired compound **3** with a 18 % yield. The reaction products were identified by ESI-MS and compared by TLC with all products which have been prepared separately. Our desired compound **3** was confirmed by the means of ^{1}H-NMR, ESI-MS and IR spectra, where the ^{1}H-NMR of **3**, indicated the singlet signal for 2-protons at 5.79 ppm for two aliphatic CH protons. The extended spirocyclic structure (**4**) was synthesized in a better yield of 52 %, by the way of MeOH and conc.H_2SO_4 using BIM ($\mathbf{1_a}$) 2 moles equivalent and 1,4-cyclohexanedione, 1 mole equivalent. The reaction solution turned from pink colour to dark violet by leaving it stirring for one hour under reflux. The product detected, purified and confirmed by means of ESI-MS (m/z: 719.29[M^+-H], EI-MS (720[M^+] 32 %). Its ^{1}H-NMR spectrum showed singlet signal at δ = 5.91 ppm for 2 protons (2CH), and 2 triplet signals every one for 4 protons ($2CH_2$) at δ = 2.03 ppm and 2.27 ppm, also the four NH indole protons appeared at 9.94 ppm as a broad signal. These data proved the similarity

of the structure which confirmed additionally by its ^{13}C-NMR spectrum.

***In vitro* cancer screen**

All the submitted compounds BIMs (**$1_{e,f,h,i,n}$**) and the indolocarbazoles **($2_{e,f,h,i,j}$)** to National Cancer Institute (NCI), USA, have been selected by the NCI for anticancer screening. The tumour growth inhibition properties of the ten compounds **1_e, 1_f, 1_h, 1_i, 1_n, 2_e, 2_f, 2_h, 2_i** and **2_j** with the NCI codes NSC D-755521/1, D-755518/1, D-755517/1, D-755519/1, D-755520/1, D-758513/1, D-758511/1, D-758510/1, D-758512/1 and D-758514/1. The selected compounds were screened on 60-human tumour cell lines at 10^{-5} M at the 60-Cell-Line Screenings of the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI, Bethesda, Maryland, USA) under the drug discovery program of the NCI. Among the selected 10 compounds the two compounds **1_h** (NSC D- 755517/1) and **2_e** (D-758513/1) were further screened for five-log dose molar range as they have shown prominent cell growth inhibition at 10^{-5} M concentration against variety of cancers cell lines. The 60-cell-line-screening of the NCI includes 60 different tumour cell lines, the nine various organs and tumour types derived (leukaemia, non-small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer).

All the five selected BIMs (**$1_{e,f,h,i,n}$**), by the NCI for *in vitro* anticancer assay were evaluated for their anticancer activity. Primary *in vitro* One dose anticancer assay was performed in full NCI 60 cell panel representing leukaemia, melanoma and cancers of lung, colon, brain breast, ovary, kidney and prostate in accordance with the protocol of the NCI, USA. The compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforhodamine B. Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. After obtaining the results for one dose assay, analysis of historical Development Therapeutics Programme (DTP) was performed and compound **1_h** (**NSC D-755517/1**) which satisfied predetermined as effective inhibition criteria was selected for NCI full panel 5 dose assays. The tested BIMs showed a distinctive pattern of selectivity with regard to sensitivity against individual cell lines all the percent growth inhibition and the mean growth percent has been collected in table (1). Compound **1_h** (**NSC D-755517/1**) exhibited broad spectrum cell growth inhibition against leukaemia cancer cell MOLT-4 (growth inhibition 20.53 %), non small lung cancer cell NCI-H460 (growth inhibition 9.25 %), colon cancer cells HCT-116 and HT29 with recorded growth inhibition values 19.91 % and 20.89% respectively, melanoma cancer cell M14 (growth inhibition 19.50 %), ovarian cancer cell IGROV1 (growth inhibition 23.79 %), and renal cancer cells (CAKI-1 and UO-31) with growth inhibition 15.65 % and 18.10 % respectively. This data confirmed that as a result of a Single dose assay concentration of 10^{-5} M the average highest cytostatic effects were recorded for the compound **1_h** (**NSC D-755517/1**) that showed the lowest over all mean value (47.39 %), see maen graph figures in the supporting information file. The two substituted derivatives **1_g** and **1_i** were observed as moderate cytostatic properties with over all mean values 75.51 % and 86.38 % respectively, especially for the cancer cell lines "leukaemia MOLT-4, non small lung cancer NCI-H460, ovarian cancer cell lines IGROVI and OVCAR-3 and renal cancer cell lines CAKI-1 and UO-31" with growth percent values in a range from 58.65 % to 34.07 %. Compounds **1_e** and **1_l** were shown as inactive cytostatics against all selected cell lines with a mean values 101.60 % and 92.63 % respectively. Table (1) showed the sixty human tumour cell line anticancer screening data at single dose assay (10^{-5} M) as percent

growth inhibition of BIMs (**1$_{e,f,h,i,n}$**), see all the figures of the one dose mean graph for all the tested compounds in the supporting information file.

Compound under investigation **1$_h$** (**NSC D-755517/1**) exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between ''1.20 – 9.56 µM'' as shown in table (2). Whereas three cell lines of non small lung cancer cell subpanel namely HOP-62, melanoma cancer cell line MALME-3M and breast cancer cell line HS 578T were found to be insensitive at the highest tested concentration 100 µM therefore a sign of ''>'' is used as prefix to the concentration. With regard to the sensitivity against some individual cell lines, compound **1$_h$** (**NSC D-755517/1**) showed obvious activity toward CNS cancer cell lines SNB-7 and U251, Melanoma cell lines MDA-MB-43 and UACC-62, renal cancer cell lines A498 and RXF 393 and breast cancer cell line MDA-MB-468, (GI_{50} value ranging from 1.20 to 1.87 µM). The criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines toward the test agent) by their individual subpanel MID (the average sensitivity of all cell lines of a particular subpanel toward the test agent). Ratios between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria rated non-selective. As per this criterion, compound under investigation was found to be non selective toward all the cell panels, table (2). The five dose screening for **1$_h$** gave the parameters log GI_{50}, log TGI and log LC_{50}, see mean graph of the five dose in the supporting information file, which are summarized in table (2). In the full NCI screening data report, three additional numbers are printed at the base of each of the three respective mean-graphs provided. These numbers are the MG-MID (Average), the *Delta* and the *Range*. The MG-MID or the average is the calculated logarithmic of a mean panel of GI_{50}, TGI or LC_{50}. The *Delta* is the differences of concentrations with parameters between the most sensitive cell line and the mean. Similarly, the *Range* is the number of log_{10} units by which the delta of the most sensitive line(s) of the panel differs from the delta of the least sensitive lines. On the other hand the given Delta and Range values quite accurately reflect a true range of differential sensitivity among the full panel of cell lines to the compound under investigation. Likewise, the given MG-MID (Average) value quite accurately reflects a true overall panel-average sensitivity of the cell lines to this agent, and therefore is a useful basis for comparison of overall potency of the given agent with related or unrelated compounds. Compound **1$_h$** has average GI_{50} responses at micromolar concentrations (11 µM), cytostatic effects at micromolar concentrations (95.5 µM) and the average cytotoxic effects on cancer cell lines at micromolar concentrations "LC_{50} (100 µM) value which is 10 fold higher than GI_{50} (11 µM) value". Based on all these data compound **1$_h$** showed a high degree of variability in its response. Compound **1$_h$** with GI_{50} values in the micromolar range is more effective than the cytostatic drugs Etoposid, Melphalan and Irinotecan (GI_{50} values of 38.9 µM, 14.5 µM and 14.1 µM respectively) [49$_h$].

We further developed the series of the substituted bis(indolyl)phenylmethanes (BIMs) with the synthesis of new structures as aryl substituted tetrahydroindolo[2,3-*b*]carbazoles to constrain the flexibility of the molecule. The NCI selected five derivatives of these substituted indolocarbazoles for the one-dose screening program at a concentration of 10^{-5} µM. The selected substances (**2$_{e,f,h,i,j}$**) showed a distinctive pattern of selectivity with regard to sensitivity against individual cell lines all the percent growth inhibition and the mean growth percent has been collected in table (3). Compound **2$_e$** exhibited broad spectrum cell growth inhibition against non

small lung cancer cell NCI-H23 (growth inhibition 5.55 %), colon cancer cell lines HCT-116 and SW-620 (growth inhibition 7.08 % and 6.72 %), renal cancer cell line ACHN, CAKI-1 and UO-31 (growth inhibition 9.61 %, 13.72 % and 12.35 % respectively) and breast cancer cell line BT-549 (growth inhibition 8.92 %) at single dose assay concentration of 10^{-5} M. The average highest anticancer activity were scored for the compound **2_e** that showed the lowest mean value (21.63 %). The two substituted derivatives **2_h** and **2_i** were observed as moderate cytostatic properties with mean values of 84.67 % and of 76.84 % respectively and the other two derivatives **2_f** and **2_j** were shown as inactive cytostatics.

Compound under investigation **2_e** (**D-758513/1**) exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between 1.07 and 5.65 µM except the two cancer cell lines non small cell lung cancer NCI-H460 and breast cancer cell line MCF7 with GI_{50} values of 6.22 and 9.29 µM respectively, table (4). From the five- dose screening for **2_e** and similar to compound **1_h** the criterion for selectivity of a compound **2_e** indicated that it also non selective toward the cancer subpanels with selectivity ratio in range of 0.62 - 1.46, table (4). Compound **2_e** has average GI_{50} responses at a micromolar concentrations (2 µM), cytostatic effects at micromolar concentrations (48.9 µM) and the average cytotoxic effects on cancer cell lines at micromolar concentrations (34.6 µM) which is 17 fold higher than GI_{50}. Furthermore, the basically substituted derivative **2_e** gave the highest antiproliferative activity being "nanomolar active" towards the selected cancer cell lines which is non small lung cancer cell line NCI-H460 with GI_{50} = 616 nM and the ovarian cancer cell line OVCAR-4 with GI_{50} = 562 nM with non critical cytotoxic properties. Based on these data we observed that compound **2_e** is more effective than the cytostatic drugs etoposid, melphalan and irinotecan (GI_{50} values of 38.9 µM, 14.5 µM and 14.1 µM respectively).

Structure activity relationship (SAR)

Structure-activity correlation of the synthesized BIMs revealed that, by a

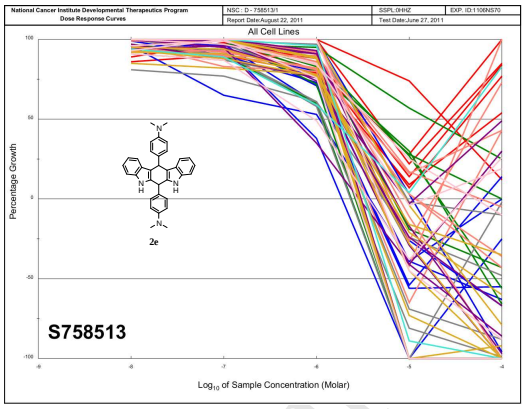

Figure (2): Superposition of all growth curves for compound **2e (nanomolar active).**

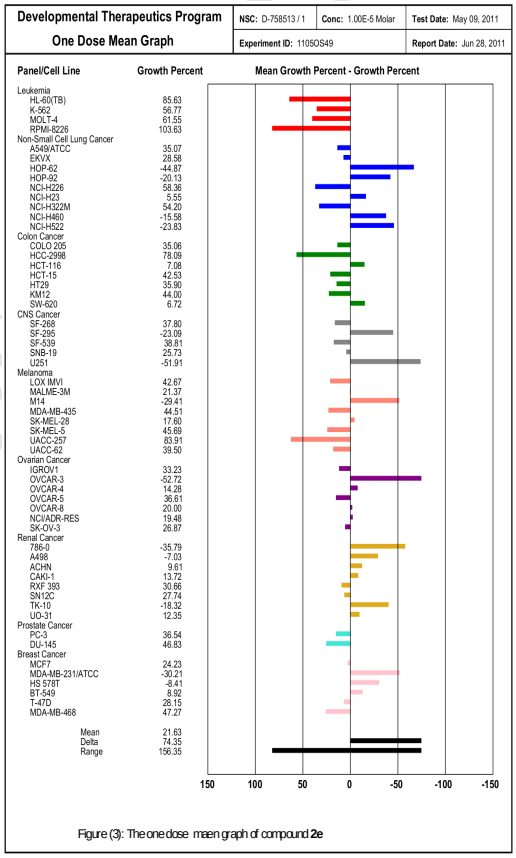

Figure (3): The one dose mean graph of compound **2e**

comparison of **1_i** and **1_h** the position of the functional groups is of great importance of being either *meta* or *para*. Where the *para*-methoxy is much more favourable than *meta* substituent. Moreover the comparison of **1_h** with **1_f** indicated that the methoxy function in addition to a benzyloxy group ensures mainly increased activity. If the methoxy function is positioned in *meta* position the effect is similar concerning no favour of a *meta* methoxy function as indicated by a comparison of **1_i** and **1_f**. The lipophilic fixed substituent in the naphthyl derivative **1_n** is not favourable compared to the rotable benzyloxy substituent in compound **1_f**. BIM **1_e** containing the basic substituent (NMe_2) which was unfavourable

concerning the all over anticancer activities whereas, its activities in a renal cancer cells indicated different anticancer activities comparable to compound **1f**. In conclusion, all BIMs (**1e,f,h,i,n**) showed best activities in the same cell lines MOLT-4 as a leukaemia cell line, IGROV1 and as an ovarian cell line and the cell lines CKAI-1 and UO-31 renal cancer cell lines. Also the basically substituted derivative demonstrates good activity for leukaemia cell line MOLT-4, non small cell lung cancer NCI-H460, colon cancer cell lines HCT-116 and HT29, melanoma cell line M14, ovarian cancer cell line IGROV1, the renal cancer cell lines CAKI-1 and UO-31, and the breast cancer cell line MCF7. Moreover compound **1h** showed non critical cytotoxic properties.

The structure activity relationship our synthesized indolocarbazoles indicated that, the basicly substituted derivative has the highest activity which recorded the very potent and broad spectrum of activity against several cancers cell lines indicated with the negative values of percent growth (-7.03 % to -52.72 %) promoted at one dose with GI_{50} value of (1.07 µM to 5.65 µM) against almost all the selected cell lines at five dose assay. The chloro-substitution on the indole phenyl ring is unfavorable with a main loss of activity in the selected cancer cell lines. Comparing compound **2h** with **2i** a *para*-benzyloxy substituent increases the activity in some novel sensitive cell line (NCI-H522 as non small lung cancer cell lines and CAKI-1 and UO-31 as renal cancer cell. By comparison of compound **2h** and **2f** a *para*-methoxy substituent ensures the activity, especially in selected cancer cell lines. The *para*-benzyloxy compound **2i** is more active than the *meta*-benzyloxy compound **2f**. The basically substituted derivative **2e** gave the highest antipoliferative activity in a nanomolar ranges in selected cell lines (non small lung cancer cell NCI-H460 and ovarian cancer cell OVCAR-4) with non critical cytotoxic properties because LC_{50} value (34.6 µM) is 17 fold higher than GI_{50} (2 µM) value. However, this compound was found to be non selective toward the cancer subpanels. See figure (2) and (3) illustrating the superposition of all growth curves for compound **2e** (nanomolar active) and its one dose mean graph.

Experimental

The melting points were measured on a Boetius-Mikroheiztisch the company "VEB weighing, Rapido Radebeul/VEB NAGEMA "measured and are uncorrected. The carbon, hydrogen and nitrogen content of the substances was performed on a "CHNS-932" automatic analyzer of the company "LECO Corporation" in the automatic Micro chemical determined. The halogen content was determined by titration in semi micro method determined. For the analyzes TLC were with aluminium foil fluorescent indicator from Merck KGaA (silica gel 60 F254, layer thickness 0.2 mm) used. R_f -values (run level relative to the solvent front), The separations were with column chromatography at atmospheric pressure on silica gel 60 (Grain size from 0.063 to 0.200 mm) from Merck KGaA. The NMR spectra were recorded on a "Gemini 2000" (400/100 MHz). The ATR spectra were recorded on a FT-IR spectrometer "IFS 28" by "Bruker", the KBr spectra on a FT-IR Spectrometer "Spectrum BX" "the Company "Perkin-Elmer" measured. The ESI mass spectra were recorded on a "Finnigan LCQ Classic" by "thermal Electron measured" the sample was injected directly. The 60-Cell-Line Screenings of the Developmental Therapeutics Program (DTP) were examined in the National Cancer Institute (USA) on a possible human tumor cell lines.

General procedure for the preparation of compounds 1_{a-n}:

In a flask containing 5 ml of glacial acetic acid and 2 mmol of indole (0.234 gm) or 5-chloroindole 0.303 gm or 6-chloroindole 0.303 gm was added under stirring until all the indole was dissolved. Then 1 mmol of the appropriate aromatic or heterocyclic aldehyde was added under vigorous stirring. The reaction mixture was allowed to stir over 4 to 6 h, where the reaction solution turned from light yellow to light pink to dark red colour. The product was detected by TLC (100 % CH_2Cl_2), and when the reaction was finished 10 ml of water were added and the solution

was extracted with ethylacetate, washed with water and brine, dried over anhydrous sodium sulfate and concentrated in vacuum. The product was purified by passing over a column and eluted with dichloromethane.

3,3'-(Phenylmethylene)bis(1*H*-indole) (1_a) [39-45]**:** pink powder, 90 % yield, $C_{23}H_{18}N_2$, 322.40 g/mol, mp:126-127^{0}C, ESI-MS: 321.32[M^+-H], IR (ATR,cm^{-1})3141(NH), ^{1}H-NMR(400 MHz,acetone-d_6) δ (ppm): 5.90(s, 1H, CH), 6.79(d, 2H, J=1.5Hz), 6.87(t, 2H, J=7.2Hz), 7.04(t, 2H, J=7.6Hz), 7.16(d, 1H, J=7.3Hz), 7.25(t, 2H, J=7.5Hz), 7.32-7.39(m, 6H), 9.99(s, 2H, 2NH), ^{13}C-NMR(100 MHz,$CDCl_3$) δ (ppm): 40.26(CH), 110.94, 119.68, 120.59, 121.79, 121.85, 123.49, 123.99, 125.99, 126.98, 128.08, 128.59, 136.55, 143.88, EA: Calcd. C, 85.68, H, 5.63, N, 8.69, Found C, 85.72, H, 5.58, N, 8.66, R_f 0.76(CH_2Cl_2).

3,3'-((4-Chlorophenyl)methylene)bis(1H-indole) (1_b) [39-45]**:** pink powder, Mp:104-106 ^{0}C, in 99% yield, $C_{23}H_{17}ClN_2$, 356.85 g/mol, ESI-MS: 355.11[M^+-H], IR(ATR,cm^{-1}): 3410(NH), ^{1}H-NMR(400 MHz,DMSO-d_6)δ (ppm): 5.85(s, 1H, CH), 6.83(d, 2H, J=7.2Hz), 6.86(t, 2H, J=7.4Hz), 7.04(t, 2H, J=7.6Hz), 7.28(d, 2H, J=7.9Hz), 7.29-7.36(m, 6H), 10.83(s, 2H, 2NH), ^{13}C-NMR(100 MHz,DMSO-d_6): 59.65(CH), 111.38, 117.48, 118.14, 118.89, 119.85, 123.48, 124.99, 127.84, 129.97, 130.16, 136.49, 143.87. EA. Calcd. C, 77.41; H, 4.80; Cl, 9.94; N, 7.85, found C, 77.50, H, 5.01, Cl, 10.00, N, 7.89. R_f. 0.87(CH_2Cl_2)

3,3'-((4-bromophenyl)methylene)bis(1H-indole) (1_c) [39-45]**:** yellow crystals, Mp 100-103 ^{0}C, in yield 76%, $C_{23}H_{17}BrN_2$, 401.30 g/mol, ESI-MS: 402 [M^++H], IR(ATR,cm^{-1}): 4356(NH), ^{1}H-NMR(400 MHz,acetone-d_6)δ(ppm): 5.91(s, 1H, CH), 6.79(d, 2H, J=7.2Hz), 6.87(t, 2H, J=7.5Hz), 7.07(t, 2H, J=7.4Hz), 7.28(d, 2H, J=8Hz), 7.36-7.40(m, 6H), 10.93(s, 2H, 2NH), ^{13}C-NMR (100MHz, acetone-d_6): 57.50(CH), 111.40, 117.48, 118.14, 118.99, 119.89, 120.80, 120.99, 123.48, 124.99, 127.89, 129.99, 136.50, 144.02, R_f 0.65(CH_2Cl_2).

3,3'-((3-Bromophenyl)methylene)bis(1H-indole) (1_d) [39-45]**:** red crystals, Mp. 93-95 ^{0}C, yield 98%, $C_{23}H_{17}BrN_2$, 401.30 g/mol, ESI-MS: 401.26[M^++H], 399.31 [M^+-H], IR (ATR,cm^{-1}): 3405(NH), ^{1}H-NMR(400 MHz,DMSO-d_6): δ (ppm): 5.86(s, 1H, CH), 6.85-6.86(m, 3H), 7.03(t, 2H, J=7.6Hz), 7.22(t, 1H, J=7.8Hz), 7.28(d, 2H, J=7.9Hz), 7.34-7.37(m, 5H), 7.49(s, 1H), 10.84(s, 2H, 2NH), ^{13}C-NMR (100 MHz,DMSO-d_6)δ (ppm): 39.16(CH), 111.38, 117.20, 118.16, 118.80, 120.84, 121.25, 123.51, 126.32, 127.23, 128.54, 130.08, 130.69, 136.42, 147.78, EA. Calcd. C, 68.84, H, 4.27, Br, 19.91, N, 6.98, found C, 68.90, H, 4.30, Br, 19.95, N, 7.00, R_f 0.74(CH_2Cl_2).

4-Di(1*H*-indol-3-yl)methyl-*N*,*N*-dimethylaniline (1_e) [39-45]**:** pink powder, Mp. 225-226 ^{0}C, yield 91, $C_{25}H_{23}N_3$, 365.47 g/mol, ESI-MS: 366.25[M^++H], 364.38[M^+-H], IR(ATR,cm^{-1}): 3314(NH), ^{1}H-NMR(400 MHz,DMSO-d_6)δ (ppm): 4.60(s,br., 6H, $2CH_3$), 5.89(s, 1H, CH), 6.84-6.88(m, 4H), 7.03(t, 2H, J=7.99Hz), 7.28(d, 2H, J=7.9Hz), 7.34(d, 2H, J=8.1Hz), 7.49(t, 4H, J=10.6Hz), 10.84(s, 2H, 2NH), ^{13}C-NMR(100 MHz, DMSO-d_6) δ(ppm): 40.13(CH_3), 43.62(CH_3), 45.07(CH), 111.39, 114.52, 117.43, 118.13, 118.85, 119.08, 120.83, 121.40, 123.47, 124.23, 126.37, 129.47, 136.46, 141.84, EA calcd. C, 82.16; H, 6.34; N, 11.50, found C, 82.20, H, 6.37, N, 11.53, R_f 0.29(CH_2Cl_2).

3,3'-(3-Benzyloxy)phenyl)methylene)bis(1*H*-indole (1_f): white powder, Mp. 190-192^{0}C, yield 87%, $C_{30}H_{24}N_2O$, 428.52g/mol, ESI-MS. 428.24[M^+-H], IR(ATR,cm^{-1}): 3425(NH), ^{1}H-NMR(400 MHz,acetone-d_6)δ(ppm): 5.01(s, 2H, CH_2), 5.90(s, 1H, CH), 6.82(d, 2H, J=7.5Hz), 6.85(d, 2H, J=7.2Hz), 6.90(t, 2H, J=7.5Hz), 7.00-7.11(m, 4H), 7.18(t, 1H, J=7.9Hz), 7.26-7.33(m, 2H), 7.37-7.39(m, 6H), 9.95(s, br., 2H, 2NH), ^{13}C-NMR(100 MHz, acetone-d_6)δ(ppm): 41.18(CH),

70.31(CH_2-O), 112.06, 112.91, 116.41, 119.26, 119.63, 120.21, 121.98, 122.13, 123.51, 124.45, 128.04, 128.32, 128.37, 129.07, 129.23, 129.69, 137.98, 138.38, 147.55, 159.66, EA calcd. C, 84.08; H, 5.65; N, 6.54, found C, 84.12, H, 5.55, N, 6.58, R_f 0.79(CH_2Cl_2).

4-(Di(1*H*-indol-3-yl)methyl)benzene-1,2-diol (1_g): light brown powder, Mp 105-107^{0}C, yield 73%, $C_{23}H_{18}N_2O_2$, 354.40 g/mol, ESI-MS: 392.89[M^++K], 354.25[M^+], 353.24[M^+-H], IR(ATR,cm^{-1}): broad 3400(NH and OH), ^{1}H-NMR(400 MHz,acetone-d_6)δ(ppm): 5.77(s, 1H, CH), 6.45(s, 1H), 6.76(d, 2H, J=8.9Hz), 6.86-6.89(m, 2H), 7.04(s, 2H), 7.29(s, 1H), 7.35(s, 4H), 7.55(s, 1H), 9.89(s, 2H, 2NH), EA calcd. C, 77.95; H, 5.12; N, 7.90, found C, 78.01, H, 5.20, N, 7.96, R_f 0.62(CH_2Cl_2).

3,3′-(3-Benzyloxy)-4-methoxyphenyl)methylene)bis(1*H*-indole (1_h): orange crystals, Mp 75-78^{0}C, yield 89%, $C_{31}H_{26}N_2O_2$, 458.55g/mol, ESI-MS 481.16 [M^++Na], 457.24[M^+-H], IR (ATR,cm^{-1}): 3398 (NH), ^{1}H-NMR (400 MHz, DMSO-d_6)δ(ppm): 3.71(s, 3H, OMe), 4.95(s, 2H, CH_2), 5.71(s, 1H, CH), 6.74-6.76(m, 2H), 6.81-6.86(m, 4H), 7.02(t, 2H, J=7.5Hz), 7.06(s, 1H), 7.23(d, 2H, J=7.9Hz), 7.29-7.31(m, 6H), 7.34(s, 1H), 10.73(s, 2H, 2NH), ^{13}C-NMR (100 MH, DMSO-d_6): 55.59(CH), 59.70(OMe), 70.08(OCH_2), 111.29, 111.98, 114.94, 118.00, 118.24, 119.03, 120.71, 123.29, 126.24, 126.56, 127.63, 127.75, 127.86, 128.18, 128.35, 136.49, 137.09, 137.39, 147.14, 147.38, EA calcd.C, 81.20; H, 5.72; N, 6.11, found C, 81.22, H, 5.75, N, 6.14, R_f 0.79 (CH_2Cl_2).

3,3′-((4-Benzyloxy)-3-methoxyphenyl)methylene)bis(1*H*-indole (1_i): light orange crystals, Mp 215-219^{0}C, yield 92%, $C_{31}H_{26}N_2O_2$, 458.55 g/mol, ESI-MS: 457.20[M^+-H], IR(ATR,cm^{-1}): 3416(NH), ^{1}H-NMR(400 MHz,acetone-d_6)δ(ppm): 3.70(s, 3H, OMe), 5.04(s, 2H, CH_2), 5.85(s, 1H, CH), 6.81(s, 2H), 6.85-6.92(m, 4H), 7.04(t, 2H, J=7.6Hz), 7.09(s, 1H), 7.29(d, 1H, J=7.5Hz), 7.33-7.37(m, 6H), 7.47(d, 2H, J=7.7Hz), 9.95(s, 2H, 2NH), ^{13}C-NMR(100 MHz,acetone-d_6)δ(ppm): 40.75(CH), 56.19(OMe), 71.61(OCH_2), 112.05, 112.10, 114.33, 114.93, 119.23, 120.09, 120.32, 121.47, 121.98, 124.32, 124.47, 128.12, 128.45, 128.49, 129.12, 138.08, 138.83, 139.31, 147.72, 150.64, EA calcd. C, 81.20; H, 5.72; N, 6.11, found C, 81.02, H, 5.90, N, 6.22, R_f 0.71 (CH_2Cl_2).

3,3′-((3-Benzyloxy)-4-methoxyphenyl)methylene)bis(5-chloro-1*H*-indole (1_j): yellow powder, Mp 82-85^{0}C, yield 91%, $C_{31}H_{24}Cl_2N_2O_2$, 527.44 g/mol, ESI-MS: 528.18[M^++H], IR(ATR,cm^{-1}): 3369(NH), ^{1}H-NMR(400 MHz, $CDCl_3$)δ (ppm): 3.77(s, 3H, OMe), 4.93(s, 2H, CH_2), 5.52(s, 2H, CH), 6.41(d, 2H, J=7.6Hz), 6.73(t, 4H, J=7.3Hz), 7.02(d, 2H, J=7Hz), 7.13(d, 2H, J=8.6Hz), 7.18(dd, 6H, J=3.1, 7.1Hz), 7.88(s, 2H, 2NH), ^{13}C-NMR(100 MHz, acetone-d_6)δ(ppm): 39.39(CH), 55.99(OMe), 71.03(OCH_2), 111.51, 111.76, 112.12, 115.37, 119.15, 121.20, 122.31, 124.77, 124.99, 126.91, 127.46, 127.50, 127.66, 127.96, 128.64, 135.04, 135.74, 137.10, 147.63, 148.36, EA calcd. C, 70.59; H, 4.59; Cl, 13.44; N, 5.31, found C, 70.62, H, 4.55, Cl, 13.55, N, 5.51, R_f 0.68 (CH_2Cl_2).

3,3′-((3-(Benzyloxy)-4-methoxyphenyl)methylene)bis(6-chloro-1*H*-indole (1_k): light orange crystals, Mp 85-87^{0}C, yield 93%, $C_{31}H_{24}Cl_2N_2O_2$, 527.44 g/mol, ESI-MS 526.14[M^+-H], IR(ATR,cm^{-1}): 1253(C-O), 2866, 2928(CH), 3420(NH), ^{1}H-NMR(400 MHz,DMSO-d_6):δ (ppm): 3.70(s, 3H, OMe), 4.94(s, 2H, OCH_2), 5.69(s, 1H, CH), 6.77(d, 2H, J=2Hz), 6.79(d, 1H, J=1.9Hz), 6.84(t, 2H, J=7.9Hz), 7.00(d, 1H, J=2Hz), 7.17(d, 2H, J=8.6Hz), 7.30(t, H, J=5.7Hz), 7.37(d, 2H, J=1.6Hz), 10.91(s, 2H, 2NH), ^{13}C-NMR(100 MHz,DMSO-d_6) δ(ppm): 26.78(CH), 55.99(OMe), 70.41(OCH_2), 111.47, 112.41, 115.11, 118.79, 118.95, 120.83, 121.08, 125.02, 125.79, 126.10, 127.02, 128.17, 128.29, 128.71, 128.89, 137.22, 137.40,

137.59, 147.70, 147.99, EA calcd. C, 70.59; H, 4.59; Cl, 13.44; N, 5.31, found C, 70.63, H, 4.72, Cl, 13.53, N, 5.34, R_f 0.68 (CH_2Cl_2).

3,3′-(Pyridin-3-ylmethylene)bis(1*H*-indole (1_l): light pink powder, Mp 98-101^{0}C, yield 95%, $C_{22}H_{17}N_3$, 323.39 g/mol, ESI-MS: 324.16[M^++H], IR(ATR,cm^{-1}): 3403(NH), ^{1}H-NMR(400 MHz,DMSO-d_6)δ(ppm): 5.70(s, 1H, CH), 5.88(s, 1H, CH), 6.84(t, 4H, J=7.1Hz), 7.01(t, 2H, J=7.6Hz), 7.22-7.29(m, 3H), 7.32(d, 2H, J=8.1Hz), 7.65(d, 1H, J=7.9Hz), 8.34-8.37(m, 1H), 8.58(d, 1H, J=7.9Hz), 10.84(s, 2H, 2NH), ^{13}C-NMR (100 MHz, DMSO-d_6)δ (ppm): 54.79(CH), 54.78(CH), 111.45, 117.07, 118.24, 118.83, 120.94, 123.15, 123.56, 126.29, 135.47, 136.51, 140.15, 146.99, 149.50, EA C, 81.71; H, 5.30; N, 2.99, found C, 81.90, H, 5.35, N, 13.02, R_f 0.46 (7%MeOH/CH_2Cl_2).

Tri(1*H*-indol-3-yl)methane (1_m): light yellow powder, Mp 235-240 ^{0}C, $C_{25}H_{19}N_3$, 361.44 g/mol, yield 98%, ESI-MS: 360.32[M^+-H], IR(ATR,cm^{-1}): 3424(NH), ^{1}H-NMR(400 MHz,acetone-d_6): δ (ppm): 6.19(s, 1H, CH), 6.85-6.93(m, 6H), 7.03(t, 4H, J=7.6Hz), 7.37(t, 3H, J=7.8Hz), 7.48(t, 2H, J=7.4Hz), 9.88(s, 3H, 3NH), ^{13}C-NMR(100 MHz,acetone-d_6): δ (ppm): 31.33(CH), 111.13, 118.95, 119.08, 120.12, 121.09, 123.17, 124.60, 127.35, 128.17, 137.19, EA calcd. C, 83.08; H, 5.30; N, 11.63, found C, 83.09, H, 5.33, N,11.71, R_f 0.73(CH_2Cl_2).

3,3′-(Naphthalen-1-ylmethylene)bis(1*H*-indole (1_n): white powder, Mp 252-255 ^{0}C, yield 97%, $C_{27}H_{20}N_2$, 372.46 g/mol, ESI-MS 371.30[M^+-H], IR(ATR,cm^{-1}): 3407(NH), ^{1}H-NMR(400 MHz,DMSO-d_6)δ(ppm): 5.71(s, 1H, CH), 6.59(s, 1H), 6.68(d, 2H, J=7Hz), 6.81(t, 2H, J=7.5Hz), 6.99(t, 2H, J=7.6Hz), 7.23(d, 4H, J=8.1Hz), 7.32(t, 2H, J=9Hz), 7.41(t, 2H, J=7.7Hz), 7.73(d, 1H, J=8Hz), 7.88(d, 1H, J=7.5Hz), 8.22(d, 1H, J=8Hz), 10.74(s, 2H, 2NH), ^{13}C-NMR(100 MHz,DMSO-d_6)δ(ppm): 35.33(CH), 111.41, 117.62, 118.15, 118.84, 120.77, 123.84, 124.13, 125.15, 125.19, 125.42, 125.68, 126.43, 126.54, 128.42, 131.23, 133.49, 136.56, 140.18, EA calcd. C, 87.07; H, 5.41; N, 7.52, R_f 0.87 (CH_2Cl_2).

General procedure for the preparation of compounds 2_{a-m}

In a round bottom flask containing 1mmol of BIMs derivatives $\mathbf{1_{a-n}}$ was stirred with 50ml MeOH under heating until it completely dissolved. The aromatic or heterocyclic aldehyde 1mmol which has been used for the synthesis of the BIMs was added and the reaction mixture was stirred under heating until the reaction solution became clear. Then a few drops of conc. H_2SO_4 were added. The reaction solution became pink turned to dark red by refluxing for about 1h. Upon the reaction completion, as monitored by TLC (100%CH_2Cl_2) the reaction was worked up by adding 50ml water, which was neutralized by NH_4OH addition, extracted with ethylacetate 100ml for two times, washed with water and then brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude reaction mixture was purified via column chromatography on silica gel using (30%EtAc/hexane) as a solvent to afford the alternative carbazole derivatives $\mathbf{2_{a-n}}$ **[4]**.

2,8-Diphenyl-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (2_a) [46]: Color and shape: light brown powder, Mp 352-355^{0}C, yield 81%, $C_{30}H_{22}N_2$, 410.51g/mol, ESI-MS: 409.35[M^+-H], IR(ATR,cm^{-1}): 3389(NH), ^{1}H-NMR(400 MHz, DMSO-d_6)δ(ppm): 5.66 (s, 2H, 2CH), 6.74(t, 2H, J=7.5Hz), 6.74(t, 2H, J=7.5Hz), 6.91(t, 2H, J=7.6Hz), 7.05(d, 2H, J=7.9Hz), 7.15-7.26(m, 8H), 7.30(d, 4H, J=7.1Hz), 10.63(s, 2H, 2NH), ^{13}C-NMR(100 MHz,DMSO-d_6)δ (ppm): 30.57(CH), 39.40(CH), 109.67, 110.85, 117.93, 118.24, 120.31, 125.46, 126.14, 128.01, 128.23, 128.98, 129.79, 136.38, 136.88, 143.84, EA calcd. C, 87.77; H, 5.40; N, 6.82, found C, 87.79, H, 5.36, N, 6.86, R_f 0.89 (CH_2Cl_2)

2,8-Bis(4-Chlorophenyl)-1,2,3,8-tetrahydro-indolo[2,3-*b*]carbazole (2_b) [46]: light green powder, Mp 339-342 ^{0}C, yield 56%, $C_{30}H_{20}Cl_2N_2$, 479.40 g/mol, ESI-MS: 478.27[M^+-H], IR(ATR,cm^{-1}): 3414(NH), ^{1}H-NMR (400 MHz, DMSO-d_6)δ(ppm): 5.69(s, 2H, 2CH), 6.78(t, 2H, J=7.5Hz), 6.84(t, 2H, J=7.6Hz), 6.93(t, 2H, J=7.4Hz), 7.06(dd, 2H, J=8, 15.2Hz), 7.21(d, 2H, J=8Hz), 7.24-7.32(m, 3H), 7.40(d, 1H, J=8Hz), 7.67(d, 1H, J=8.3Hz), 7.75(d, 1H, J=8.3Hz), 10.57(s, 1H, NH), 10.72(s, 1H, NH), ^{13}C-NMR(100 MHz, DMSO-d_6)δ (ppm): 38.66(CH), 40.17(CH), 109.54, 111.10, 118.32, 120.73, 125.40, 128.18, 129.26, 130.23, 130.88, 131.93, 132.68, 136.19, 137.09, 142.89, EA calcd. C, 75.16; H, 4.21; Cl, 14.79; N, 5.84, found C, 75.18, H, 4.24, Cl, 14.82, N, 5.79, R_f 0.96 (CH_2Cl_2).

2,8-Bis(4-bromophenyl)-1,2,3,8-tetrahydro-indolo[2,3-*b*]carbazole (2_c): yellow powder, Mp >350 ^{0}C, yield 87%, $C_{30}H_{20}Br_2N_2$, 568.30 g/mol, ESI-MS: 569.19[M^++H], 567.12[M^+-H], IR(ATR,cm^{-1}): 3436(NH), ^{1}H-NMR(400 MHz, DMSO-d_6)δ(ppm): 5.66(s, 2H, 2CH), 6.78(t, 2H, J=7.5Hz), 6.94(t, 2H, J=7.5 Hz), 7.05(d, 2H, J=7.9 Hz), 7.12-7.29(m, 6H), 7.43(d, 4H, J=8Hz), 10.71(s, 2H, 2NH), ^{13}C-NMR(100 MHz, DMSO-d_6)δ(ppm): 39.02(CH), 39.99(CH), 110.22, 112.34, 119.05, 120.22, 126.40, 128.80, 130.55, 136.40, 137.62, 139.00, 143.01, EA calcd. C, 63.40; H, 3.55; Br, 28.12; N, 4.93, found C, 63.42, H, 3.58, Br, 28.16, N, 4.98, R_f 0.87 (CH_2Cl_2).

4,4′-(8,3,2,1-Tetrahydroindolo[2,3-*b*]carbazole-2,8-diyl)bis(*N*,*N*-dimethyl aniline) (2_d) [46]: dark gray powder, Mp 324-325^{0}C, yield 91 %, $C_{34}H_{32}N_4$, 496.64g/mol, ESI-MS 497.21[M^++H], IR: (ATR, cm^{-1}):3304(NH),^{1}H-NMR(400 MHz,DMSO-d_6)δ(ppm): 3.05(s, 12H, 4Me), 5.73(s, 2H, 2CH), 6.78(t, 2H, J=7.5Hz), 6.94(t, 2H, J=7.5Hz), 7.09(d, 2H, J=7.9Hz), 7.23(d, 2H, J=8.1Hz), 7.35-7.41(m, 8H), 10.73(s, 2H, 2NH), ^{13}C-NMR(100 MHz,DMSO-d_6)δ (ppm): 38.59(Me), 44.58(Me), 52.81(CH), 109.59, 111.06, 118.27, 118.34, 120.66, 125.39, 127.80, 129.61, 133.32, 136.24, 137.01, EA calcd. C, 82.22, H, 6.49, N, 11.28, found C, 82.25, H, 6.51, N, 11.38, R_f 0.66 (CH_2Cl_2).

2,8-Bis(3-bromophenyl)-1,2,3,8-tetrahydro-indolo[2,3-*b*]carbazole (2_e): light green powder, Mp 255-257 ^{0}C, yield 54 %, $C_{30}H_{20}Br_2N_2$, 568.30 g/mol, ESI-MS: 569.16[M^++H], 567.01[M^+-H], IRATR,cm^{-1}): 3390(NH), ^{1}H-NMR(400 MHz,DMSO-d_6): δ (ppm): 5.75(s, 2H, 2CH), 6.82(t, 4H, J=7.4Hz), 6.97(t, 2H, J=7.3Hz), 7.09(d, 2H, J=7.9Hz), 7.26(d, 2H, J=7.5Hz), 7.27-7.34(m, 4H), 7.47(s, 2H), 10.81(s, 2H, 2NH), ^{13}C-NMR(100 MHz,DMSO-d_6): 40.12(CH), 109.39, 111.15, 118.31, 120.82, 121.53, 125.32, 127.55, 128.07, 129.32, 130.88, 136.03, 137.06, 146.41, 146.68, EA calcd. C, 63.40; H, 3.55; Br, 28.12; N, 4.93, found C, 63.40, H, 3.58, Br, 28.18, N, 5.00, R_f 0.92 (CH_2Cl_2)

2,8-Bis(3-(benzyloxy)phenyl)-1,2,3,8-tetra-hydroindolo[2,3-*b*]carbazole (2_f): white powder, Mp 275-279 ^{0}C, yield 72 %, $C_{44}H_{34}N_2O_2$, 622.75 g/mol, ESI-MS: 623.26[M^++H], 621.31[M^+-H], IR(ATR,cm^{-1}): 3390(NH), ^{1}H-NMR(400 MHz,DMSO-d_6): δ (ppm): 5.00(s, 4H, $2CH_2$), 5.62(s, 2H, 2CH), 6.76(t, 4H, J=7.3Hz), 6.82(d, 2H, J=7.9Hz), 6.93(t, 2H, J=7.2Hz), 7.03(d, 2H, J=7.7Hz), 7.14(t, 2H, J=8Hz), 7.23(d, 2H, J=7.9Hz), 7.24-7.32(m, 6H), 7.37(d, 4H, J=6.7Hz), 10.62(s, 2H, 2NH), EA calcd. C, 84.86; H, 5.50; N, 4.50, found C, 84.89, H, 5.54, N, 4.53, R_f 0.85(CH_2Cl_2).

4,4-(1,2,3,8-Tetrahydroindolo[2,3-*b*]carbazole-2,8-diyl)dibenzene-1,2-diol (2_g): dark brown powder, Mp 273-275 ^{0}C, yield 45 %, $C_{30}H_{22}N_2O_4$, 474.51 g/mol, ESI-MS: 475.10[M^++H], 473.09[M^+-H], IR(ATR,cm^{-1}): 3250(OH), 3430(NH), ^{1}H-NMR(400 MHz, acetone-d_6): δ(ppm): 5.53(s, 2H, 2CH), 6.65(d, 2H, J=2Hz), 6.74(d, 7H, J=7.9Hz), 6.78-6.92(m, 4H), 6.94(t, 2H, J=7Hz), 7.18(d, 2H,

J=7.9Hz), 7.25(d, 2H, J=8Hz), 7.59(s,br., 4H, 4OH), 9.75(s, 2H, 2NH), ^{13}C-NMR(100 MHz, acetone-d_6): δ(ppm):40.67(CH), 111.42, 111.79, 115.93, 116.39, 119.32, 119.97, 121.05, 121.69, 127.63, 136.81, 138.28, 138.55, 144.82, 145.94, EA calcd. C, 75.94; H, 4.67; N, 5.90, found C, 75.99, H, 4.69, N, 5.93, R_f 0.54 (10%MeOH/CH_2Cl_2).

2,8-Bis(3-(benzyloxy)-4-methoxyphenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (2_h): white powder, Mp 310-313^{0}C, yield 84 %, $C_{46}H_{38}N_2O_4$, 682.80g/mol, Mp 310-313^{0}C, ESI-MS: 705.19[M^++Na], 681.41[M^+-H], IR (ATR,cm^{-1}): 3389(NH), ^{1}H-NMR(400 MHz,DMSO-d_6): δ (ppm): 3.66(s, 6H, 2OMe), 5.53(s, 4H, 2CH_2), 5.69(s, 2H, 2CH), 6.69-6.78(m, 4H), 6.82(d, 2H, J=8.3Hz), 6.92(t, 2H, J=7.3Hz), 6.98(d, 2H, J=7.9Hz), 7.06(d, 2H, J=7.7Hz), 7.19(d, 2H, J=10.4Hz), 7.22-7.26(m, 4H), 7.32(dd, 4H, J=3, 6.6Hz), 7.41(d, 4H, J=7.9Hz), 10.51(s, 2H, 2NH), ^{13}C-NMR(100 MHz,DMSO-d_6): δ (ppm):38.97(CH), 55.63(OMe), 70.09(OCH_2), 109.68, 110.00, 111.55, 112.44, 114.04, 118.35, 119.21, 119.44, 120.55, 121.00, 123.50, 127.20, 127.55, 127.61, 128.24, 137.19, 137.96, 138.40, 146.83, 149.75, EA calcd.C, 80.92; H, 5.61; N, 4.10, found C, 80.95, H, 5.62, N, 4.16, R_f 0.71(CH_2Cl_2).

2,8-Bis(4-(benzyloxy)-3-methoxyphenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (2_i): Color and shape: dark green powder, Mp 289-291 ^{0}c,yield 88%, $C_{46}H_{38}N_2O_4$, 682.80 g/mol, ESI-MS 683.20[M^++H], IR(ATR,cm^{-1}): 3301(NH), ^{1}H-NMR(400 MHz,acetone-d_6): δ (ppm): 3.70(s, 6H, 2OMe), 5.04(s, 4H, 2CH_2), 5.84(s, 2H, 2CH), 6.81(d, 4H, J=1.7Hz), 6.84-6.92(m, 4H), 7.04(t, 4H, J=8Hz), 7.09(d, 2H, J=1.9Hz), 7.28(d, 2H, J=7.3Hz), 7.33-7.37(m, 4H), 7.46(d, 4H, J=7Hz), 9.95(s, 2H, 2NH), ^{13}C-NMR(100 MHz,acetone-d_6): δ (ppm): 39.87(CH), 55.31(OMe), 70.72(OCH_2), 110.98, 111.22, 113.44, 113.98, 114.04, 118.35, 119.20, 119.44, 120.58, 121.10, 123.59, 127.23, 127.56, 127.61, 128.24, 137.19, 137.94, 138.42, 146.83, 149.75, EA calcd. C, 80.92; H, 5.61; N, 4.10, found C, 80.95, H, 5.64, N, 4.17, R_f 0.65(CH_2Cl_2).

2,8-Di(pyridin-3-yl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (2_j): light pink powder, Mp 129-132 ^{0}C, yield 58 %, $C_{28}H_{20}N_4$, 412.49 g/mol, ESI-MS 413[M^++H], EI-MS 412[M^+]10%, 334[M^+-pyridine]5%, 323[M^+-pyridine.CH]100%, 45[indolyl.CH.indolyl]80%, IR(ATR,cm^{-1}): 1338(C=N), 3398(NH), ^{1}H-NMR(400 MHz,DMSO-d_6): δ (ppm): 5.43(s, 1H, CH), 5.89(s, 1H, CH), 6.86(d, 4H, J=7.3Hz), 7.02(t, 2H, J=7.5Hz), 7.22-7.25(m, 1H), 7.28(d, 2H, J=8Hz), 7.35(d, 2H, J=7.5Hz), 7.66-7.69(m, 2H), 8.35(d, 1H, J=7.6Hz), 8.51(d, 1H, J=7.6Hz), 8.57(dd, 1H, J=1.6, 11.6Hz), 10.86(s, 2H, 2NH), ^{13}C-NMR(100 MHz,DMSO-d_6)δ(ppm):37.72(CH), 53.34(CH), 101.78, 112.04, 117.67, 118.85, 119.42, 121.55, 123.85, 124.15, 126.89, 134.13, 134.75, 136.09, 137.12, 140.75, 147.56, 148.39, 150.04, 150.06, EA calcd. C, 81.53; H, 4.89; N, 13.58, found C, 81.50, H, 4.95, N, 13.62, R_f 0.49 (7% MeOH /CH_2Cl_2).

2,8-Di(1*H*-indol-3-yl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (2_k): light yellow powder, Mp 190-193 ^{0}C, yield 47 %, $C_{34}H_{24}N_4$, 488.58 g/mol, ESI-MS 489.18[M^++H], IR(ATR,cm^{-1}): 3406(NH), ^{1}H-NMR(400 MHz,acetone-d_6): δ (ppm): 5.85(s, 2H, 2CH), 6.81-6.89(m, 2H), 7.00-7.07(m, 2H), 7.14(t, 2H, J=7Hz), 7.31-7.37(m, 4H), 7.46(d, 2H, J=7.2Hz), 7.48-7.55(m, 4H), 7.74(s, 1H), 8.16(s, 1H), 9.95(s, 2H, 2NH), 10.13(s, 2H, 2NH), ^{13}C-NMR(100 MHz, acetone-d_6): δ (ppm):27.42(CH), 111.22, 112.00, 115.23, 118.55, 119.38, 120.28, 120.55, 121.89, 122.10, 124.52, 124.61, 129.00, 130.32, 138.00, 138.26, 142.55, EA calcd. C, 83.58; H, 4.95; N, 11.47, found C, 83.55, H, 5.01, N, 11.49, R_f 0.65 (CH_2Cl_2).

2,8-Bis(3-(benzyloxy)-4-methoxyphenyl)-6,10-dichloro-1,2,3,8-tetra-hydroindolo[2,3-*b*]carbazole (2_l): brown powder, Mp 320-322 ^{0}C, yield 89 %, $C_{46}H_{36}Cl_2N_2O_4$, 751.70 g/mol,

ESI-MS: 752.10[M^++H], 749.17[M^+-H], IR(ATR,cm^{-1}): 3304(NH), ^{1}H-NMR(400 MHz,DMSO-d_6): δ (ppm): 3.70(s, 6H, 2OMe), 4.97(s, 4H, 2OCH_2), 5.60(s, 2H, 2CH), 6.82(dd, 2H, J=1.9, 8.2Hz), 6.90(d, 2H, J=8.2Hz), 6.97(dd, 2H, J=2, 8.6Hz), 7.05(dd, 4H, J=1.9, 8.6Hz), 7.23-7.27(m, 8H), 7.33(dd, 4H, J=2.6, 6.7Hz), 10.81(s, 2H, 2NH), ^{13}C-NMR (100 MHz, DMSO-d_6)δ(ppm): 26.29(CH), 55.53(OMe), 70.13(OCH_2), 109.45, 112.19, 112.54, 114.48, 117.67, 120.44, 120.85, 122.64, 126.86, 127.72, 127.89, 128.19, 128.34, 133.00, 135.56, 135.81, 136.89, 138.57, 147.56, 148.00, EA calcd. C, 73.50; H, 4.83; Cl, 9.43; N, 3.73, found C, 73.52, H, 4.85, Cl, 9.45, N, 3.75, R_f 0.85(CH_2Cl_2).

2,8-Bis(3-(benzyloxy)-4-methoxyphenyl)-5,11-dichloro-1,2,3,8-tetra hydroindolo[2,3-*b*]carbazole (2_m): white powder, Mp 322-324^{0}C, yield 90%, $C_{46}H_{36}C_{l2}N_2O_4$, 751.70 g/mol, ESI-MS: 751.27[M^+], 752.30[M^++H], 750.26[M^+-H], IR(ATR,cm^{-1}): 3348(NH), ^{1}H-NMR(400 MHz,acetone-d_6): δ (ppm): 3.69(s, 6H, 2OMe), 4.94(s, 4H, 2OCH_2), 5.52(s, 2H, 2CH), 6.71(dd, 2H, J=1.9, 8.6Hz), 6.81(s, 4H), 6.96(d, 4H, J=7.2Hz), 7.17-7.21(m, 8H), 7.30(dd, 4H, J=2, 7.5Hz), 10.31(s, 2H, 2NH), ^{13}C-NMR(100 MHz,acetone-d_6) δ(ppm): 30.55(CH), 56.00(OMe), 74.05(OCH_2), 108.99, 112.89, 112.99, 114.50, 115.20, 117.68, 120.00, 120.95, 122.90, 126.58, 127.72, 127.89, 128.18, 128.34, 135.56, 135.81, 136.89, 138.55, 147.56, 148.05, EA calcd. C,73.50; H, 4.83; Cl, 9.43; N, 3.73, found C,73.49, H, 4.88, Cl, 9.39, N, 3.80, R_f 0.79 (CH_2Cl_2).

Procedure for the preparation of 4-(8-(3-(Benzyloxy)-4-methoxyphenyl)-1,2,3,8-tetra hydroindolo[2,3-*b*]carbazol-2-yl)-*N*,*N*-dimethylaniline (3): BIM (1_i) 1mmol, 0.5 gm was dissolved in 25 ml of MeOH, and 1mmol 0.149 mg of *p*-*N*,*N*-dimethylaminobenzaldehyde was added to the reaction mixture. The reaction was allowed to stir under reflux until all the reactants had dissolved. After that few drops of conc. H_2SO_4 were dropwisly added. Then the reaction was allowed to stirring under reflux for about one hour. TLC of the reaction mixture showed the formation of four products. The reaction was worked up by adding 10 ml of water, neutralization with a solution of NH_4OH, extracted by CH_2Cl_2, dried over anhydrous Na_2SO_4, evaporated and purified by column chromatography eluted with CH_2Cl_2 to separate the four products that were identified as compound $\mathbf{1_e}$ as a main product with a 30 % yield, compound $\mathbf{1_d}$ in a 15 % yield, compound $\mathbf{1_h}$ with a 10 % yield and our desired compound **3** in a 18 % yield, as light pink powder, $C_{40}H_{35}N_3O_2$, 589.72 g/mol, Mp 299-301^{0}C, ESI-MS: 590.26[M^++H], IR(ATR,cm^{-1}): 3416(NH), ^{1}H-NMR(100 MHz,acetone-d_6): δ (ppm): 2.03(s, 6H, 2Me), 3.78(s, 3H, OMe), 4.98(s, 2H, CH_2), 5.79(s, 2H, 2CH), 6.73(s, 2H), 6.85-6.91(m, 4H), 7.03(t, 2H, J=7.2Hz), 7.09(d, 2H, J=7.9Hz), 7.27-7.37(m, 8H), 9.93(s, br., 2H, 2NH), R_f 0.55 (CH_2Cl_2).

Procedure of the preparation of the Spirocyclic structure (4):

In a round bottom flask containing 50ml of MeOH 2 mmol (0.65 mg) of BIMs derivatives $\mathbf{1_a}$ was added under stirring until it completely dissolved. Cyclohexane-1,4-dione (1mmol, 0.112 mg) was added to the reaction mixture. When the reaction solution became clear, few drops of conc. H_2SO_4 were added slowly. The reaction solution became pink and the colour turned to dark violet by leaving it stirring under reflux for one hour. Upon the reaction completion as monitored by TLC (100 % CH_2Cl_2) the reaction was worked up by added of 50 ml of water, neutralized by NH_4OH, extracted with ethylacetate 200 ml two times washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluted with (30 % EtAc/hexane) to afford compound **4** in a moderate yield 52%, as light

pink powder, Mp 149-152 ^{0}C, $C_{52}H_{40}N_4$, 720.90 g/mol, ESI-MS 719.29[M^+-H], EI-MS: 720 [M^+] 32 %, 322 [3,3'-(phenylmethylene)bis(1H-indole)] 100 %, 245 [indolyl.CH.indolyl] 75 %, 117 [indolyl] 75%, 90 [Ph.CH] 31 %, IR(ATR,cm^{-1}): 3409 (NH), ^{1}H-NMR(400 MHz,acetone-d_6): δ (ppm): 2.03(t, 4H, 2CH_2, J=7Hz), 2.27(t, 4H, 2CH_2, J=11.4Hz), 5.91(s, 2H, 2CH), 6.79(s, 2H), 6.88(t, 2H, J=7.5Hz), 7.04(t, 4H, J=7.6Hz), 7.11-7.20(m, 4H), 7.25(t, 4H, J=7.5Hz), 7.35(dd, 4H, J=7.9, 15.8Hz), 7.38(d, 4H, J=8Hz), 7.47(t, 2H, J=8.8Hz), 9.94(s, br., 2H, 2NH), ^{13}C-NMR(100 MHz,acetone-d_6)δ(ppm):26.69(CH_2), 26.96(CH_2), 29.66(CH), 29.69(C), 110.22, 111.73, 117.21, 118.48, 120.39, 122.24, 123.27, 125.58, 125.65, 126.72, 127.82, 128.22, 128.50, 128.82, 128.84, 129.46, 130.09, 130.86, 130.89, 134.11, 137.03, 140.96, R_f 0.97 (CH_2Cl_2).

In-vitro Cancer Screen

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10^{-5} M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels. The human tumor cell lines of the cancer-screening panel are grown in RPMI 1640 medium containing 5 % fetal bovine serum and 2 µM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 mL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 C, 5 % CO, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethylsulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is dissolved and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 ml of these different drug dilutions are added to the appropriate microtiter wells already containing 100 ml of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 ^{0}C, 5 % CO, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 ml of cold 50 % TCA (final concentration, 10 % TCA) and incubated for 60 min at 4 C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 ml) at 0.4% in 1 % acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 µM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nM. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 ml of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as: [(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti > / = Tz , [(Ti-Tz)/Tz] x 100 for concentrations for

which Ti < Tz. Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from (Ti = Tz). The LC_{50} (concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested [49].

Conclusion:

The present research proved that, all synthesized BIMs (**$1_{e,f,h,i,n}$**) showed best activities in the same cell lines MOLT-4 in leukaemia cell line and in IGROV1 in an ovarian cancer cell line. Also the basically substituted derivative demonstrates good activity in the renal cancer cell lines CAKI-1 and UO-31. The TGI and LC_{50} values were higher than 100 µM so the compound **1_h** showed noncritical cytotoxic properties. The basically substituted derivative **2_e** gave the highest antiproliferative activity in a nanomolar ranges in selected cell lines with noncritical cytotoxic properties (17 fold higher LC_{50} "34.6 µM" than GI_{50} "2 µM" values). Further SAR modifications in compounds **1_h** and **2_e** that are under investigation in our lab wishing to discover more potent antitumor agents.

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Tables:

Table (1): 60 human tumour cell line anticancer screening data at single dose assay (10^{-5} M) as percent growth inhibition of BIMs.

Panel/Cell Line	Growth Percent				
	BIM (**1_h**)	BIM (**1_e**)	BIM (**1_g**)	BIM (**1_i**)	BIM (**1_l**)

Leukaemia					
CCRF-CEM	41.24	94.29	65.84	82.70	90.92
HL-60(TB)	47.74	128.59	85.73	97.61	100.22
K-562	37.52	91.47	63.92	71.05	76.69
MOLT-4	20.53	96.36	52.47	58.65	75.09
RPMI-8226	42.97	94.00	75.01	94.76	93.10
SR	38.00	100.85	67.99	77.82	89.79
Non-Small Cell Lung Cancer					
A549/ATCC	44.24	98.14	70.37	80.66	78.08
EKVX	33.06	94.88	70.13	75.34	92.09
HOP-62	85.39	103.12	117.07	89.05	96.56
HOP-92	37.86	97.98	77.26	74.21	65.77
NCI-H226	75.60	103.98	96.26	86.74	95.12
NCI-H23	48.28	105.48	83.58	83.17	90.04
NCI-H322M	53.46	89.81	76.91	72.67	94.19
NCI-H460	9.25	111.93	40.92	87.36	90.80
NCI-H522	44.03	103.14	81.51	90.05	88.29
Colon Cancer					
COLO 205	28.69	104.13	98.17	102.68	103.48
HCC-2998	52.61	107.97	89.58	102.35	101.88
HCT-116	19.91	89.91	49.68	63.15	78.21
HCT-15	43.97	100.56	71.42	80.96	93.30
HT29	20.89	100.05	67.05	80.75	92.07
KM12	28.51	102.97	61.54	88.20	83.31
SW-620	37.08	93.36	66.72	84.23	87.23
CNS Cancer					
SF-268	52.76	116.79	67.22	89.60	99.12
SF-295	41.84	98.27	90.26	86.27	75.79
SF-539	75.91	91.54	90.70	97.53	104.56
SNB-19	56.58	109.25	88.91	101.55	113.06
SNB-75	64.21	92.69	69.63	80.78	76.20
U251	39.48	111.50	71.87	94.01	91.64
Melanoma					
LOX IMVI	35.11	96.58	70.34	84.68	91.70
MALME-3M	76.44	108.26	86.23	95.57	104.86
M14	19.50	102.85	60.84	94.58	91.67
MDA-MB-435	39.23	100.64	66.55	97.07	104.96
SK-MEL-2	62.89	108.23	87.10	106.14	117.60
SK-MEL-28	75.02	115.84	94.00	107.74	110.32
SK-MEL-5	53.71	102.49	69.90	89.22	104.38
UACC-257	64.60	113.36	88.90	97.97	97.73
UACC-62	67.03	92.49	65.78	75.87	90.79
Ovarian Cancer					
IGROV1	23.79	99.14	51.22	53.76	92.57
OVCAR-3	42.79	108.57	57.39	95.78	89.75
OVCAR-4	68.99	108.38	85.86	93.36	105.91
OVCAR-5	52.85	102.74	84.10	77.30	92.79
OVCAR-8	54.18	100.41	84.14	99.85	98.97
NCI/ADR-RES	43.84	105.48	83.51	95.00	95.63
SK-OV-3	82.74	105.24	99.10	92.84	100.06
Renal Cancer					
786-0	66.10	101.34	93.63	96.46	105.42
A498	63.36	106.51	104.66	89.10	95.52
ACHN	37.06	93.95	63.05	74.33	86.38
CAKI-1	15.65	74.45	34.27	57.43	53.07
RXF 393	71.08	115.46	90.57	110.45	94.67
SN12C	45.51	103.75	71.34	87.36	103.59
UO-31	18.10	64.28	42.30	51.55	66.86
Prostate Cancer					
PC-3	31.31	88.06	58.42	67.79	69.02
DU-145	63.31	120.00	74.41	109.45	105.82
Breast Cancer					
MCF7	21.93	105.91	52.85	76.47	104.09
MDA-MB-231/ATCC	50.81	104.98	86.20	68.39	79.28
BT-549	80.58	109.82	102.59	99.13	102.05
T-47D	43.17	89.79	65.05	72.49	99.38
MDA-MB-468	56.56	110.54	97.31	119.11	101.32
Mean	47.39 % Selected for 5-dose	101.60 % Non selected	75.51 % Non selected	86.38 % Non selected	92.63 % Non selected

Table (2): NCI in vitro testing result of compound **1_h (NSC D-755517/1)** at five dose level in µM.

Panel/Cell Line	GI_{50} Concentration per cell line	MID[b]	selectivity ratio (MID^a:MID^b)	TGI	LC_{50}	$LogGI_{50}$	Log TGI	Log LC_{50}

Leukemia	.....	6.44	0.79	.....	.....	.....	....	.....
CCRF-CEM	6.91			> 1.00	> 1.00	-5.16	> -4.00	> -4.00
HL-60(TB)	8.67			> 1.00	> 1.00	-5.06	> -4.00	> -4.00
K-562	6.49			> 1.00	> 1.00	-5.19	> -4.00	> -4.00
MOLT-4	5.51			4.83	> 1.00	-5.26	-4.32	> -4.00
RPMI-8226	3.83			> 1.00	> 1.00	-5.42	> -4.00	> -4.00
SR	7.20			> 1.00	> 1.00	-5.14	> -4.00	> -4.00
Non-Small Cell Lung Cancer	.....	5.35	0.96	.....	.....	.....	.....	.....
A549/ATCC	7.18			> 1.00	> 1.00	-5.14	> -4.00	> -4.00
EKVX	5.83			> 1.00	> 1.00	-5.23	> -4.00	> -4.00
HOP-62	> 1.00			> 1.00	> 1.00	> -4.00	> -4.00	> -4.00
HOP-92	3.03			> 1.00	> 1.00	-5.52	> -4.00	> -4.00
NCI-H226	3.49			> 1.00	> 1.00	-4.46	> -4.00	> -4.00
NCI-H23	8.10			> 1.00	> 1.00	-5.09	> -4.00	> -4.00
NCI-H322M	7.51			> 1.00	> 1.00	-4.12	> -4.00	> -4.00
NCI-H460	4.54			> 1.00	> 1.00	-5.34	> -4.00	> -4.00
NCI-H522	3.15			> 1.00	> 1.00	-5.50	> -4.00	> -4.00
Colon Cancer	.....	6.56	0.78	.....	.....	.....	.....	.....
COLO 20	9.47			> 1.00	> 1.00	-5.02	> -4.00	> -4.00
HCC-2998	9.31			> 1.00	> 1.00	-5.03	> -4.00	> -4.00
HCT-116	5.05			> 1.00	> 1.00	-5.30	> -4.00	> -4.00
HCT-1	4.53			> 1.00	> 1.00	-5.34	> -4.00	> -4.00
HT29	4.74			> 1.00	> 1.00	-5.32	> -4.00	> -4.00
KM12	5.12			> 1.00	> 1.00	-5.29	> -4.00	> -4.00
SW-620	7.71			> 1.00	> 1.00	-5.11	> -4.00	> -4.00
CNS Cancer	.....	3.58	1.42	.....	.....	.....	.....	.....
SF-268	2.79			> 1.00	> 1.00	-4.55	> -4.00	> -4.00
SF-29	9.07			> 1.00	> 1.00	-5.04	> -4.00	> -4.00
SF-539	4.12			> 1.00	> 1.00	-4.39	> -4.00	> -4.00
SNB-19	2.35			> 1.00	> 1.00	-4.63	> -4.00	> -4.00
SNB-7	1.87			> 1.00	> 1.00	-4.73	> -4.00	> -4.00
U251	1.28			> 1.00	> 1.00	-4.89	> -4.00	> -4.00
Melanoma.....	.....	4.09	1.24	.....	.....	.....	.....	.....
LOX IMVI	5.76			> 1.00	> 1.00	-5.24	> -4.00	> -4.00
MALME-3M	> 1.00			> 1.00	> 1.00	>-4.00	> -4.00	> -4.00
M14	3.91			5.28	> 1.00	-5.41	-4.28	> -4.00
MDA-MB-43	1.50			> 1.00	> 1.00	-4.82	> -4.00	> -4.00
SK-MEL-2	3.78			7.87	> 1.00	-5.42	-4.10	> -4.00
SK-MEL-28	6.12			> 1.00	> 1.00	-4.21	> -4.00	> -4.00
SK-MEL-	3.81			> 1.00	> 1.00	-4.42	> -4.00	> -4.00
UACC-257	6.40			> 1.00	> 1.00	-4.19	> -4.00	> -4.00
UACC-62	1.42			> 1.00	> 1.00	-4.85	> -4.00	> -4.00
Ovarian Cancer	.....	6.93	0.73	.....	.....	.....	.....	.....
IGROV1	6.98			> 1.00	> 1.00	-4.16	> -4.00	> -4.00
OVCAR-3	6.95			> 1.00	> 1.00	-5.16	> -4.00	> -4.00
OVCAR-4	6.22			> 1.00	> 1.00	-5.21	> -4.00	> -4.00
OVCAR-	5.04			> 1.00	> 1.00	-5.30	> -4.00	> -4.00
OVCAR-8	6.81			> 1.00	> 1.00	-4.17	> -4.00	> -4.00
NCI/ADR-RES	9.56			> 1.00	> 1.00	-5.02	> -4.00	> -4.00
Renal Cancer	.....	3.5	1.45	.....	.....	.....	.....	.....
A498	1.46			> 1.00	> 1.00	-4.83	> -4.00	> -4.00
ACHN	3.01			> 1.00	> 1.00	-5.52	> -4.00	> -4.00
CAKI-1	4.78			> 1.00	> 1.00	-5.32	> -4.00	> -4.00
RXF 393	1.20			> 1.00	> 1.00	-4.92	> -4.00	> -4.00
SN12C	5.22			> 1.00	> 1.00	-5.28	> -4.00	> -4.00
TK-10	5.47			> 1.00	> 1.00	-5.26	> -4.00	> -4.00
UO-31	3.52			> 1.00	> 1.00	-5.45	> -4.00	> -4.00
Prostate Cancer	.....	2.1	2.42	.....	.....	.....	.....	.....
PC-3	4.47			> 1.00	> 1.00	-5.35	> -4.00	> -4.00
DU-14	3.81			> 1.00	> 1.00	-4.42	> -4.00	> -4.00
Breast Cancer	.....	4.78	1.07	.....	.....	.....	.....	.....
MCF7	5.67			> 1.00	> 1.00	-5.25	> -4.00	> -4.00
MDA-MB-231/ATCC 0.594	2.84			5.57	> 1.00	-5.55	-4.25	> -4.00
HS 578T	>1.00			> 1.00	> 1.00	> - 4.00	> -4.00	> -4.00
T-47D	9.32			> 1.00	> 1.00	-5.03	> -4.00	> -4.00
MDA-MB-468	1.27			> 1.00	> 1.00	-4.90	> -4.00	> -4.00
MID[a]	5.09							
Average:						-4.96 (11 µM)	-4.02 (95.5 µM)	-4.0 (>100µM)
Delta:						0.59	0.30	0.00
Range:						1.55	0.32	0.00

MID[a]: Average sensitivity of all cell line in µM. MID[b]: Average sensitivity of all cell line of a particular subpanel in µM.

Table (3): 60 cell line anticancer screening data at single dose assay (10^{-5} M) as percent growth inhibition of indolo-carbazoles.

Panel/Cell Line.....	Growth Percent (%).....				
	2_e	**2_f**	**2_h**	**2_i**	**2_l**

Leukaemia	.....	.....	.....	.....	.....
HL-60(TB)	85.63	93.01	93.83	92.43	71.69
K-562	56.77	93.77	90.31	69.13	91.85
MOLT-4	61.55	87.83	89.45	57.59	100.00
RPMI-8226	103.63	93.28	101.84	85.35	98.07
Non-Small Cell Lung Cancer.....	.....	.....	.....	.....	.....
A549/ATCC	35.07	101.29	87.05	70.47	102.55
EKVX	28.58	85.39	100.47	60.37	100.02
HOP-62	-44.87	103.65	75.37	87.43	82.44
HOP-92	-20.13	117.34	98.69	56.36	89.60
NCI-H226	58.36	113.20	121.56	95.18	113.72
NCI-H23	5.55	99.57	85.95	72.69	87.64
NCI-H322M	54.20	92.60	94.27	74.83	106.18
NCI-H460	-15.58	101.36	11.95	68.71	74.67
NCI-H522	-23.83	76.28	69.76	40.64	67.55
Colon Cancer	.....	.....	.....	.....	.....
COLO 205	35.06	102.90	46.32	89.45	104.17
HCC-2998	78.09	94.79	102.40	89.55	nd
HCT-116	7.08	93.94	72.65	58.49	92.35
HCT-15	42.53	91.90	97.68	67.40	101.24
HT29	35.90	90.98	97.62	57.82	103.79
KM12	44.00	105.92	70.71	73.25	104.52
SW-620	6.72	91.96	11.44	80.96	75.56
CNS Cancer	.....	.....	.....	.....	.....
SF-268	37.80	104.78	95.54	93.10	111.10
SF-295	-23.09	108.54	97.81	67.24	99.02
SF-539	38.81	89.59	87.95	95.85	100.18
SNB-19	25.73	104.31	101.34	89.22	109.29
U251	-51.91	97.54	44.79	75.14	91.47
Melanoma	.....	.....	.....	.....	.....
LOX IMVI	42.67	91.90	91.85	76.41	95.39
MALME-3M	21.37	89.19	70.52	95.88	101.60
M14	-29.41	87.83	86.36	78.68	102.10
MDA-MB-435	44.51	102.34	90.59	79.49	100.85
SK-MEL-28	17.60	96.34	87.37	95.93	102.29
SK-MEL-5	45.69	113.79	100.59	71.78	106.20
UACC-257	83.91	104.58	109.35	97.58	106.52
UACC-62	39.50	93.61	90.58	77.85	100.27
Ovarian Cancer	.....	.....	.....	.....	.....
IGROV1	33.23	94.67	94.15	62.03	105.11
OVCAR-3	-52.72	124.29	122.18	96.52	108.60
OVCAR-4	14.28	92.04	78.48	73.12	101.27
OVCAR-5	36.61	88.96	94.14	85.87	104.54
OVCAR-8	20.00	107.28	59.24	89.33	96.21
NCI/ADR-RES	19.48	106.83	75.38	79.55	108.71
SK-OV-3	26.87	105.46	94.19	94.58	91.66
Renal Cancer.....	.....	.....	.....	.....	.....
786-0	-35.79	97.88	84.04	76.33	92.18
A498	-7.03	113.64	86.53	78.86	118.27
ACHN	9.61	98.38	94.06	70.22	104.77
CAKI-1	13.72	80.17	84.65	41.36	95.32
RXF 393	30.66	112.55	105.50	85.59	104.21
SN12C	27.74	96.88	97.15	81.56	99.00
TK-10	-18.32	106.78	95.31	94.84	139.73
UO-31	12.35	57.58	60.84	39.78	76.77
Prostate Cancer.....	.....	.....	.....	.....	.....
PC-3	36.54	107.22	58.82	48.49	76.27
DU-145	46.83	115.99	39.16	98.24	97.18
Breast Cancer.....	.....	.....	.....	.....	.....
MCF7	24.23	89.39	77.15	59.07	94.71
MDA-MB-231/ATCC	-30.21	89.23	86.89	63.58	84.64
HS 578T	-8.41	109.72	91.24	88.18	97.29
BT-549	8.92	113.56	87.17	81.59	94.79
T-47D	28.15	85.57	92.62	59.13	96.62
MDA-MB-468	47.27	115.17	108.96	112.86	121.33
Mean	21.63 % Selected	98.65 % Non selected	84.67% Non selected	76.84% Non selected	98.24% Non selected

Table (4): NCI in vitro testing result of compound **2_e (D-758513/1)** at five dose level in µM.

Panel/Cell Line	GI_{50} Concentration	TGI	LC_{50}	Log GI_{50}	Log TGI	Log LC_{50}

	per cell line	MID[b]	selectivity ratio (MID[a]:MID[b])					
Leukemia	.....	2.41	1.0	.....	.....	.....	.....	.....
CCRF-CEM	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
HL-60(TB)	2.41			> 1.00	> 1.00	-4.62	> -4.00	> -4.00
K-562	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
MOLT-4	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
RPMI-8226	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
SR	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
Non-Small Cell Lung Cancer	.....	2.29	1.06	.....	.....	.....	.....	.....
A549/ATCC	1.51			nd	nd	-5.82	nd	nd
EKVX	1.92			5.72	3.90	-5.72	-5.24	-4.41
HOP-62	1.16			2.38	nd	-5.94	-5.62	nd
HOP-92	1.07			3.73	2.83	-5.97	-5.43	-4.55
NCI-H226	3.28			1.08	3.42	-5.48	-4.97	-4.47
NCI-H23	1.69			4.59	> 1.00	-5.77	-5.34	> -4.00
NCI-H322M	1.46			3.63	9.01	-5.83	-5.44	-5.05
NCI-H460	6.22			1.88	4.34	-6.21	-5.72	-5.36
Colon Cancer	.....	2.68	0.9	.....	.....	.....	.....	.....
COLO 20	4.22			2.05	6.77	-5.38	-4.69	-4.17
HCC-2998	1.69			> 1.00	> 1.00	-4.77	>-4.00	> -4.00
HCT-116	1.34			2.61	5.11	-5.87	-5.58	-5.29
HCT-1	4.24			2.12	8.44	-5.37	-4.67	-4.07
HT29	2.36			6.72	> 1.00	-4.63	-5.17	> -4.00
KM12	3.77			> 1.00	> 1.00	-5.42	>-4.00	> -4.00
SW-620	1.15			2.36	4.86	-4.94	-5.63	-5.31
CNS Cancer	.....	1.66	1.46	.....	.....	.....	.....	.....
SF-268	1.79			5.35	1.99	-5.75	-5.27	-4.70
SF-29	1.19			2.69	6.06	-5.92	-5.57	-5.22
SF-539	3.00			9.53	> 1.00	-5.52	-5.02	> -4.00
SNB-19	1.52			3.35	7.40	-5.82	-5.47	-5.13
SNB-7	1.34			5.24	> 1.00	-5.87	-5.28	> -4.00
U251	1.13			2.33	nd	-5.95	-5.63	nd
Melanoma.....	.....	2.51	0.96	.....	.....	.....	.....	.....
LOX IMVI	2.56			1.21	> 1.00	-5.59	-4.92	> -4.00
MALME-3M	nd			nd	> 1.00	nd	nd	> -4.00
M14	1.67			3.03	5.51	-5.78	-5.52	-5.26
MDA-MB-43	3.10			> 1.00	> 1.00	-5.51	>-4.00	> -4.00
SK-MEL-2	nd			nd	> 1.00	nd	nd	> -4.00
SK-MEL-28	1.62			nd	nd	-5.79	nd	nd
SK-MEL-	2.57			7.81	2.74	-5.59	-5.11	-4.56
UACC-257	3.54			5.93	> 1.00	-5.45	-4.23	>-4.00
UACC-62	2.48			1.15	> 1.00	-5.61	-4.94	> -4.00
Ovarian Cancer	.....	2.5	0.97	.....	.....	.....	.....	.....
IGROV1	2.06			nd	> 1.00	-5.69	nd	> -4.00
OVCAR-3	1.67			3.03	5.51	-5.78	-5.52	-5.26
OVCAR-4	5.65			2.89	1.58	-6.25	-5.54	-4.80
OVCAR-	2.39			6.97	2.60	-5.62	-5.16	-4.58
OVCAR-8	1.72			nd	> 1.00	-5.76	Nd	> -4.00
NCI/ADR-RES	1.82			5.77	3.99	-5.74	-5.24	-4.40
SK-OV-3	2.19			5.72	1.97	-5.66	-5.24	-4.70
Renal Cancer	.....	1.94	1.25	.....	.....	.....	.....	.....
786-0	1.60			2.95	5.43	-5.80	-5.53	-5.27
A498	1.53			3.28	7.05	-5.82	-5.48	-5.15
ACHN	1.96			4.61	1.22	-5.71	-5.34	-4.91
CAKI-1	1.90			6.11	5.51	-5.72	-5.21	-4.26
RXF 393	3.19			9.24	4.14	-5.50	-5.03	-4.38
SN12C	2.00			6.81	> 1.00	-5.70	-5.17	> -4.00
TK-10	1.52			2.85	5.34	-5.82	-5.54	-5.27
UO-31	1.82			5.41	2.01	-5.74	-5.27	-4.70
Prostate Cancer	.....	1.77	1.37	.....	.....	.....	.....	.....
PC-3	nd.			> 1.00	> 1.00	nd	>-4.00	> -4.00
DU-14	1.77			3.30	6.13	-5.75	-5.48	-5.21
Breast Cancer	.....	3.86	0.62	.....	.....	.....	.....	.....
MCF7	9.29			nd	> 1.00	-6.03	nd	> -4.00
MDA-MB-231/ATCC 0.594	1.41			2.70	5.20	-5.85	-5.57	-5.28
HS 578T	2.40			5.08	1.29	-5.62	-5.29	-4.89
T-47D	2.46			nd	> 1.00	-5.61	Nd	> -4.00
MDA-MB-468	3.74			5.23	> 1.00	-5.43	-4.28	> -4.00
_MID[a]	2.42							
Avarege:						-5.69 (2 µM)	-5.01 (10 µM)	-4.46 (34.6 µM)
Delta:						0.56	0.71	0.9
Range:						1.63	1.72	1.36

MID[a]: Average sensitivity of all cell line in µM. MID[b]: Average sensitivity of all cell line of a particular subpanel in µM.

Research Highlights

- We synthesized BIMs and tetrahydroindolocarbazoles,
- Ten compounds have selected by the NCI for anticancer screening,
- Compound **2_e** gave the highest antiproliferative activity in a nanomolar ranges.
- Compound **2_e** showed non critical cytotoxcity.

Supporting information for

Synthesis, Cytostatic Evaluation and Structure Activity Relationships of Novel Bis-indolylmethanes and their Corresponding Tetrahydroindolocarbazoles.

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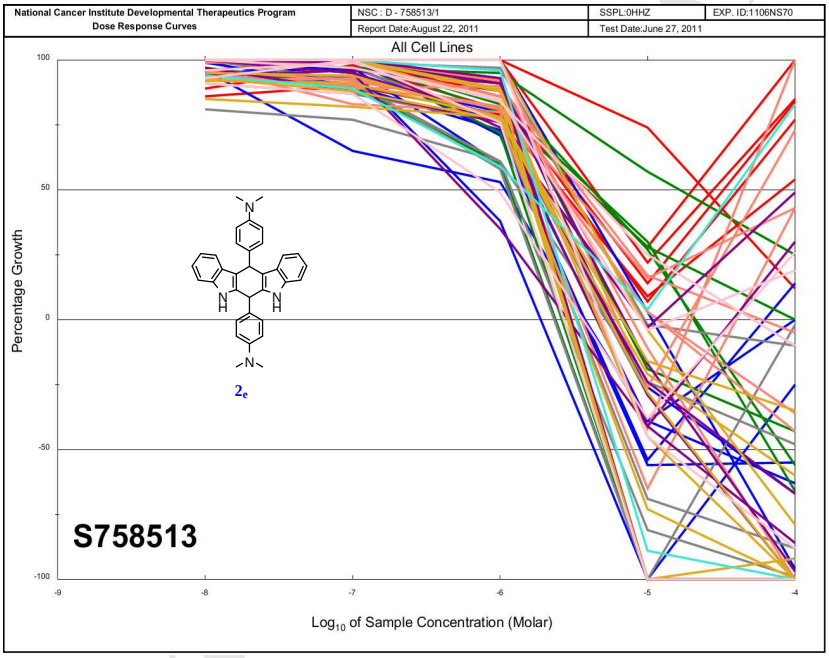
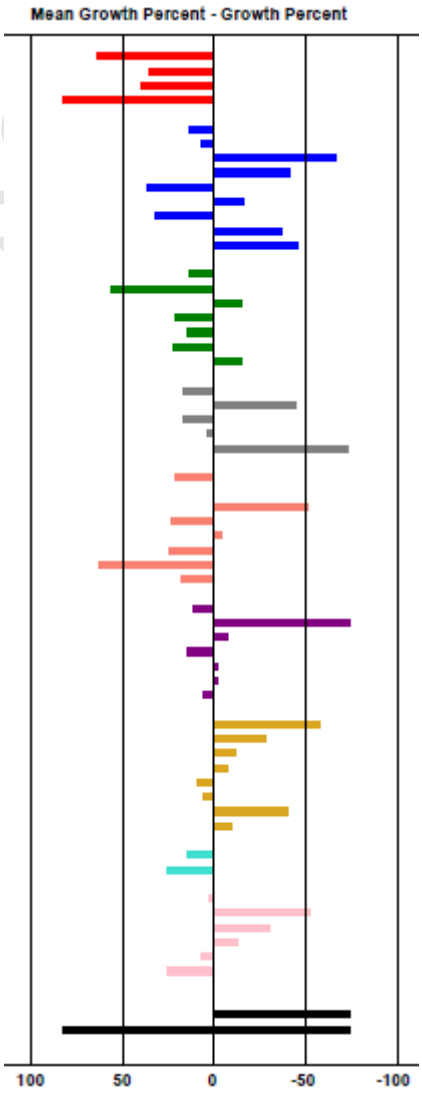




Mean graphs of One and Five dose anticancer screening

Developmental Therapeutics Program
One Dose Mean Graph

NSC: D-755518 / 1	Conc: 1.00E-5 Molar	Test Date: Jan 10, 2011
Experiment ID: 1101OS87		Report Date: Feb 03, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	65.84
HL-60(TB)	85.73
K-562	63.92
MOLT-4	52.47
RPMI-8226	75.01
SR	67.99
Non-Small Cell Lung Cancer	
A549/ATCC	70.37
EKVX	70.13
HOP-62	117.07
HOP-92	77.26
NCI-H226	96.26
NCI-H23	83.58
NCI-H322M	76.91
NCI-H460	40.92
NCI-H522	81.51
Colon Cancer	
COLO 205	98.17
HCC-2998	89.58
HCT-116	49.68
HCT-15	71.42
HT29	67.05
KM12	61.54
SW-620	66.72
CNS Cancer	
SF-268	67.22
SF-295	90.26
SF-539	90.70
SNB-19	88.91
SNB-75	69.63
U251	71.87
Melanoma	
LOX IMVI	70.34
MALME-3M	86.23
M14	60.84
MDA-MB-435	66.55
SK-MEL-2	87.10
SK-MEL-28	94.00
SK-MEL-5	69.90
UACC-257	88.90
UACC-62	65.78
Ovarian Cancer	
IGROV1	51.22
OVCAR-3	57.39
OVCAR-4	85.86
OVCAR-5	84.10
OVCAR-8	84.14
NCI/ADR-RES	83.51
SK-OV-3	99.10
Renal Cancer	
786-0	93.63
A498	104.66
ACHN	63.05
CAKI-1	34.27
RXF 393	90.57
SN12C	71.34
UO-31	42.30
Prostate Cancer	
PC-3	58.42
DU-145	74.41
Breast Cancer	
MCF7	52.85
MDA-MB-231/ATCC	86.20
BT-549	102.59
T-47D	65.05
MDA-MB-468	97.31
Mean	75.51
Delta	41.24
Range	82.80

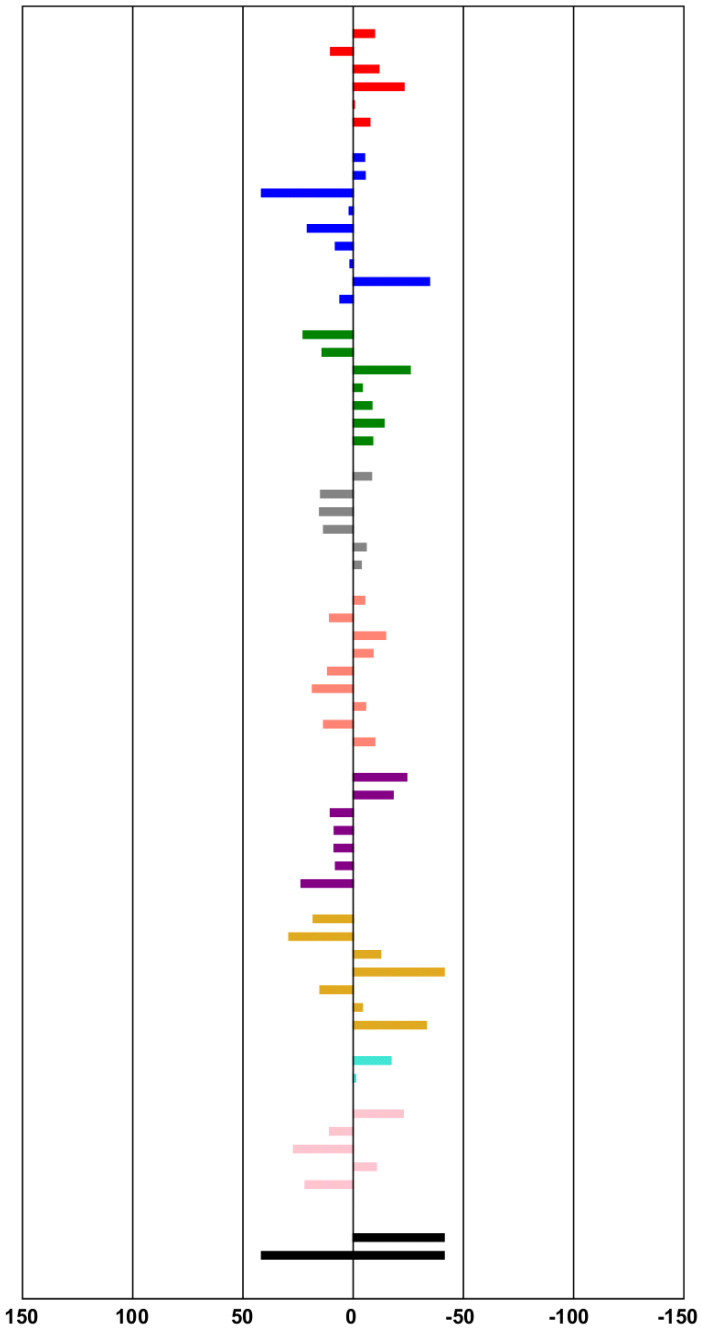


Figure (1): Maen graph one dose screening of **1g**

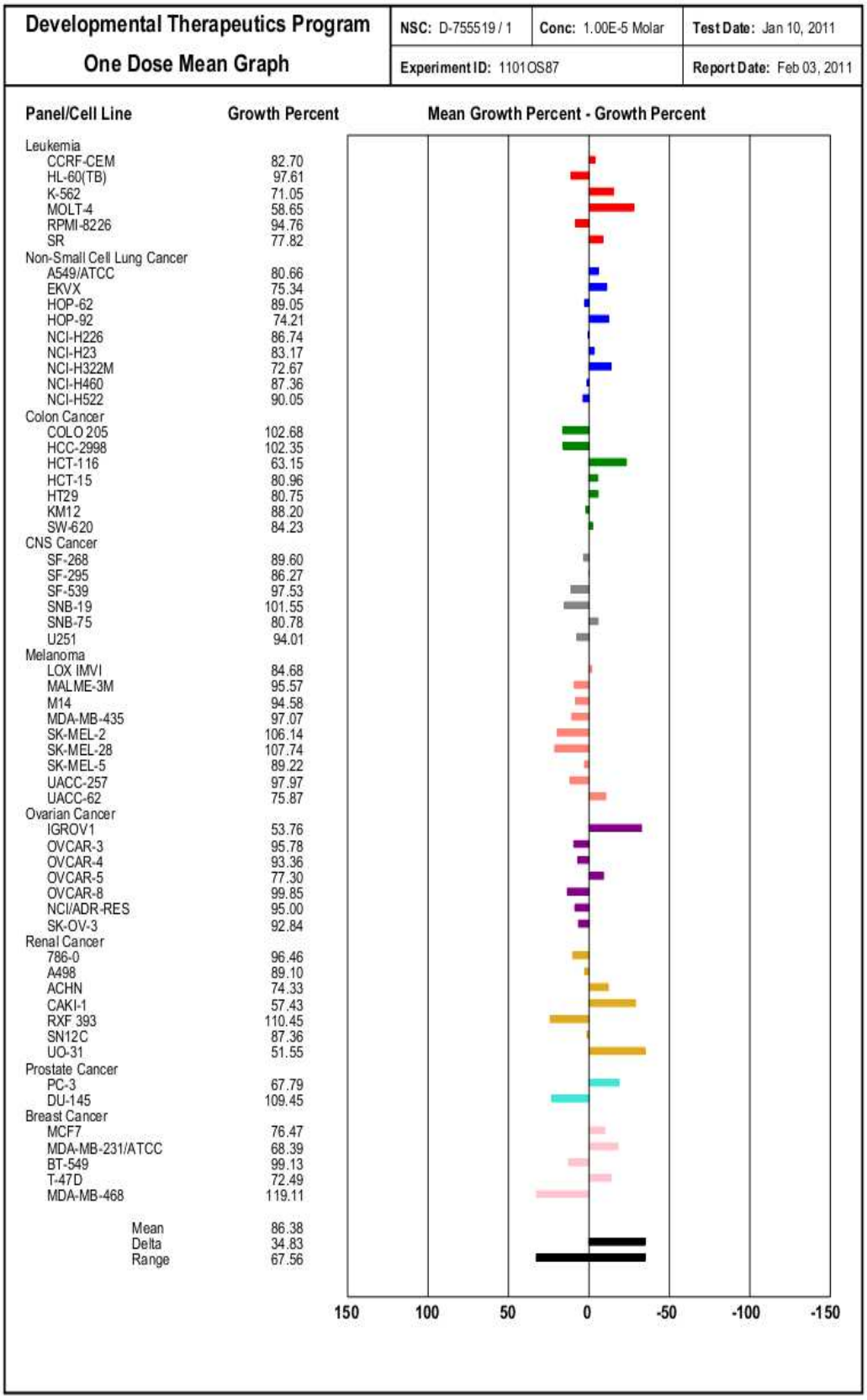


Figure (2) Maen graph one dose screening of $\mathbf{1}_{\mathbf{i}}$.

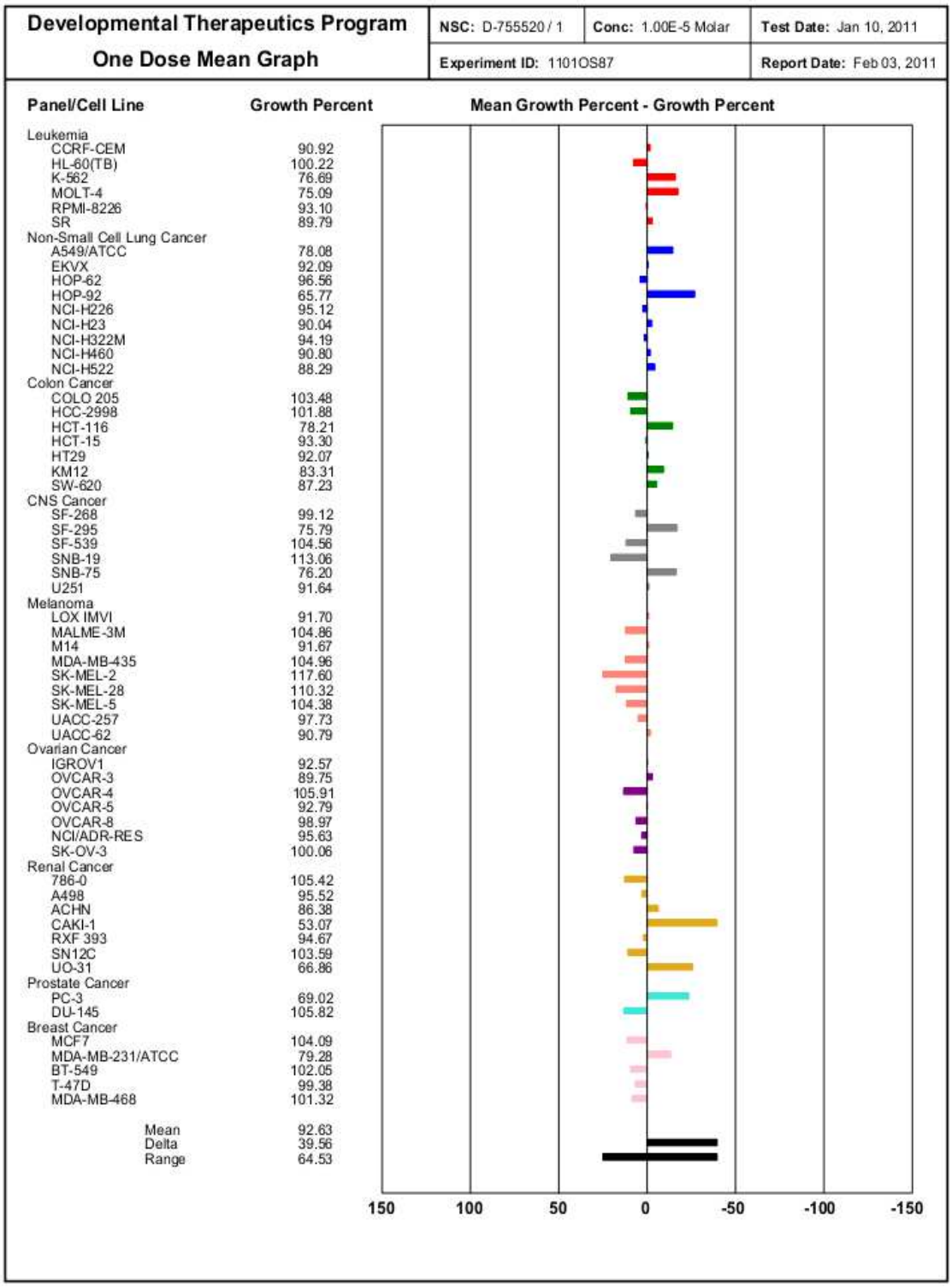


Figure (3): Maen graph one dose screening of **1**$_{l}$.

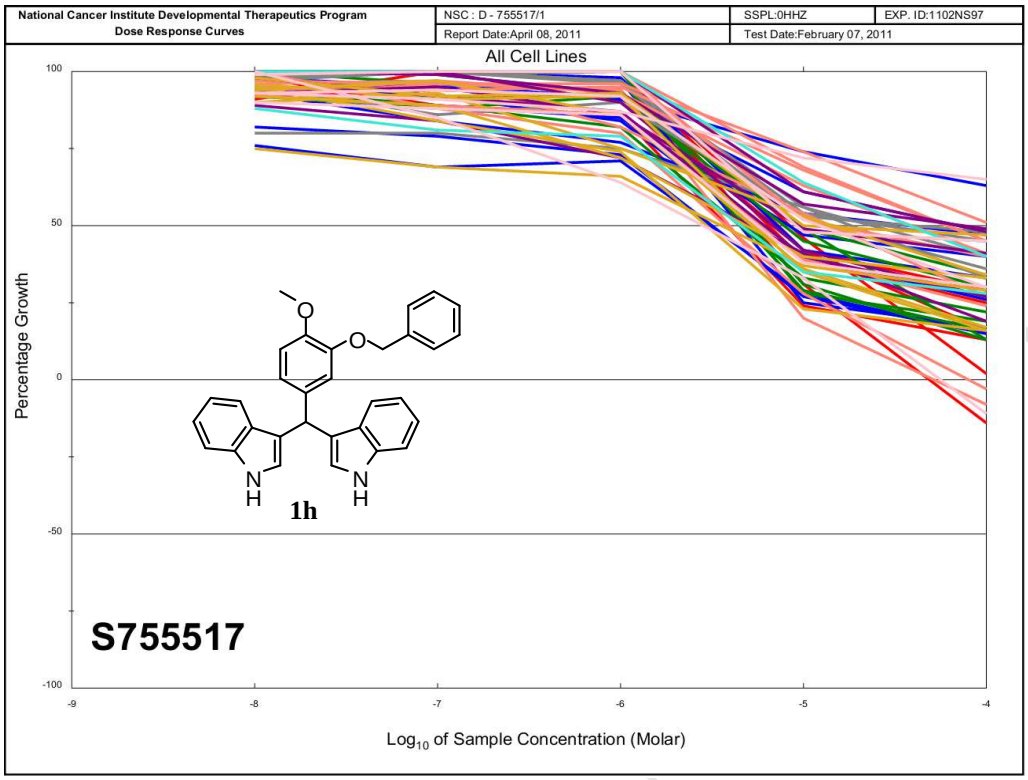


Figure (4): Superposition of all the growth curves of compound $\mathbf{1_h}$

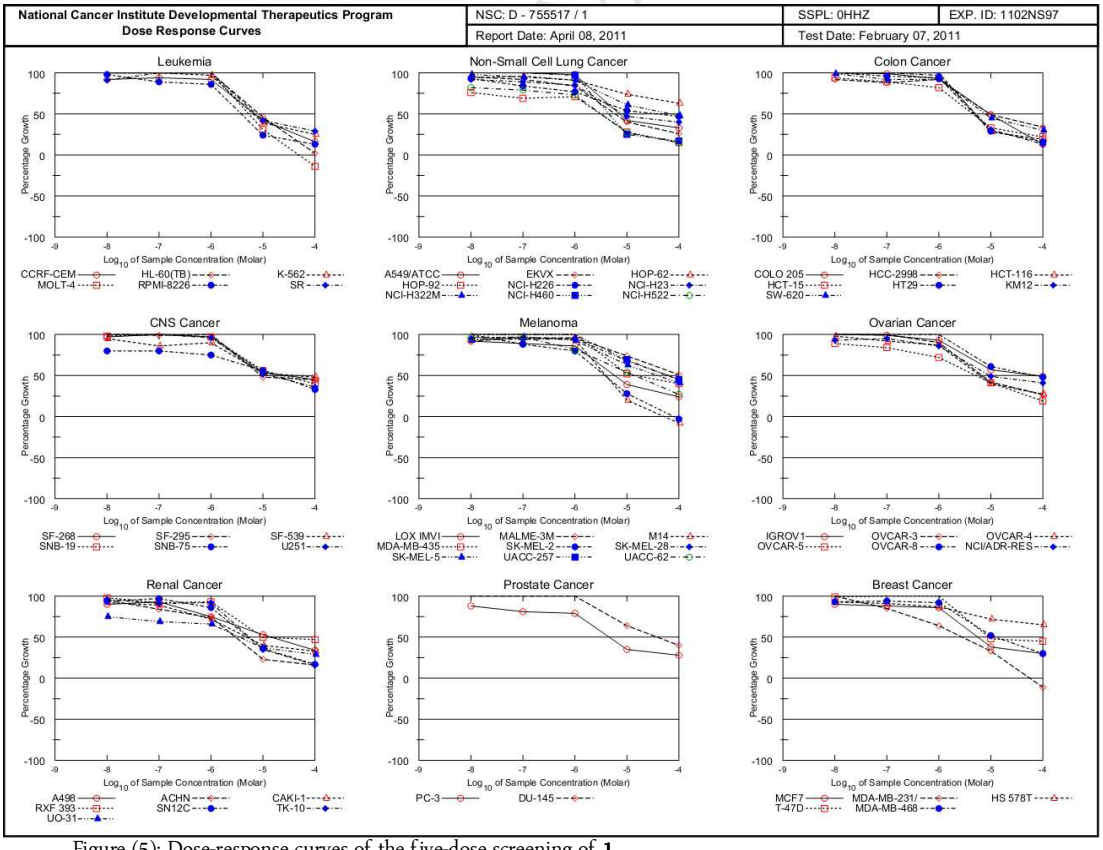


Figure (5): Dose-response curves of the five-dose screening of $\mathbf{1_h}$

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-758511 / 1	Conc: 1.00E-5 Molar	Test Date: May 09, 2011
Experiment ID: 1105OS49		Report Date: Jun 28, 2011

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	93.01
K-562	93.77
MOLT-4	87.83
RPMI-8226	93.28
Non-Small Cell Lung Cancer	
A549/ATCC	101.29
EKVX	85.39
HOP-62	103.65
HOP-92	117.34
NCI-H226	113.20
NCI-H23	99.57
NCI-H322M	92.60
NCI-H460	101.36
NCI-H522	76.28
Colon Cancer	
COLO 205	102.90
HCC-2998	94.79
HCT-116	93.94
HCT-15	91.90
HT29	90.98
KM12	105.92
SW-620	91.96
CNS Cancer	
SF-268	104.78
SF-295	108.54
SF-539	89.59
SNB-19	104.31
U251	97.54
Melanoma	
LOX IMVI	91.90
MALME-3M	89.19
M14	87.83
MDA-MB-435	102.34
SK-MEL-28	96.34
SK-MEL-5	113.79
UACC-257	104.58
UACC-62	93.61
Ovarian Cancer	
IGROV1	94.67
OVCAR-3	124.29
OVCAR-4	92.04
OVCAR-5	88.96
OVCAR-8	107.28
NCI/ADR-RES	106.83
SK-OV-3	105.46
Renal Cancer	
786-0	97.88
A498	113.64
ACHN	98.38
CAKI-1	80.17
RXF 393	112.55
SN12C	96.88
TK-10	106.78
UO-31	57.58
Prostate Cancer	
PC-3	107.22
DU-145	115.99
Breast Cancer	
MCF7	89.39
MDA-MB-231/ATCC	89.23
HS 578T	109.72
BT-549	113.56
T-47D	85.57
MDA-MB-468	115.17
Mean	98.65
Delta	41.07
Range	66.71

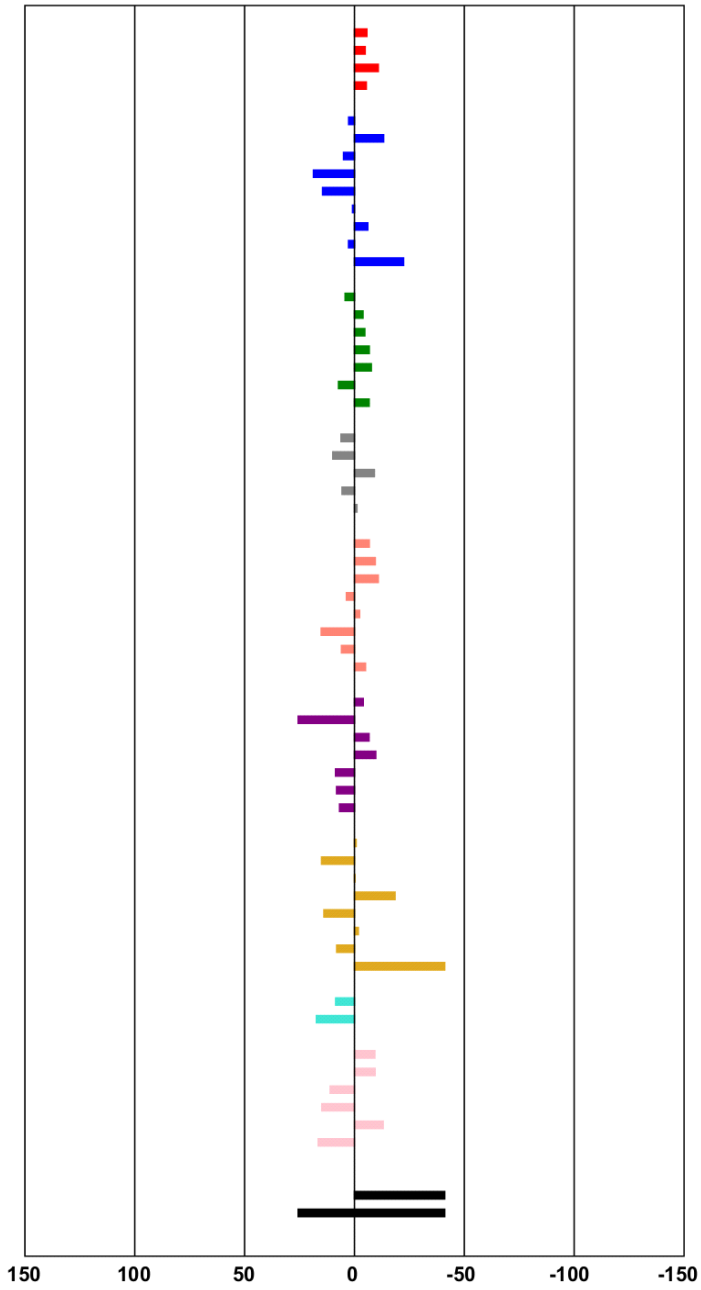


Figure (6): Maen graph one dose screening of $\mathbf{2}_{f}$

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-758512 / 1	Conc: 1.00E-5 Molar	Test Date: May 09, 2011
Experiment ID: 1105OS49		Report Date: Jun 28, 2011

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	92.43
K-562	69.13
MOLT-4	57.59
RPMI-8226	85.35
Non-Small Cell Lung Cancer	
A549/ATCC	70.47
EKVX	60.37
HOP-62	87.43
HOP-92	56.36
NCI-H226	95.18
NCI-H23	72.69
NCI-H322M	74.83
NCI-H460	68.71
NCI-H522	40.64
Colon Cancer	
COLO 205	89.45
HCC-2998	89.55
HCT-116	58.49
HCT-15	67.40
HT29	57.82
KM12	73.25
SW-620	80.96
CNS Cancer	
SF-268	93.10
SF-295	67.24
SF-539	95.85
SNB-19	89.22
U251	75.14
Melanoma	
LOX IMVI	76.41
MALME-3M	95.88
M14	78.68
MDA-MB-435	79.49
SK-MEL-28	95.93
SK-MEL-5	71.78
UACC-257	97.58
UACC-62	77.85
Ovarian Cancer	
IGROV1	62.03
OVCAR-3	96.52
OVCAR-4	73.12
OVCAR-5	85.87
OVCAR-8	89.33
NCI/ADR-RES	79.55
SK-OV-3	94.58
Renal Cancer	
786-0	76.33
A498	78.86
ACHN	70.22
CAKI-1	41.36
RXF 393	85.59
SN12C	81.56
TK-10	94.84
UO-31	39.78
Prostate Cancer	
PC-3	48.49
DU-145	98.24
Breast Cancer	
MCF7	59.07
MDA-MB-231/ATCC	63.58
HS 578T	88.18
BT-549	81.59
T-47D	59.13
MDA-MB-468	112.86
Mean	76.84
Delta	37.06
Range	73.08

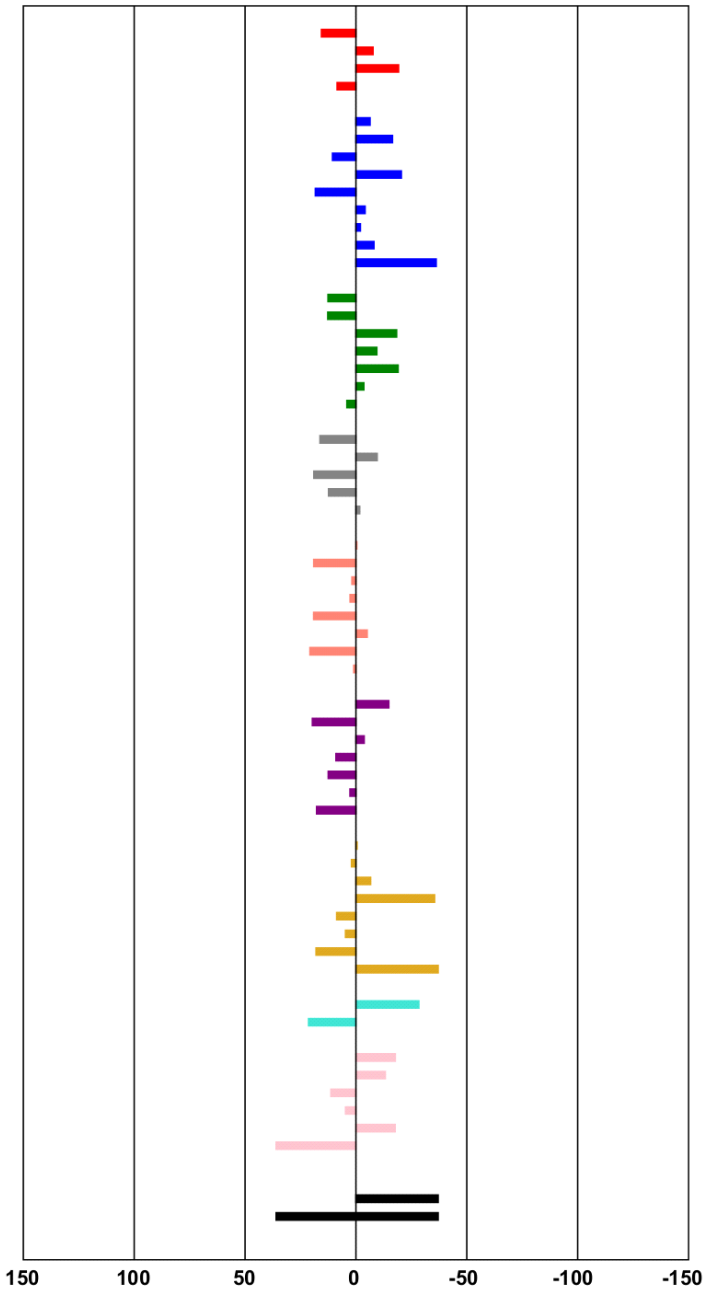


Figure (7): Maen graph one dose screening of **2i**

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-758512 / 1	Conc: 1.00E-5 Molar	Test Date: May 09, 2011
Experiment ID: 1105OS49		Report Date: Jun 28, 2011

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	92.43
K-562	69.13
MOLT-4	57.59
RPMI-8226	85.35
Non-Small Cell Lung Cancer	
A549/ATCC	70.47
EKVX	60.37
HOP-62	87.43
HOP-92	56.36
NCI-H226	95.18
NCI-H23	72.69
NCI-H322M	74.83
NCI-H460	68.71
NCI-H522	40.64
Colon Cancer	
COLO 205	89.45
HCC-2998	89.55
HCT-116	58.49
HCT-15	67.40
HT29	57.82
KM12	73.25
SW-620	80.96
CNS Cancer	
SF-268	93.10
SF-295	67.24
SF-539	95.85
SNB-19	89.22
U251	75.14
Melanoma	
LOX IMVI	76.41
MALME-3M	95.88
M14	78.68
MDA-MB-435	79.49
SK-MEL-28	95.93
SK-MEL-5	71.78
UACC-257	97.58
UACC-62	77.85
Ovarian Cancer	
IGROV1	62.03
OVCAR-3	96.52
OVCAR-4	73.12
OVCAR-5	85.87
OVCAR-8	89.33
NCI/ADR-RES	79.55
SK-OV-3	94.58
Renal Cancer	
786-0	76.33
A498	78.86
ACHN	70.22
CAKI-1	41.36
RXF 393	85.59
SN12C	81.56
TK-10	94.84
UO-31	39.78
Prostate Cancer	
PC-3	48.49
DU-145	98.24
Breast Cancer	
MCF7	59.07
MDA-MB-231/ATCC	63.58
HS 578T	88.18
BT-549	81.59
T-47D	59.13
MDA-MB-468	112.86
Mean	76.84
Delta	37.06
Range	73.08

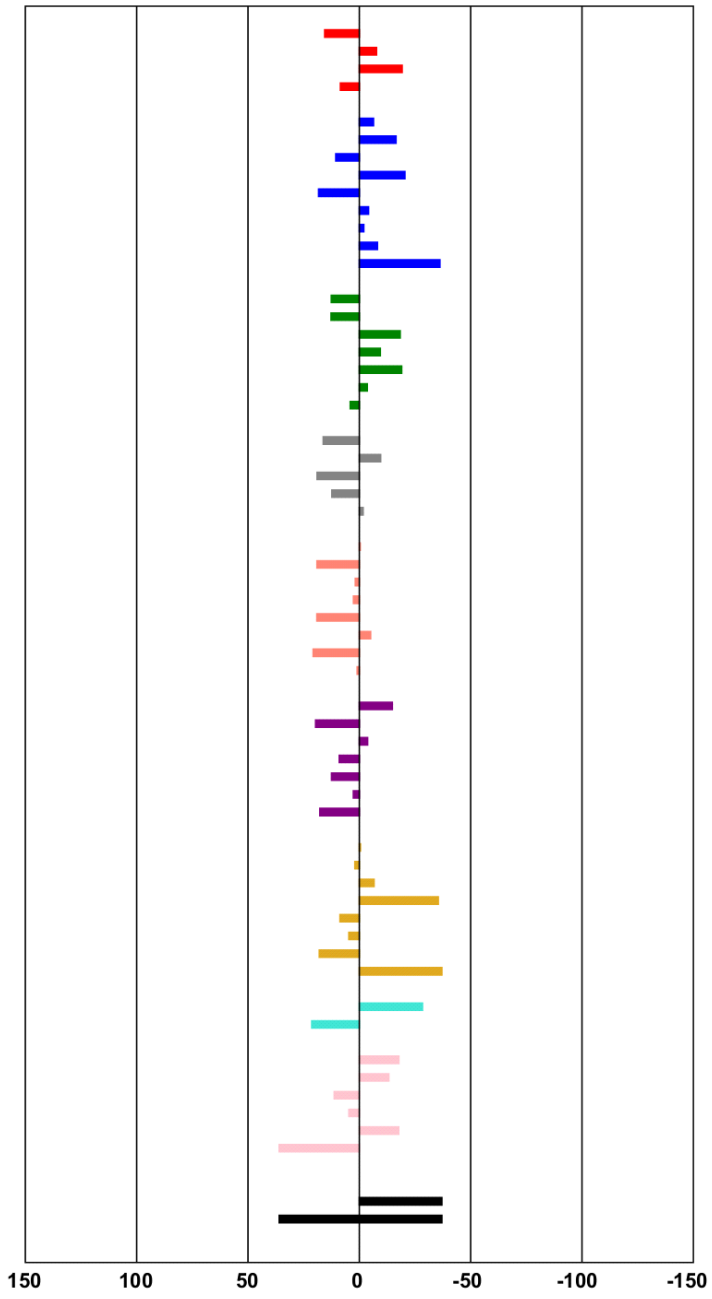


Figure (8): Maen graph one dose screening of **2**$_i$

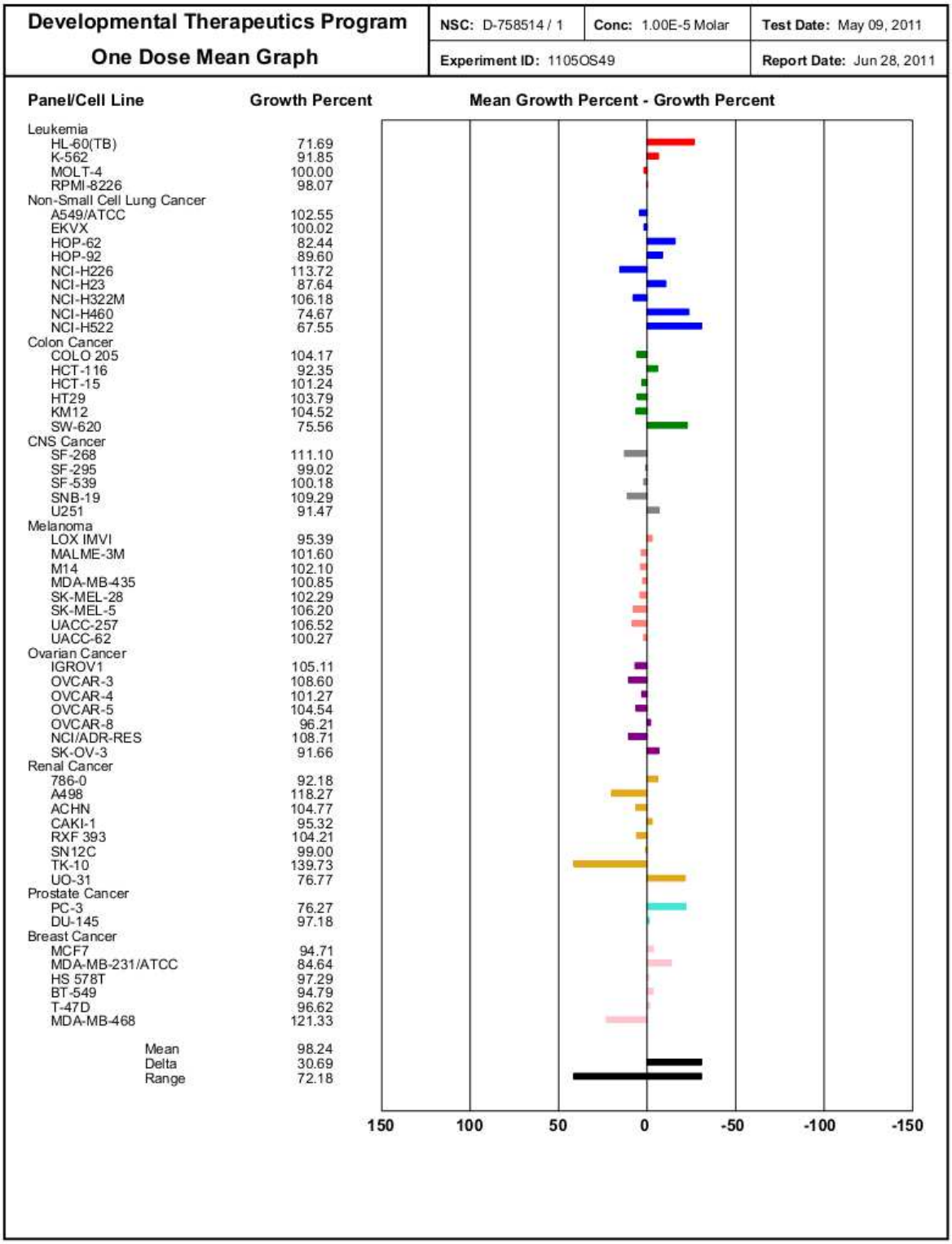


Figure (9): Maen graph one dose screening of **2**$_1$

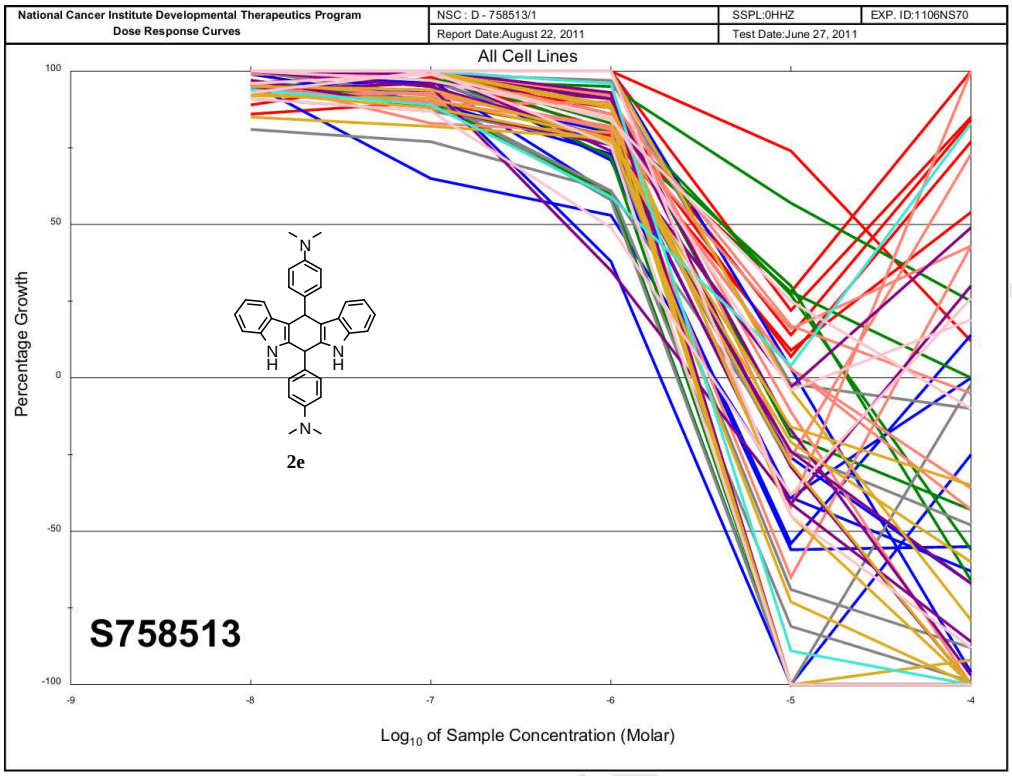


Figure (10): Superposition of all the growth curves for compound **2e**

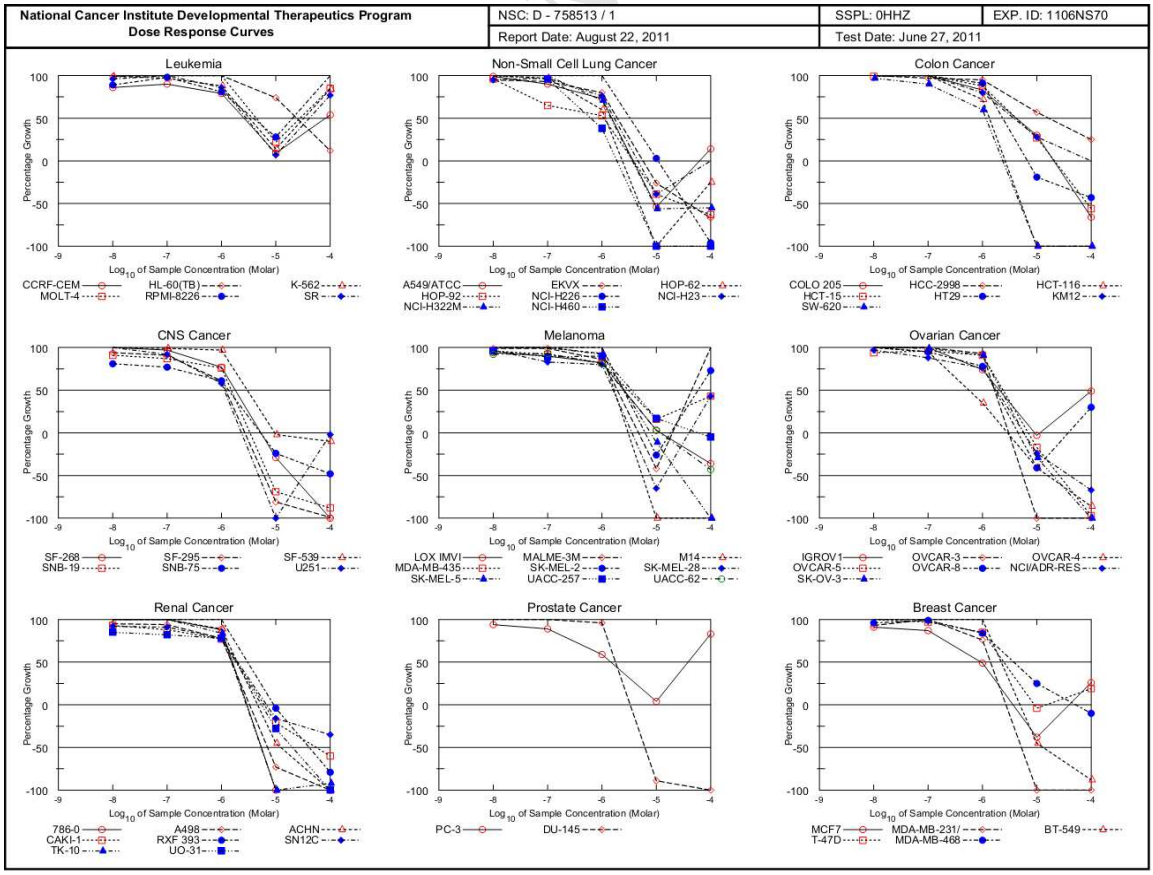


Figure (11): Dose response curves of compound **2e**

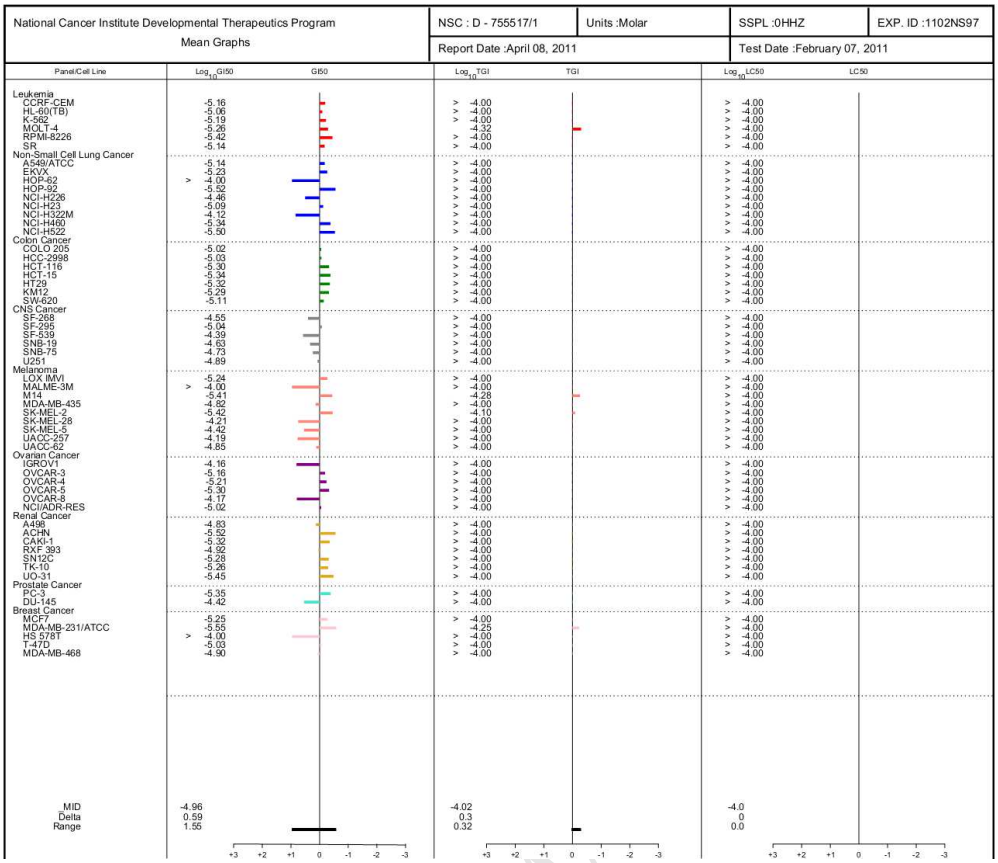


Figure (12): Five dose test results of compound $\mathbf{1_h}$

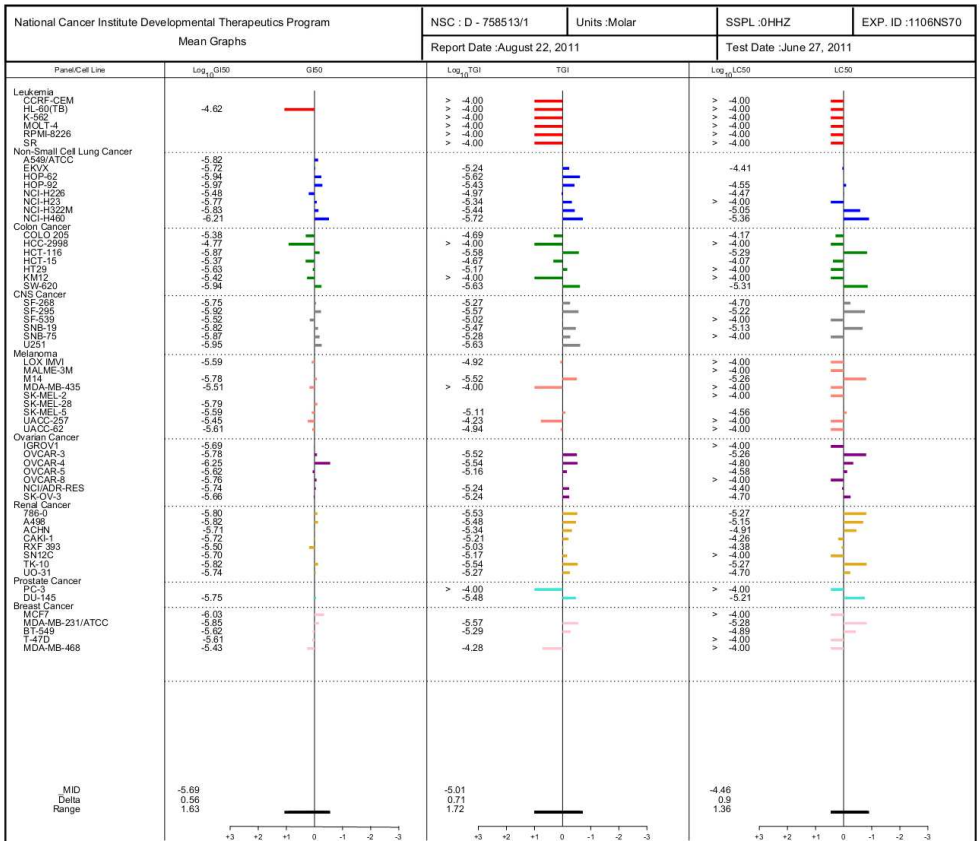


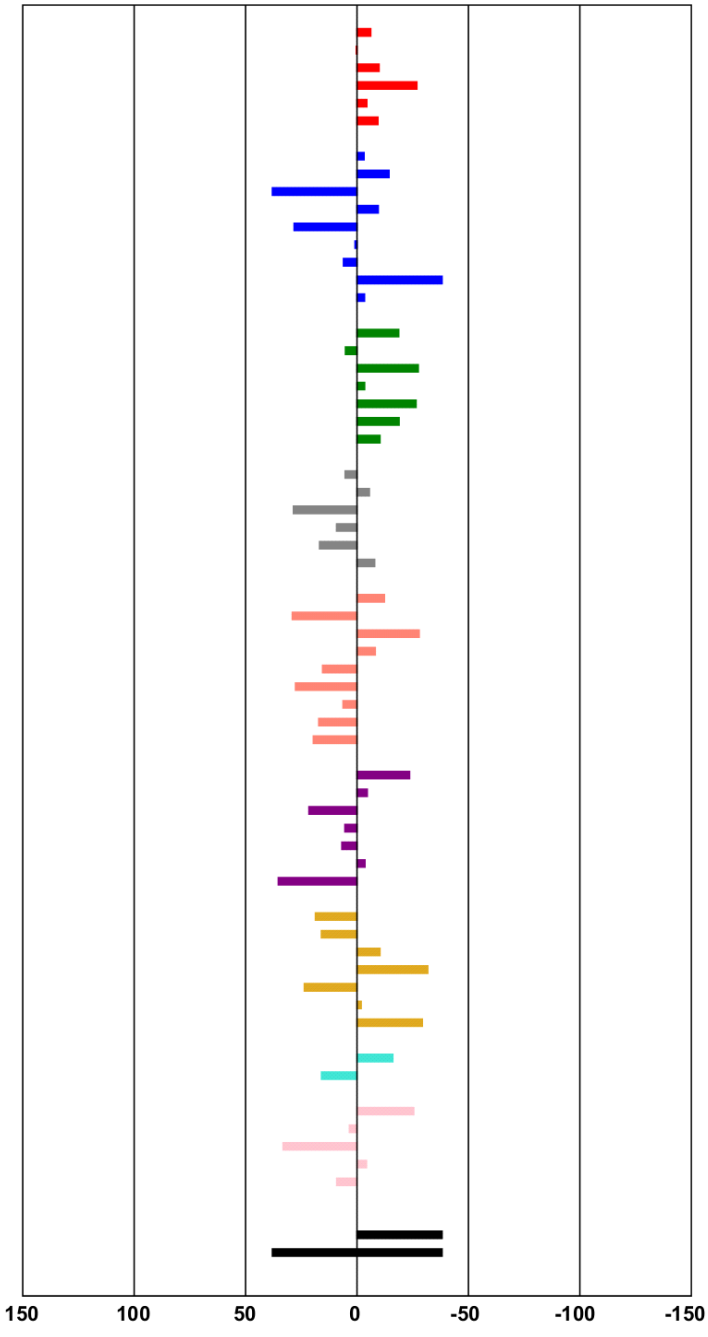
Figure (13): Five dose test results of compound $\mathbf{2_e}$

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-755517 / 1	Conc: 1.00E-5 Molar	Test Date: Jan 10, 2011
Experiment ID: 1101OS87		Report Date: Feb 03, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	41.24
HL-60(TB)	47.74
K-562	37.52
MOLT-4	20.53
RPMI-8226	42.97
SR	38.00
Non-Small Cell Lung Cancer	
A549/ATCC	44.24
EKVX	33.06
HOP-62	85.39
HOP-92	37.86
NCI-H226	75.60
NCI-H23	48.28
NCI-H322M	53.46
NCI-H460	9.25
NCI-H522	44.03
Colon Cancer	
COLO 205	28.69
HCC-2998	52.61
HCT-116	19.91
HCT-15	43.97
HT29	20.89
KM12	28.51
SW-620	37.08
CNS Cancer	
SF-268	52.76
SF-295	41.84
SF-539	75.91
SNB-19	56.58
SNB-75	64.21
U251	39.48
Melanoma	
LOX IMVI	35.11
MALME-3M	76.44
M14	19.50
MDA-MB-435	39.23
SK-MEL-2	62.89
SK-MEL-28	75.02
SK-MEL-5	53.71
UACC-257	64.60
UACC-62	67.03
Ovarian Cancer	
IGROV1	23.79
OVCAR-3	42.79
OVCAR-4	68.99
OVCAR-5	52.85
OVCAR-8	54.18
NCI/ADR-RES	43.84
SK-OV-3	82.74
Renal Cancer	
786-0	66.10
A498	63.36
ACHN	37.06
CAKI-1	15.65
RXF 393	71.08
SN12C	45.51
UO-31	18.10
Prostate Cancer	
PC-3	31.31
DU-145	63.31
Breast Cancer	
MCF7	21.93
MDA-MB-231/ATCC	50.81
BT-549	80.58
T-47D	43.17
MDA-MB-468	56.56
Mean	47.39
Delta	38.14
Range	76.14



Figure(14): Results of the one-dose screening of $\mathbf{1_h}$.

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 755517 / 1	Experiment ID : 1102NS97	Test Type : 08	Units : Molar
Report Date : April 08, 2011	Test Date : February 07, 2011	QNS :	MC :
COMI : Elm 168a (101196)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0HHZ	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.313	1.220	1.147	1.169	1.148	0.694	0.456	92	94	92	42	16	6.91E-6	> 1.00E-4	> 1.00E-4
HL-60(TB)	0.835	2.419	2.537	2.518	2.480	1.571	0.865	107	106	104	46	2	8.67E-6	> 1.00E-4	> 1.00E-4
K-562	0.221	1.713	1.724	1.758	1.672	0.804	0.594	101	103	97	39	25	6.49E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.527	1.800	1.874	1.938	1.866	0.919	0.452	106	111	105	31	-14	5.51E-6	4.83E-5	> 1.00E-4
RPMI-8226	0.750	1.982	1.961	1.844	1.815	1.046	0.915	98	89	86	24	13	3.83E-6	> 1.00E-4	> 1.00E-4
SR	0.351	1.070	1.007	1.108	1.072	0.651	0.557	91	105	100	42	29	7.20E-6	> 1.00E-4	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.380	1.540	1.560	1.595	1.519	0.866	0.761	102	105	98	42	33	7.18E-6	> 1.00E-4	> 1.00E-4
EKVX	0.709	1.863	1.805	1.772	1.677	1.167	1.007	95	92	84	40	26	5.83E-6	> 1.00E-4	> 1.00E-4
HOP-62	0.346	0.976	0.936	0.952	0.918	0.815	0.745	94	96	91	74	63	> 1.00E-4	> 1.00E-4	> 1.00E-4
HOP-92	1.088	1.583	1.464	1.429	1.438	1.225	1.164	76	69	71	28	15	3.03E-6	> 1.00E-4	> 1.00E-4
NCI-H226	0.896	1.877	1.812	1.721	1.649	1.422	1.357	93	84	77	54	47	3.49E-5	> 1.00E-4	> 1.00E-4
NCI-H23	0.696	1.953	1.854	1.820	1.759	1.281	1.196	92	89	85	47	40	8.10E-6	> 1.00E-4	> 1.00E-4
NCI-H322M	0.515	1.214	1.203	1.180	1.150	0.940	0.854	98	95	91	61	48	7.51E-5	> 1.00E-4	> 1.00E-4
NCI-H460	0.201	1.575	1.625	1.616	1.538	0.549	0.438	104	103	97	25	17	4.54E-6	> 1.00E-4	> 1.00E-4
NCI-H522	0.538	1.262	1.136	1.114	1.067	0.733	0.650	82	79	73	27	15	3.15E-6	> 1.00E-4	> 1.00E-4
Colon Cancer															
COLO 205	0.277	1.275	1.329	1.261	1.209	0.766	0.410	105	99	93	49	13	9.47E-6	> 1.00E-4	> 1.00E-4
HCC-2998	0.490	1.731	1.627	1.584	1.630	1.094	0.918	92	88	92	49	34	9.31E-6	> 1.00E-4	> 1.00E-4
HCT-116	0.251	1.594	1.645	1.667	1.614	0.631	0.504	104	105	101	28	19	5.05E-6	> 1.00E-4	> 1.00E-4
HCT-15	0.315	1.662	1.577	1.513	1.419	0.763	0.613	94	89	82	33	22	4.53E-6	> 1.00E-4	> 1.00E-4
HT29	0.225	1.096	1.093	1.061	1.047	0.475	0.364	100	96	94	29	16	4.74E-6	> 1.00E-4	> 1.00E-4
KM12	0.405	1.671	1.701	1.682	1.631	0.796	0.575	102	101	97	31	13	5.12E-6	> 1.00E-4	> 1.00E-4
SW-620	0.226	1.502	1.491	1.394	1.398	0.796	0.611	99	92	92	45	30	7.71E-6	> 1.00E-4	> 1.00E-4
CNS Cancer															
SF-268	0.544	1.747	1.708	1.743	1.714	1.194	1.085	97	100	97	54	45	2.79E-5	> 1.00E-4	> 1.00E-4
SF-295	0.706	2.433	2.454	2.418	2.426	1.532	1.487	101	99	100	48	45	9.07E-6	> 1.00E-4	> 1.00E-4
SF-539	0.818	2.032	1.972	1.862	1.907	1.463	1.401	95	86	90	53	48	4.12E-5	> 1.00E-4	> 1.00E-4
SNB-19	0.425	1.187	1.174	1.209	1.166	0.849	0.733	98	103	97	56	40	2.35E-5	> 1.00E-4	> 1.00E-4
SNB-75	0.713	1.161	1.072	1.072	1.048	0.965	0.861	80	80	75	56	33	1.87E-5	> 1.00E-4	> 1.00E-4
U251	0.237	1.113	1.112	1.110	1.075	0.690	0.556	100	100	96	52	36	1.28E-5	> 1.00E-4	> 1.00E-4
Melanoma															
LOX IMVI	0.259	1.776	1.655	1.610	1.568	0.845	0.623	92	89	86	39	24	5.76E-6	> 1.00E-4	> 1.00E-4
MALME-3M	0.698	1.499	1.436	1.468	1.470	1.292	1.107	92	96	96	74	51	> 1.00E-4	> 1.00E-4	> 1.00E-4
M14	0.403	1.198	1.177	1.169	1.150	0.560	0.373	97	96	94	20	-8	3.91E-6	5.28E-5	> 1.00E-4
MDA-MB-435	0.537	2.111	2.109	2.200	2.153	1.358	1.165	100	106	103	52	40	1.50E-5	> 1.00E-4	> 1.00E-4
SK-MEL-2	0.899	1.312	1.316	1.264	1.229	1.016	0.870	101	88	80	28	-3	3.78E-6	7.87E-5	> 1.00E-4
SK-MEL-28	0.542	1.335	1.298	1.288	1.296	1.082	0.900	95	94	95	68	45	6.12E-5	> 1.00E-4	> 1.00E-4
SK-MEL-5	0.534	2.357	2.227	2.303	2.222	1.679	1.277	93	97	93	63	41	3.81E-5	> 1.00E-4	> 1.00E-4
UACC-257	0.613	1.100	1.120	1.142	1.142	0.950	0.834	104	109	109	69	45	6.40E-5	> 1.00E-4	> 1.00E-4
UACC-62	0.735	1.842	1.797	1.807	1.642	1.335	1.029	96	97	82	54	27	1.42E-5	> 1.00E-4	> 1.00E-4
Ovarian Cancer															
IGROV1	0.518	1.792	1.851	1.783	1.706	1.248	1.138	105	99	93	57	49	6.98E-5	> 1.00E-4	> 1.00E-4
OVCAR-3	0.405	1.215	1.215	1.214	1.138	0.749	0.627	100	100	90	42	27	6.95E-6	> 1.00E-4	> 1.00E-4
OVCAR-4	0.453	1.309	1.291	1.245	1.200	0.798	0.687	98	92	87	40	27	6.22E-6	> 1.00E-4	> 1.00E-4
OVCAR-5	0.575	1.425	1.330	1.291	1.186	0.922	0.735	89	84	72	41	19	5.04E-6	> 1.00E-4	> 1.00E-4
OVCAR-8	0.313	1.208	1.229	1.224	1.218	0.855	0.742	102	102	101	61	48	6.81E-5	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.581	1.885	1.798	1.816	1.697	1.224	1.112	93	95	86	49	41	9.56E-6	> 1.00E-4	> 1.00E-4
Renal Cancer															
A498	0.939	1.720	1.645	1.667	1.521	1.355	1.201	90	93	75	53	34	1.46E-5	> 1.00E-4	> 1.00E-4
ACHN	0.424	1.224	1.187	1.093	1.019	0.612	0.551	95	84	74	23	16	3.01E-6	> 1.00E-4	> 1.00E-4
CAKI-1	0.844	2.467	2.340	2.292	2.013	1.487	1.373	92	89	72	40	33	4.78E-6	> 1.00E-4	> 1.00E-4
RXF 393	0.711	1.373	1.362	1.317	1.324	1.044	1.021	98	92	93	50	47	1.20E-5	> 1.00E-4	> 1.00E-4
SN12C	0.650	1.718	1.657	1.681	1.565	1.035	0.836	94	97	86	36	17	5.22E-6	> 1.00E-4	> 1.00E-4
TK-10	0.640	1.124	1.106	1.078	1.087	0.809	0.715	96	91	92	35	16	5.47E-6	> 1.00E-4	> 1.00E-4
UO-31	0.562	1.821	1.503	1.431	1.396	1.023	0.921	75	69	66	37	29	3.52E-6	> 1.00E-4	> 1.00E-4
Prostate Cancer															
PC-3	0.450	1.300	1.199	1.142	1.118	0.745	0.690	88	81	79	35	28	4.47E-6	> 1.00E-4	> 1.00E-4
DU-145	0.392	1.368	1.407	1.401	1.428	1.015	0.783	104	103	106	64	40	3.81E-5	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.480	2.257	2.088	2.044	2.015	1.157	1.012	90	88	86	38	30	5.67E-6	> 1.00E-4	> 1.00E-4
MDA-MB-231/ATCC	0.594	1.170	1.229	1.083	0.965	0.782	0.528	110	85	64	33	-11	2.84E-6	5.57E-5	> 1.00E-4
HS 578T	0.509	1.289	1.235	1.220	1.186	1.073	1.013	93	91	87	72	65	> 1.00E-4	> 1.00E-4	> 1.00E-4
T-47D	0.580	1.280	1.276	1.305	1.299	0.919	0.893	99	104	103	48	45	9.32E-6	> 1.00E-4	> 1.00E-4
MDA-MB-468	0.606	1.367	1.317	1.319	1.307	1.004	0.835	93	94	92	52	30	1.27E-5	> 1.00E-4	> 1.00E-4

Figure (16): Five dose testing results of compound $\mathbf{1_h}$.

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-758513 / 1	**Conc:** 1.00E-5 Molar	**Test Date:** May 09, 2011
Experiment ID: 1105OS49		**Report Date:** Jun 28, 2011

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	85.63
K-562	56.77
MOLT-4	61.55
RPMI-8226	103.63
Non-Small Cell Lung Cancer	
A549/ATCC	35.07
EKVX	28.58
HOP-62	-44.87
HOP-92	-20.13
NCI-H226	58.36
NCI-H23	5.55
NCI-H322M	54.20
NCI-H460	-15.58
NCI-H522	-23.83
Colon Cancer	
COLO 205	35.06
HCC-2998	78.09
HCT-116	7.08
HCT-15	42.53
HT29	35.90
KM12	44.00
SW-620	6.72
CNS Cancer	
SF-268	37.80
SF-295	-23.09
SF-539	38.81
SNB-19	25.73
U251	-51.91
Melanoma	
LOX IMVI	42.67
MALME-3M	21.37
M14	-29.41
MDA-MB-435	44.51
SK-MEL-28	17.60
SK-MEL-5	45.69
UACC-257	83.91
UACC-62	39.50
Ovarian Cancer	
IGROV1	33.23
OVCAR-3	-52.72
OVCAR-4	14.28
OVCAR-5	36.61
OVCAR-8	20.00
NCI/ADR-RES	19.48
SK-OV-3	26.87
Renal Cancer	
786-0	-35.79
A498	-7.03
ACHN	9.61
CAKI-1	13.72
RXF 393	30.66
SN12C	27.74
TK-10	-18.32
UO-31	12.35
Prostate Cancer	
PC-3	36.54
DU-145	46.83
Breast Cancer	
MCF7	24.23
MDA-MB-231/ATCC	-30.21
HS 578T	-8.41
BT-549	8.92
T-47D	28.15
MDA-MB-468	47.27
Mean	21.63
Delta	74.35
Range	156.35

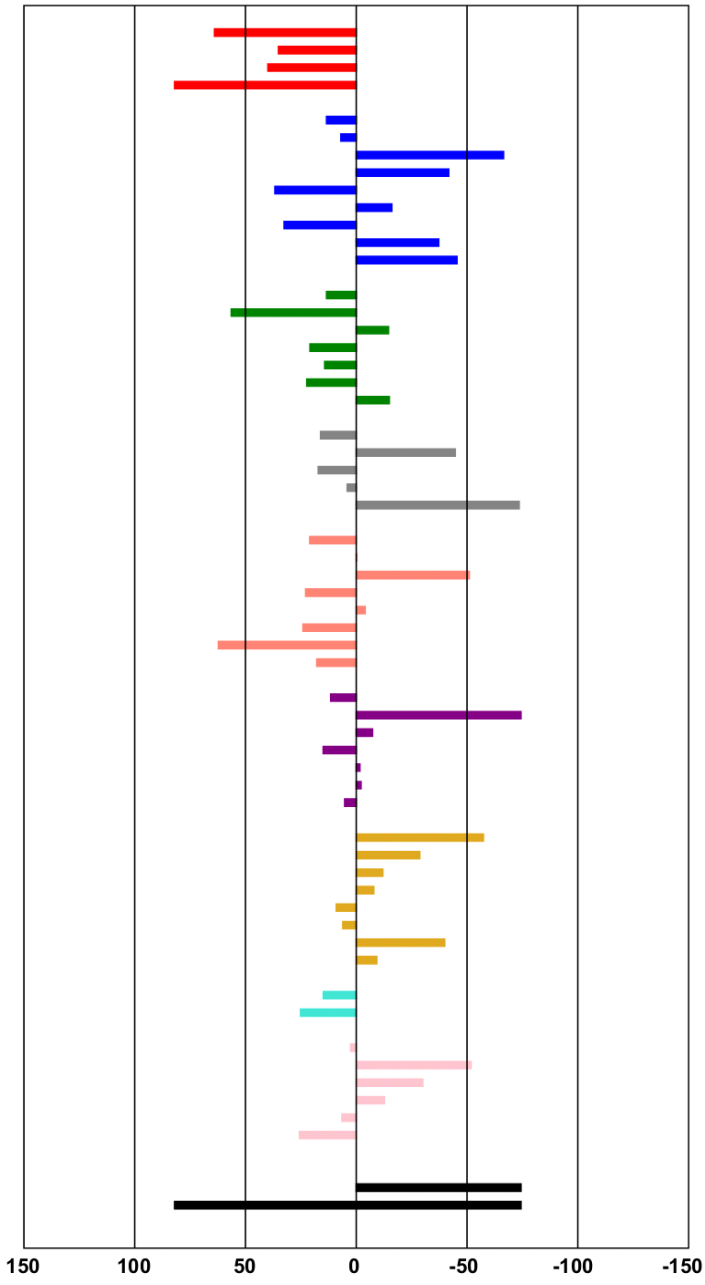


Figure (17): Results of the one dose screening of **2e**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 758513 / 1	Experiment ID : 1106NS70	Test Type : 08	Units : Molar
Report Date : August 22, 2011	Test Date : June 27, 2011	QNS :	MC :
COMI : Elm-213a (105821)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0HHZ	

Log10 Concentration

Panel/Cell Line	Time Zero	Mean Optical Densities: Ctrl	-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															
CCRF-CEM	0.557	1.688	1.525	1.571	1.455	0.663	1.164	86	90	79	9	54	.	> 1.00E-4	> 1.00E-4
HL-60(TB)	0.977	2.710	2.699	2.783	2.718	2.252	1.182	99	104	100	74	12	2.41E-5	> 1.00E-4	> 1.00E-4
K-562	0.168	1.073	1.064	1.049	0.965	0.299	0.930	99	97	88	14	84	.	> 1.00E-4	> 1.00E-4
MOLT-4	0.522	1.548	1.592	1.584	1.574	0.748	1.393	104	104	103	22	85	.	> 1.00E-4	> 1.00E-4
RPMI-8226	0.384	1.035	0.960	1.024	0.914	0.564	1.193	89	98	81	28	124	.	> 1.00E-4	> 1.00E-4
SR	0.374	1.380	1.341	1.387	1.236	0.440	1.145	96	101	86	7	77	.	> 1.00E-4	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.333	1.611	1.593	1.488	1.263	0.153	0.509	99	90	73	-54	14	1.51E-6	.	.
EKVX	0.707	1.743	1.692	1.674	1.537	0.526	0.235	95	93	80	-26	-67	1.92E-6	5.72E-6	3.90E-5
HOP-62	0.364	1.094	1.111	1.075	0.804	-0.031	0.274	102	97	60	-100	-25	1.16E-6	2.38E-6	.
HOP-92	1.098	1.382	1.370	1.282	1.247	0.666	0.407	96	65	53	-39	-63	1.07E-6	3.73E-6	2.83E-5
NCI-H226	0.752	1.456	1.470	1.453	1.455	0.775	0.027	102	100	100	3	-96	3.28E-6	1.08E-5	3.42E-5
NCI-H23	0.627	1.698	1.645	1.662	1.446	0.383	0.626	95	97	76	-39	.	1.69E-6	4.59E-6	> 1.00E-4
NCI-H322M	0.347	0.757	0.770	0.776	0.638	0.154	0.155	103	105	71	-56	-55	1.46E-6	3.63E-6	9.01E-6
NCI-H460	0.224	1.797	1.843	1.739	0.821	-0.071	-0.173	103	96	38	-100	-100	6.22E-7	1.88E-6	4.34E-6
Colon Cancer															
COLO 205	0.246	1.305	1.395	1.343	1.130	0.563	0.083	109	104	83	30	-66	4.22E-6	2.05E-5	6.77E-5
HCC-2998	0.650	2.205	2.233	2.159	2.133	1.541	1.045	102	97	95	57	25	1.69E-5	> 1.00E-4	> 1.00E-4
HCT-116	0.170	1.128	1.170	1.138	0.856	-0.016	-0.350	104	101	72	-100	-100	1.34E-6	2.61E-6	5.11E-6
HCT-15	0.310	1.842	1.822	1.864	1.664	0.727	0.136	99	101	88	27	-56	4.24E-6	2.12E-5	8.44E-5
HT29	0.223	1.076	1.199	1.134	1.001	0.181	0.126	114	107	91	-19	-43	2.36E-6	6.72E-6	> 1.00E-4
KM12	0.398	1.756	1.753	1.777	1.482	0.779	0.405	100	102	80	28	.	3.77E-6	> 1.00E-4	> 1.00E-4
SW-620	0.189	1.094	1.067	1.004	0.729	-0.029	-0.316	97	90	60	-100	-100	1.15E-6	2.36E-6	4.86E-6
CNS Cancer															
SF-268	0.327	1.134	1.133	1.114	0.946	0.234	-0.437	100	97	77	-29	-100	1.79E-6	5.35E-6	1.99E-5
SF-295	0.783	2.216	2.125	2.085	1.655	0.151	0.005	94	91	61	-81	-99	1.19E-6	2.69E-6	6.06E-6
SF-539	0.824	2.025	2.073	2.019	1.995	0.807	0.740	104	99	97	-2	-10	3.00E-6	9.53E-6	> 1.00E-4
SNB-19	0.436	1.503	1.403	1.363	1.252	0.135	0.052	91	87	76	-69	-88	1.52E-6	3.35E-6	7.40E-6
SNB-75	0.617	1.459	1.295	1.261	1.128	0.471	0.319	81	77	61	-24	-48	1.34E-6	5.24E-6	> 1.00E-4
U251	0.291	1.233	1.237	1.153	0.839	-0.039	0.285	100	92	58	-100	-2	1.13E-6	2.33E-6	.
Melanoma															
LOX IMVI	0.314	1.879	1.783	1.716	1.604	0.364	0.202	94	90	82	3	-36	2.56E-6	1.21E-5	> 1.00E-4
MALME-3M	0.544	0.860	0.858	0.857	0.816	0.313	1.138	99	99	86	-42	188	.	.	> 1.00E-4
M14	0.369	1.251	1.278	1.305	1.189	-0.034	-0.418	103	106	93	-100	-100	1.67E-6	3.03E-6	5.51E-6
MDA-MB-435	0.384	1.689	1.634	1.591	1.461	0.598	0.945	96	92	82	16	43	3.10E-6	> 1.00E-4	> 1.00E-4
SK-MEL-2	0.846	1.603	1.700	1.730	1.611	0.624	1.396	113	117	101	-26	73	.	.	> 1.00E-4
SK-MEL-28	0.321	1.063	1.042	0.939	0.917	0.112	0.641	97	83	80	-65	43	1.62E-6	.	.
SK-MEL-5	0.644	1.929	1.991	1.940	1.831	0.573	-0.221	105	101	92	-11	-100	2.57E-6	7.81E-6	2.74E-5
UACC-257	0.691	1.412	1.386	1.332	1.343	0.812	0.657	96	89	90	17	-5	3.54E-6	5.93E-5	> 1.00E-4
UACC-62	0.566	2.336	2.193	2.204	1.996	0.616	0.322	92	93	81	3	-43	2.48E-6	1.15E-5	> 1.00E-4
Ovarian Cancer															
IGROV1	0.420	1.215	1.279	1.268	1.011	0.408	0.814	108	107	74	-3	49	2.06E-6	.	> 1.00E-4
OVCAR-3	0.433	1.091	1.111	1.103	1.045	-0.017	-0.409	103	102	93	-100	-100	1.67E-6	3.03E-6	5.51E-6
OVCAR-4	0.396	1.499	1.497	1.440	0.785	0.233	0.057	100	95	35	-41	-86	5.65E-7	2.89E-6	1.58E-5
OVCAR-5	0.467	1.181	1.137	1.154	1.114	0.389	0.016	94	96	91	-17	-97	2.39E-6	6.97E-6	2.60E-5
OVCAR-8	0.311	1.275	1.290	1.231	1.065	0.183	0.601	102	95	78	-41	30	1.72E-6	.	> 1.00E-4
NCI/ADR-RES	0.532	1.620	1.585	1.487	1.360	0.405	0.174	97	88	76	-24	-67	1.82E-6	5.77E-6	3.99E-5
SK-OV-3	0.566	1.510	1.546	1.499	1.423	0.402	-0.003	104	99	91	-29	-100	2.19E-6	5.72E-6	1.97E-5
Renal Cancer															
786-0	0.607	1.874	1.890	1.892	1.729	-0.113	-0.606	101	101	88	-100	-100	1.60E-6	2.95E-6	5.43E-6
A498	0.970	1.418	1.397	1.392	1.318	0.264	-0.462	95	94	78	-73	-100	1.53E-6	3.28E-6	7.05E-6
ACHN	0.438	1.657	1.672	1.675	1.526	0.240	-0.192	101	101	89	-45	-100	1.96E-6	4.61E-6	1.22E-5
CAKI-1	0.599	2.093	1.993	1.917	1.756	0.473	0.239	93	88	77	-21	-60	1.90E-6	6.11E-6	5.51E-5
RXF 393	0.605	0.934	0.950	0.947	0.949	0.583	0.129	105	104	104	-4	-79	3.19E-6	9.24E-6	4.14E-5
SN12C	0.469	1.740	1.639	1.627	1.462	0.396	0.304	92	91	78	-16	-35	2.00E-6	6.81E-6	> 1.00E-4
TK-10	0.590	1.158	1.177	1.185	1.065	-0.044	0.045	103	105	84	-100	-92	1.52E-6	2.85E-6	5.34E-6
UO-31	0.674	1.480	1.361	1.336	1.299	0.484	-0.280	85	82	78	-28	-100	1.82E-6	5.41E-6	2.01E-5
Prostate Cancer															
PC-3	0.524	1.626	1.563	1.504	1.176	0.572	1.439	94	89	59	4	83	.	> 1.00E-4	> 1.00E-4
DU-145	0.342	1.031	1.107	1.057	1.004	0.036	-0.553	111	104	96	-89	-100	1.77E-6	3.30E-6	6.13E-6
Breast Cancer															
MCF7	0.212	1.153	1.073	1.026	0.671	0.133	0.458	91	87	49	-38	26	9.29E-7	.	> 1.00E-4
MDA-MB-231/ATCC	0.437	1.064	1.018	1.065	0.914	-0.136	-0.537	93	100	76	-100	-100	1.41E-6	2.70E-6	5.20E-6
BT-549	0.905	1.618	1.667	1.692	1.680	0.495	0.111	107	110	109	-45	-88	2.40E-6	5.08E-6	1.29E-5
T-47D	0.448	1.150	1.179	1.131	1.043	0.430	0.580	104	97	85	-4	19	2.46E-6	.	> 1.00E-4
MDA-MB-468	0.682	1.377	1.352	1.370	1.263	0.856	0.615	96	99	84	25	-10	3.74E-6	5.23E-5	> 1.00E-4

Figure (18): Five dose testing results of compound $\mathbf{2_e}$.

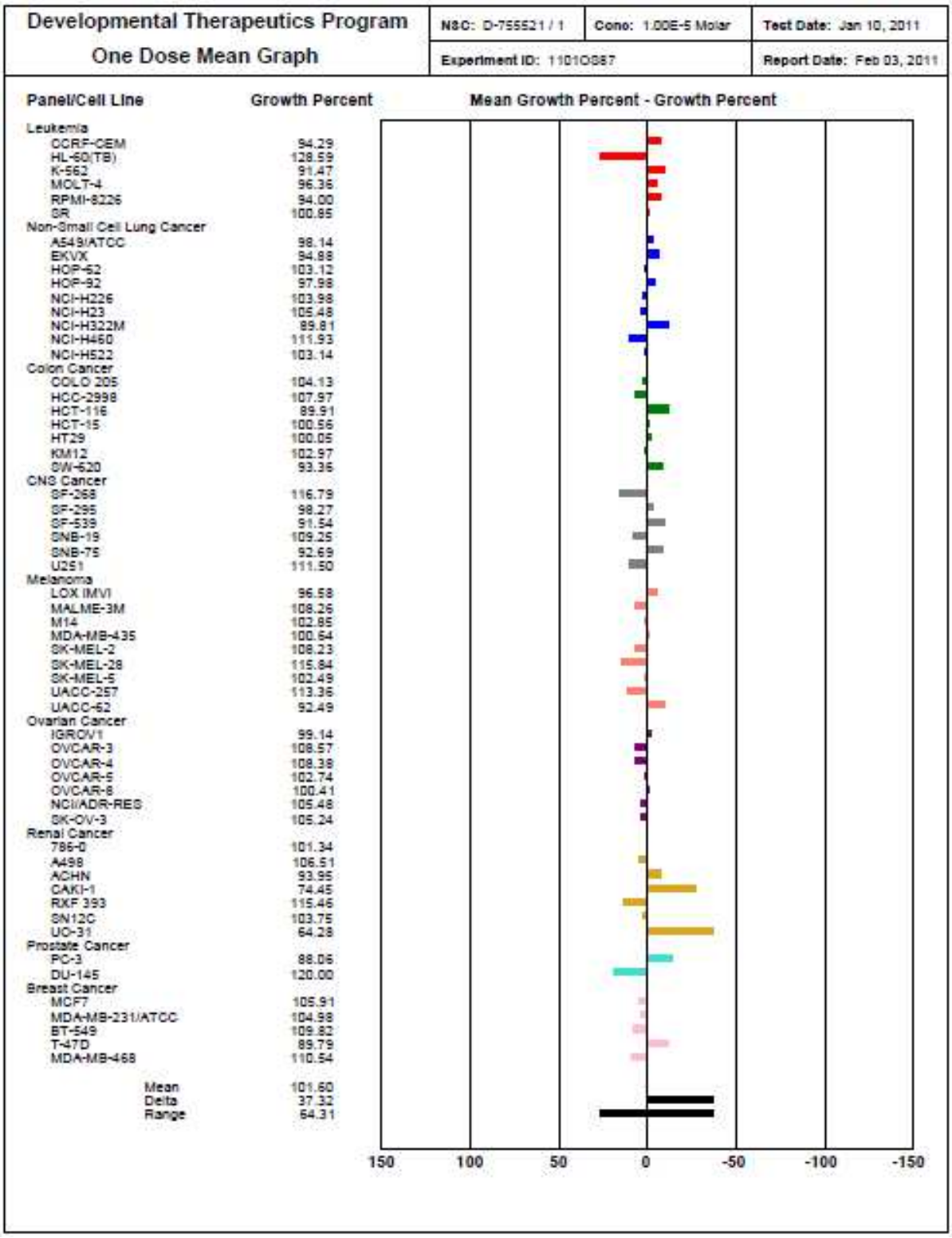


Figure (19): Results of the one dose screening of **1e**