

Synthesis, characterization and evaluation of some benzothiazole derivatives bearing oxindole moiety as potential anticancer agents

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Abstract

This study was aimed at investigating the antitumor activity of novel 5-substituted 2-oxindole derivatives. A series of oxindole derivatives of N-(1,3,4-thiadiazol-2-yl)benzo[d]thiazol-2-amine were prepared and confirmed by ¹H NMR, mass and IR data. These compounds were evaluated for their anticancer activity at National Cancer Institute (NCI), USA by single High dose (10⁻⁵ M) in full NCI 60 cell panel. 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. Among them compound **1[D]** has showed moderate anticancer activity while compound **3[A-2]** has showed significant anticancer activity with overall mean growth percent 38.25. These findings prompt us to further explore the oxindole structure as leading scaffold to design new molecules with potent antitumor activity.

Keywords: 2-Oxindole, 1,3,4-thiadiazole, Benzothiazole, Anticancer, NCI

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Introduction

Cancer is a group of diseases in which cells can be aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues) and/or metastatic (spread to other locations in body). These three malignant properties of cancer differentiate them from benign tumors, which are self-limited in their growth and do not invade or metastasize. Cancer may affect people at all ages, even fetuses, but risk for the more common varieties tends to increase with age^[1]. Cancer causes about 13% of all deaths^[2]. According to the American Cancer Society, 7.6 million people died from cancer in the world during 2007^[3]. Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells^[4]. With the current chemotherapy, lack of selectivity of chemotherapeutic agents against cancerous cells is a significant problem.

Unlike normal cells, cancer cells lose the ability to control their cell cycle and continue growing unchecked. In most forms of cancer deregulation of the cell signalling pathways that are intimately involved in regulating and governing cell cycle status leads to altered response to growth stimuli. In today's era of personalized medicine clinicians need to type cancers based on which pathways are activated and which are inactive to aid selection of the right type of modern

anti-cancer drugs which specifically target and shut down overactive pathways^[5].

The search for novel anticancer agents with more selectivity and lower toxicity continues to be an area of intensive investigation. The unique structural features of Oxindoles together with diverse biological activities have made them privileged structures in new drug discovery^[6]. Oxindole-core synthetic molecules are currently being developed as anticancer drugs that target protein tyrosine kinases associated with growth factor receptors^[7,8,9].

The search for novel anticancer agents with more selectivity and lower toxicity continues to be an area of intensive investigation. The unique structural features of Oxindoles together with diverse biological activities of 1, 3, 4-thiadiazole^[10,11,12] and Benzothiazole^[13,14,15] can prove the area of research quite unique. All three core nucleus has been found to have potential anticancer activity. So there is a need to combine these molecules in a single structural composition to explore its activity.

In this study, 5 derivatives of oxindole with Substituted benzothiazole coupled by 1,3,4-thiadiazole were synthesized and evaluated for anticancer activity on the NCI 60 cell panel.

Materials and Methods

- Synthesis
- Reagents

All the chemicals and solvents used were of AR-grade and LR-grade and obtained from Sigma-Aldrich, Sisco Research Laboratories, Qualigens, Rankem, S.D. Fine, Hi-Media and Merck and were used without further purification.

Equipment's: Melting points were measured on an electrothermal melting point apparatus Toshniwal and are uncorrected. Infrared (IR) spectra were recorded as KBr pellets with an FTIR-8300 spectrophotometer (Shimadzu). Proton magnetic resonance (¹H NMR) spectra were recorded in DMSO-d₆ (Merck) on an Avance bruker II-400 NMR spectrometer. Mass spectra were recorded on a LCMS (Shimadzu Corporation, Japan).

Synthesis

- Synthesis of 5-(2-chloroacetyl) indolin-2-one **1[C]**^[16]

To a 250 ml round-bottomed flask equipped with condenser and N₂ inlet were added 30.7 g (230 mmol) aluminium chloride, 150 ml carbon disulfide, and 3.8 ml (48 mmol) chloroacetyl chloride. To the stirring mixture was added 5.0 g (37 mmol) of oxindole portionwise over 15 minutes. The reaction was stirred a further 10 minutes, then refluxed 2 hours. The reaction was cooled, added to ice, stirred thoroughly, and the beige precipitate filtered, washed with water, and dried to afford 7.67 g (97%) of 5-chloroacetyloxindole.

- Synthesis of 5-(5-amino-1,3,4-thiadiazol-2-yl)indolin-2-one **1[D]**

A mixture of 5-chloroacetyl oxindole (0.01 mol), thiosemicarbazide (0.01 mol) and POCl₃ (10 mol) reflux at 75 °C for 45 min. After cooling down to room temperature, sufficient water was added. The reaction mixture was then reflux at 75 °C for 4-5 hrs. After cooling reaction mixture was basified to PH 8 by drop wise addition of 50% NaOH solution under stirring. The precipitate was filtered and recrystallized with ethanol to get yield.

- Synthesis of substituted Isothiocyanates. **2[B]**

To the solution of thiophosgene (0.01 mol) in CH₂Cl₂, substituted primary amine (0.01 mol) was added quickly at room temperature under stirring. Then a 25 ml saturated solution of NaHCO₃ was added slowly and stirred for 3h. The CH₂Cl₂ layer was dried over anhydrous sod. sulphate and removed under reduced pressure. Completions of reaction were confirmed by TLC.

Table 1: Physical data of the intermediates 2B [1-5]

Intermediates	Molecular formula	Molecular weight	% yield (%W/W)	Melting point (°C)	Rf value ^a
2 [B-1]	C ₉ H ₉ NS	163.24	62.80	124-126	0.76
2 [B-2]	C ₈ H ₆ ClNS	183.66	55.28	130-132	0.42
2 [B-3]	C ₇ H ₃ Cl ₂ NS	204.08	68.31	123-126	0.56
2 [B-4]	C ₇ H ₂ Cl ₃ NS	238.52	45.69	120-122	0.47
2 [B-5]	C ₇ H ₃ Cl ₂ NS	204.08	51.90	110-112	0.42

^a Chloroform: Methanol 9:1

- Synthesis of different substituted Phenylthiourea Derivatives. **3[A]**

To a solution of optically active substituted isothiocyanates (**2[B-1]-2[B-5]**) (0.01 mol) in dry MeOH (10 ml), an equimolar quantity of 5-(5-amino-1,3,4-thiadiazol-2-yl)indolin-2-one (**1[D]**) (0.01 mol) was added with stirring. The reaction mixture was heated on a steam bath at 70°C for about 2-3 h and then the solvent was distilled off. The solid residue that separated was washed with water and dried at 40 °C and recrystallized by methanol.

Table 2: Physical data of the intermediates 3A [1-5]

Intermediates	Molecular formula	Molecular weight	% yield (%W/W)	Melting point (°C)	Rf value ^a
3 [A-1]	C ₁₉ H ₁₇ N ₅ OS ₂	395.50	48.66	168-172	0.56
3 [A-2]	C ₁₈ H ₁₄ ClN ₅ OS ₂	415.91	38.91	210-214	0.64
3 [A-3]	C ₁₇ H ₉ Cl ₂ N ₅ OS ₂	434.32	55.45	220-224	0.46
3 [A-4]	C ₁₇ H ₁₀ Cl ₃ N ₅ OS ₂	470.78	41.61	228-232	0.60
3 [A-5]	C ₁₇ H ₉ Cl ₂ N ₅ OS ₂	434.32	59.44	220-222	0.48

^a Chloroform: Methanol 9:1

- Synthesis of different substituted Benzothiazole Derivatives. **3[B]**

Substituted Phenylthiourea (**3[A]**) (0.01 mol) were dissolved in Chloroform. (15 ml), the reaction mixture was cooled in an ice bath and then bromine: Chloroform (1:9) mixture was added drop wise. The reaction was monitored by TLC and after an hour, was poured on to crushed ice. The solid that separated was filtered, dried in each case and recrystallized by methanol.

Table 3: Physical data of the intermediates 3B [1-5]

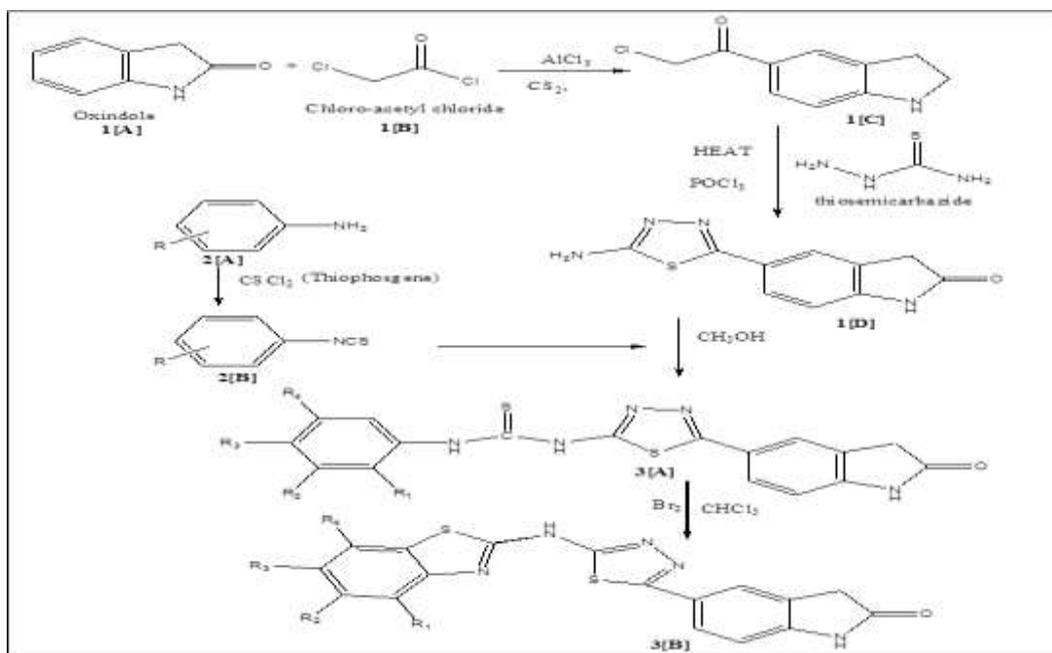
Intermediates	Molecular formula	Molecular weight	% yield (%W/W)	Melting point (°C)	Rf value ^a
3 [B-1]	C ₁₉ H ₁₅ N ₅ OS ₂	393.49	77.00	200-204	0.52
3 [B-2]	C ₁₈ H ₁₂ ClN ₅ OS ₂	413.9	72.10	222-224	0.44
3 [B-3]	C ₁₇ H ₉ Cl ₂ N ₅ OS ₂	434.32	61.88	228-230	0.46
3 [B-4]	C ₁₇ H ₈ Cl ₃ N ₅ OS ₂	468.77	70.55	240-244	0.71
3 [B-5]	C ₁₇ H ₉ Cl ₂ N ₅ OS ₂	434.32	62.34	230-234	0.42

^a Chloroform: Methanol 9:1

- **3[B-1]** 5-(5-(4,7-dimethylbenzo [d]thiazol-2-ylamino)-1,3,4-thiadiazol-2-yl)indolin-2-one
IR (KBr): 3434.37 (N–H), 1713.81 (C=O), 1257.63 (C=N), 682.82 (C–S)cm⁻¹. ¹H NMR (DMSO): δ ppm; 2.28(s, 2H, CH₂), 3.89(br s, 1H, Ar-NH), 8.07 (s, 1H, 2°-NH), 2.35(s, 6H- Ar-CH₃), 7.63-8.00 (m, 5H, Ar-H). LC–MS: 394.2 (M+1).
- **3[B-2]** 5-(5-(5-chloro-4-methyl benzo[d]thiazol-2-ylamino)-1,3,4-thiadiazol-2-yl)indolin-2-one
IR (KBr): 3375.54 (N–H), 1697.41 (C=O), 1256.67 (C=N), 682.82 (C–S)cm⁻¹. ¹H NMR (DMSO): δ ppm; 3.47(s, 2H, CH₂), 6.55(br s, 1H, Ar-NH), 9.18 (s, 1H, 2°-NH), 2.35(s, 3H- Ar-CH₃), 7.06-7.99 (m, 5H, Ar-H). LC–MS: 414.2 (M+1).
- **3[B-3]** 5-(5-(4,7-dichlorobenzo[d]thiazol-2-ylamino)-1,3,4-thiadiazol-2-yl)indolin-2-one
IR (KBr): 3357.2 (N–H), 1698.38 (C=O), 1256.67 (C=N), 683.79 (C–S)cm⁻¹.
- **3[B-4]** 5-(5-(4,6,7-trichlorobenzo [d]thiazol-2-ylamino)-1,3,4 -thiadiazol-2-yl)indolin-2-one
IR (KBr): 3383.2 (N–H), 1712.85 (C=O), 1250.88 (C=N), 674.14 (C–S), 770.59 (C-Cl)cm⁻¹. ¹H NMR (DMSO): δ ppm; 3.29(s, 2H, CH₂), 5.56(br s, 1H, Ar-NH), 7.60 (s, 1H, 2°-NH), 6.55-7.29 (m, 4H, Ar-H). LC–MS: 469.3 (M+1).
- **3[B-5]** 5-(5-(4,6-dichlorobenzo [d]thiazol-2-ylamino)-1,3,4-thiadiazol-2-yl)indolin-2-one
IR (KBr): 3361.07 (N–H), 1698.38 (C=O), 1209.41 (C=N), 683.79 (C–S), 768.66 (C-Cl)cm⁻¹. ¹H NMR (DMSO): 5.72(s, 2H, CH₂), 5.08(br s, 1H, Ar-NH), 7.58 (s, 1H, 2°-NH), 6.84-7.58 (m, 5H, Ar-H). LC–MS: 434.3 (M+).

Table 4: Derivatives of 3[B-1]-3[B-5]

Intermediates	R ₁	R ₂	R ₃	R ₄
3 [B-1]	CH ₃	H	H	CH ₃
3 [B-2]	CH ₃	Cl	H	H
3 [B-3]	Cl	H	H	Cl
3 [B-4]	Cl	H	Cl	Cl
3 [B-5]	Cl	H	Cl	H



Biological evaluation

- Anticancer activity^[17]

The screening is a two stage process; it begins with the evaluation of all compounds against the 60 cell lines at a single dose of 10 μM . The output from this single dose screening is reported as a mean graph and is available for analysis by the compare program. Compounds which exhibit significant tumor growth inhibition are further evaluated against the 60 cell panel at five conc. level.

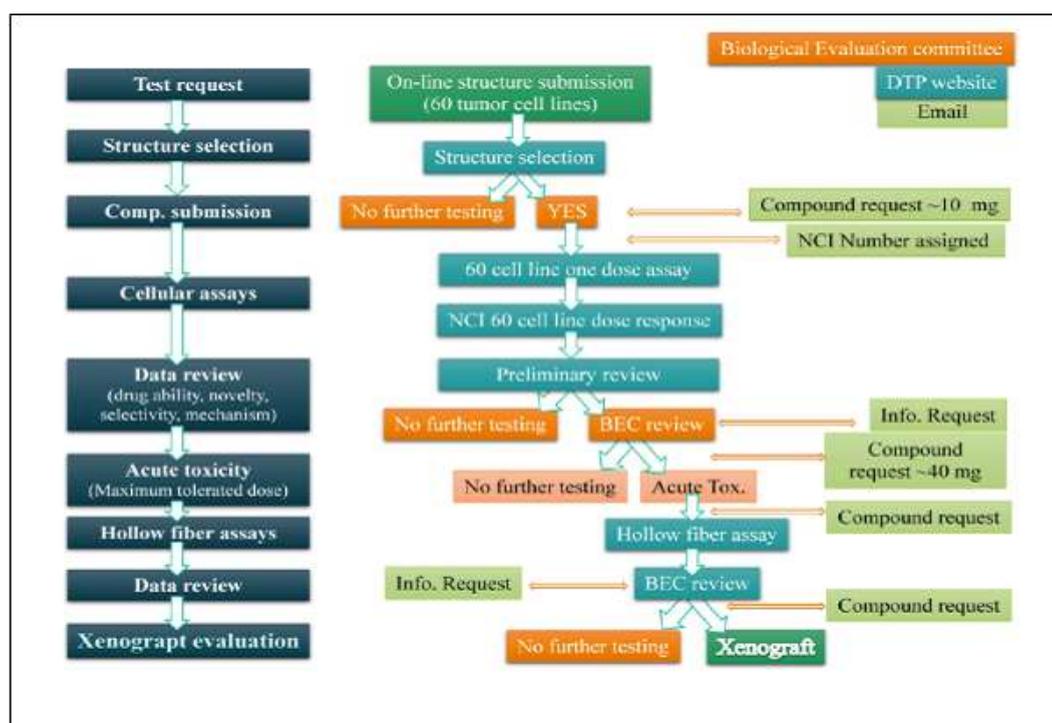


Fig. 1: NCI-60 Cell Line Assay Procedure

Results and Discussion

Among the synthesized compounds, 3 compounds

(1[D], 3[B-2], 3[B-4,]) were screened for in-vitro anticancer activity against 60 cell lines at NCI, U.S.A.

Result of compound 1[D], 3[B-2], has been obtained. Compounds have shown good anticancer activity.

The compounds were screened for anticancer activity against various cancer cell lines at National Cancer Institute (NCI, USA). All the selected compounds submitted to National Cancer Institute (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 leukemia, 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^{-1} M) and the culture was incubated for 48 hrs. 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.

Table 5: Mean graph of the one dose screening for the compound 1[D]

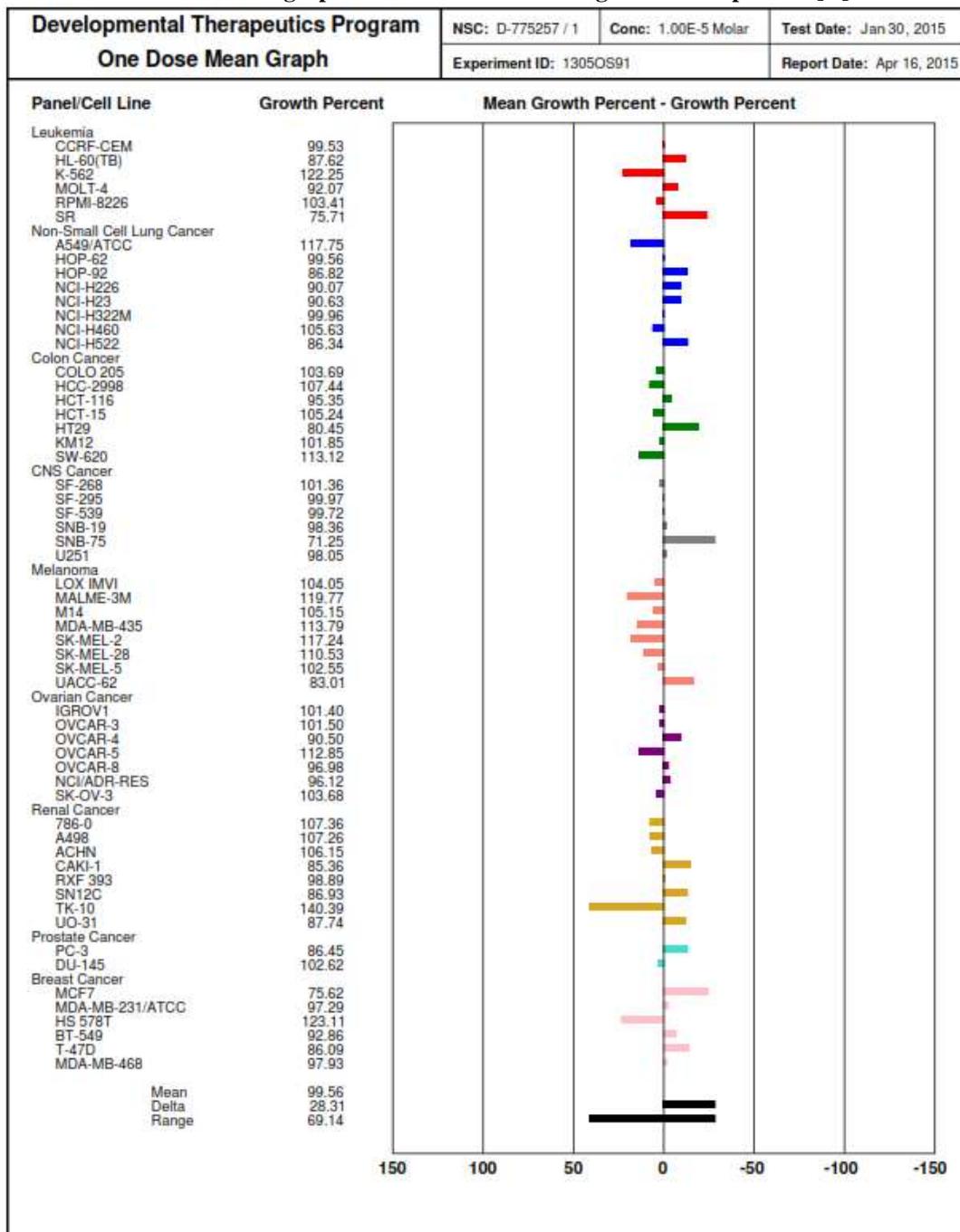
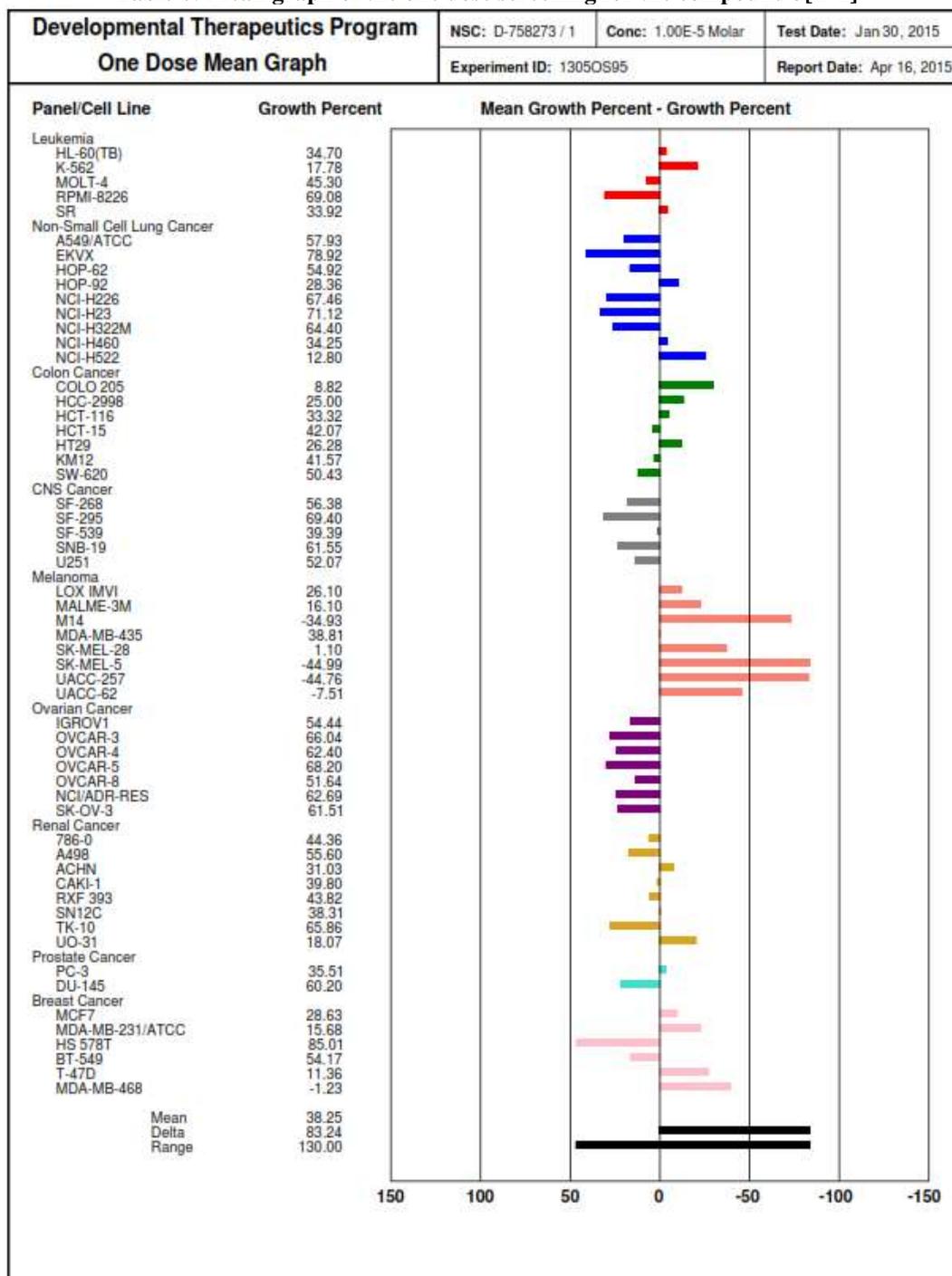


Table 6: Mean graph of the one dose screening for the compound 3[B-2]



Conclusion

The synthesized Benzothiazole derivatives have potential to act as anticancer agents and the activity of various compounds varied according to the substituent attached. These preliminary encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent anti-tumor agent.

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