



Microwave Assisted Synthesis and Anticancer Activity of Substituted Pyrimido [2,1-*b*] [1,3] benzothiazole Derivatives

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ABSTRACT

A series of pyrimido[2,1-*b*][1,3]benzothiazole derivatives were synthesized by reaction of 2-amino benzothiazole, aromatic aldehyde and active methylene compound in ethanol by conventional, as well as, microwave irradiation methods through MCR Mechanism. Multi component reactions comply with the principles of green chemistry in terms of economy of steps as well as many of the stringent criteria of an ideal organic synthesis. The microwave irradiation technique gives better yield in shorter reaction time. The compounds were evaluated at single concentration of 10^{-5} M towards the panel of 60 cancer cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, Ovarian, Renal, prostate and breast cancer. The tested compounds showed some distinctive potential pattern of selectivity and results have been reported in this work. The results showed that a change in the substitution pattern in pyrimido[2,1-*b*][1,3]benzothiazole derivatives may cause a marked effect on their anticancer activity, such as compounds (4*R*)-2-amino-7-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]Benzothiazole-3-carbonitrile (1g) showing 48.32 % growth inhibition on non small cell lung cancer (NCI-H522) cell line having chloro substitution and (4*R*)-2-amino-8-chloro-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile (1h) showing 52.55 % growth inhibition on non small cell lung cancer (NCI-H522) cell line having chloro and methoxy substitutions.

Keywords: Antitumor Activity, Multicomponent Reaction, Substituted Benzothiazoles.

INTRODUCTION

One of the main challenges in medicinal chemistry is to synthesise biologically active molecules. With high selectivity, low energy consumption. The identification of novel structures that can be potentially useful in designing new, potent selective and less toxic anticancer agents is still a major challenge to medicinal chemistry researchers. Multi-component reactions, because of their productivity, simple procedures, convergence, facile execution, and atom economy, are one of the best tools in the synthesis of diverse and complex compounds as well as small and drug like heterocycles. A multi component reaction in combination with microwave assisted synthesis is a green approach towards the synthesis of various heterocyclic compounds and for a researcher there is lot of scope to change the reaction condition, to change the catalyst or to modify the catalyst or even to develop various novel multi component reactions. Pyrimidine, iminopyrimidine and fused benzothiazole heterocycles¹⁻⁶ are reported to be effective pharmacophores. A survey of literature made it evident that very little work has been carried out on the synthesis of fused pyrimido benzothiazole possessing three to four rings⁷⁻⁸ which exhibit a wide spectrum of activities like antitumor, phosphodiesterase inhibition, anti-allergic⁹, anti-inflammatory and anti parkinsonism. Pyrazolopyrimidine derivatives have considerable potential in the field of chemotherapy, as they were found to exhibit their antitumor activity by inhibiting different types of enzymes such as cyclin-dependent kinase¹⁰⁻¹², Src and Abl tyrosine kinase¹³, glycogen synthase kinase-3¹⁴⁻¹⁶, adenosine deaminase¹⁷, and

epidermal growth factor receptor protein tyrosine kinase.¹⁸ The derivatives of pyrazolo[3,4-*d*]pyrimidine have already been discovered as antitumor agents by the NCI (National Cancer Institute, USA) on HCT-116 and other cell lines. In view of various applications of this system, synthesis of such condensed system has attracted much attention in recent years. In 1950s, a number of 2-aminobenzothiazoles have been investigated as central muscle relaxants. Since then, the quest for research on this heterocycle fused with others started with a great rigidity. Pyrimido benzothiazole, benzothiazolo quinoline derivatives¹⁹, imidazobenzothiazoles²⁰⁻²¹ as well as polymerized benzothiazole²² showed remarkable antitumor activity. The benzothiazole moiety with various substitutions shows antitumor activity for example its carbonitrile derivatives show selective growth inhibitory properties against human cancer cell lines and proliferation of cells. Review literature revealed that Chlorinated and fluorinated derivatives of this moiety exhibit good *in-vitro* antitumor activity. In the light of these observations this prompted us to synthesize a novel derivatives of Substituted pyrimido[2,1-*b*]benzothiazoles, evaluate them by spectral analysis (IR, ¹H NMR and MASS spectrometry) and screen for their anti-tumour activity.

EXPERIMENTAL SECTIONS

Step 1: General Synthesis of substituted 2-amino-1,3-benzothiazole²³⁻²⁵

Substituted 2-amino- 1,3-benzothiazole was synthesized as per the reported procedure. (Scheme I)



Step II: General Synthesis of substituted 4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile. (MCR)

A solution of substituted benzothiazole, malononitrile and substituted aldehyde in ethanol (25ml) add 4-5 drops of Triethylamine. Placed in microwave oven and irradiated for 4 min at 640 W. The reaction mixture was cooled at room temperature to give a solid mass which crystallized from ethanol. (Scheme II)

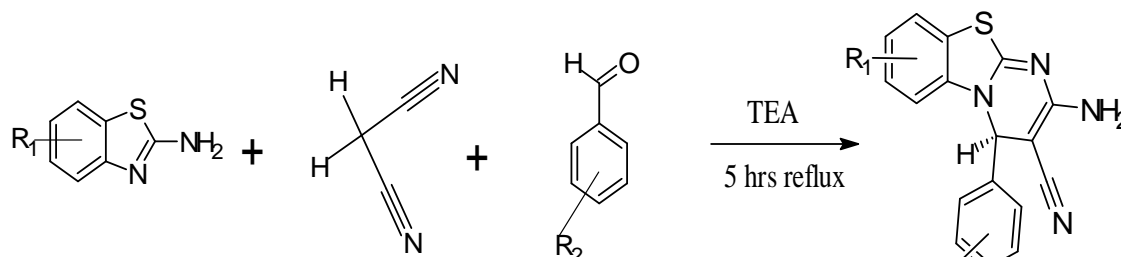
Experimental Protocols

Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC on silica gel plates. The solvent system used to carry out the TLC is Benzene. Spectral data IR spectra (cm^{-1}) were recorded in KBr on an alpha-T bruker FTIR spectrometer. ^1H NMR (ppm) [NMR (DMSO-d_6)] in DMSO using TMS as reference on Bruker AVANCE 400. Mass spectra of the compound coded were carried out. Melting point and R_f value were plotted in table 1.

Scheme I

Substituted aniline

substituted 2-amino-1,3benzothiazole

Scheme II (MCR)

substituted 2-aminobenzothiazole

malononitrile substituted benzaldehyde

Substituted
(4R)-2-amino-4-phenyl-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile**Table 1: Physical Constants of Synthesized Compounds**

| Comp. ID | Substituent's | | m.p. (°C) | Synthesis Methods | | | | | |
|----------|---------------------|------------------------|-----------|--|------|-----------|------|--------|-------|
| | R ¹ (Bt) | R ² (Ph) | | Reaction time & R _f value * | | | | %Yield | |
| | | | | Conventional | | Microwave | | Conv. | MW |
| 1a | 6-Cl | 3-NO ₂ | 98-100 | 5hrs | 0.72 | 5 min. | 0.71 | 72.20 | 91.65 |
| 1b | 6-NO ₂ | p-Br | 158-160 | 5 hrs | 0.64 | 5 min. | 0.63 | 70.54 | 90.00 |
| 1c | 6-Br | 4-F | 98-100 | 5 hrs | 0.68 | 5 min. | 0.66 | 70.42 | 92.73 |
| 1d | 6-NO ₂ | 3,4,5-OCH ₃ | 136-138 | 5hrs | 0.64 | 5 min. | 0.64 | 68.56 | 88.55 |
| 1e | 6-NO ₂ | 4-OH | 150-152 | 5hrs | 0.60 | 5 min. | 0.62 | 65.56 | 88.45 |
| 1f | 5-OCH ₃ | 3,4,5-OCH ₃ | 140-142 | 5hrs | 0.74 | 5 min. | 0.72 | 70.21 | 87.56 |
| 1g | 5-Cl | p-Cl | 158-160 | 5hrs | 0.66 | 5 min. | 0.68 | 55.25 | 74.45 |
| 1h | 6-Cl | 3,4,5-OCH ₃ | 150-152 | 5hrs | 0.66 | 5 min. | 0.68 | 70.56 | 81.25 |
| 1i | 4-Cl | p-Cl | 158-160 | 5hrs | 0.74 | 5 min. | 0.72 | 68.12 | 81.56 |
| 1j | 3-Cl,4-F | 4-Br | 142-144 | 5hrs | 0.74 | 5 min. | 0.70 | 58.25 | 77.12 |
| 1k | 6-Br | p-Cl | 158-160 | 5hrs | 0.70 | 5 min. | 0.72 | 74.12 | 90.12 |

*R_f value was determined in benzene: ethyl acetate (8:2); Bt: Benzothiazole ring substitutions; Ph: 4- Phenyl ring substitutions.

Spectral Data**(1a) (4R) 2-amino-8-chloro-4-(3-nitrophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile**

m.p. 98-100 (°C) IR : (KBr/cm⁻¹) 3381 (-NH str); 3183 (Ar-C-H); 2227 (CN str); 1678 (C=N); 1452(NO₂); 687(C-Cl); ¹H NMR DMSO-d₆ δ (ppm) 7.0(m, 8H-Ar-H), 5.5 (s, 1H) 3.3 (s, 2H -NH₂), 2.0 (1H-Py-H) Mass (TOF MS-ES) m/z: 383.81 (M⁺); RA (30 %); % Anal.(Found) For C₁₇H₁₀ClN₅O₂S; C, 52.80; H, 2.75; N, 18.37; O, 8.42; S, 8.43; Calculated : C, 53.20; H, 2.63; N, 18.25; O, 8.34; S, 8.35

(1b) (4R) 2-amino-4-(4-bromophenyl)-8-nitro-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile

m.p. 158-160 (°C) IR :(KBr/cm⁻¹); 3417(-NH str); 3032 (Ar-C-H); 2306 (CN); 1648 (C=N); 1481 (NO₂) ; ¹H NMR DMSO-d₆ δ (ppm) 6.8-8.2(m, 8H-Ar-H), 5.6 (s, 1H), 3.0 (s, 2H -NH₂), 2.0 (1H-Py-H) Mass (TOF MS-ES) m/z: 428.26 (M⁺); RA (10 %); % Anal. Found For C₁₇H₁₀BrN₅O₂S; C, 46.84; H, 2.45; N, 16.52; O, 7.49; S, 7.51; Calculated: C, 47.68; H, 2.35; N, 16.35; O, 7.47; S, 7.49

(1c) (4R)2-amino-8-bromo-4-(4-fluorophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile

m.p. 98-100 (°C) IR :(KBr/cm⁻¹); 3381(-NH str); 3035 (Ar-C-H); 2227 (CN); 1627 (C=N); 1222(C-F); ¹H NMR DMSO-d₆ δ (ppm) 7.1(m, 8H-Ar-H), 5.9 (s, 1H), 2.9 (s, 2H -NH₂), 2.0 (1H-Py-H); Mass (TOF MS-ES) m/z: 401.25 (M⁺); RA (5 %); % Anal. Found For C₁₇H₁₀BrFN₅S; C, 49.99; H, 2.92; N, 13.99; S, 7.91; Calculated: C, 50.89; H, 2.51; N, 13.96; S, 7.99

(1d) (4R) 2-amino-8-nitro-4-(3, 4, 5-trimethoxyphenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile

m.p. 136-138 (°C) IR :(KBr/cm⁻¹); 3453(-NH str); 2979 (Ar-C-H); 2217 (CN); 1640 (C=N); 1500 (NO₂); ¹H NMR DMSO-d₆ δ (ppm) 8.32(s,9H), 6.6-8.2(m, 8H-Ar-H), 5.9 (s, 1H), 2.8 (s, 2H -NH₂), 2.0 (1H-Py-H); Mass (TOF MS-ES) m/z: 439.44 (M⁺); RA (10 %); % Anal. Found For C₂₀H₁₇N₅O₅S; C, 53.81; H, 3.99; N, 16.01; O, 18.90; S, 7.29; Calculated: C, 54.66; H, 3.90; N, 15.94; O, 18.20; S, 7.30

(1e) (4R) 2-amino-4-(4-hydroxyphenyl)-8-nitro-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile

m.p. 150-152 (°C) IR :(KBr/cm⁻¹); 3457(-NH str); 3076 (Ar-C-H); 2221 (CN); 1649 (C=N); 1493 (NO₂) ¹H NMR DMSO-d₆ δ (ppm) 9.63 (1H, brs, OH), 6.7-8.3(m, 8H-Ar-H), 5.5 (s, 1H) 2.34 (s, 2H -NH₂), 2.0 (1H-Py-H); Mass (TOF MS-ES) m/z: 365.36 (M⁺); RA (10 %); % Anal. Found For C₁₇H₁₁N₅O₃S; C, 54.56; H, 3.19; N, 19.89; O, 13.88; S, 8.48; Calculated: C, 55.88; H, 3.03; N, 19.17; O, 13.14; S, 8.78

(1f) (4R)2-amino-7-methoxy-4-(3,4,5-trimethoxyphenyl)-4H-pyrimido[2,1b][1,3]benzothiazole-3-carbonitrile

m.p. 140-142 (°C) IR :(KBr/cm⁻¹); 3452(-NH str); 3018 (Ar-C-H); 2218 (CN); 1642 (C=N); ¹H NMR DMSO-d₆ δ (ppm) 8.32(s,9H),6.5-8.0(m, 8H-Ar-H), 5.5 (s, 1H)3.5 (s, 2H -NH₂), 2.0 (1H-Py-H); Mass (TOF MS-ES) m/z: 424.47 (M⁺);

RA (20 %); % Anal. Found For C₂₁H₂₀N₄O₄S; C, 59.39; H, 4.74; N, 13.22; O, 15.10; S, 7.52; Calculated: C, 59.42; H, 4.72; N, 13.20; O, 15.08; S, 7.55

(1g) (4R) 2-amino-7-chloro-4-(4-chlorophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile

m.p. 158-160 (°C) IR :(KBr/cm⁻¹); 3448 (-NH str); 3033 (Ar-C-H); 2224 (CN); 1648 (C=N); 704 (C-Cl); ¹H NMR DMSO-d₆ δ (ppm) 6.8-8.5 (m, 8H-Ar-H), 5.8 (s, 1H), 3.35 (s, 2H -NH₂), 2.0 (1H-Py-H); Mass (TOF MS-ES) m/z: 373.25 (M⁺); RA (5 %); % Anal. Found For C₁₇H₁₀Cl₂N₄S; C, 54.71; H, 2.69; N, 15.05; S, 8.55; Calculated: C, 54.70; H, 2.70; N, 15.01; S, 8.59

(1h) (4R) 2-amino-8-chloro-4-(3,4,5-trimethoxyphenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile

m.p. 150-152 (°C) IR :(KBr/cm⁻¹); 3452 (-NH str); 3030 (Ar-C-H); 2220 (CN); 1645 (C=N); 690 (C-Cl); ¹H NMR DMSO-d₆ δ (ppm) 8.32(s,9H), 6.6-8.4(m, 8H-Ar-H), 5.5 (s, 1H), 3.34 (s, 2H -NH₂), 2.0 (1H-Py-H); Mass (TOF MS-ES) m/z: 428.89 (M⁺); RA (10 %); % Anal. Found For C₂₀H₁₇ClN₄O₃S; C, 56.11; H, 4.01; N, 13.01; O, 11.14; S, 7.47; Calculated: C, 56.01; H, 4.00; N, 13.06; O, 11.09; S, 7.48

(1i) (4R) 2-amino-6-chloro-4-(4-chlorophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3-Carbonitrile

m.p. 158-160 (°C) IR :(KBr/cm⁻¹); 3454 (-NH str); 3034 (Ar-C-H); 2305 (CN); 1641 (C=N); 705 (C-Cl) ¹H NMR DMSO-d₆ δ (ppm) 7.0(m, 8H-Ar-H), 5.6 (s, 1H), 3.2 (s, 2H -NH₂), 2.1 (1H-Py-H) ; Mass (TOF MS-ES) m/z: 373.25 (M⁺); RA (5 %); % Anal. Found For C₁₇H₁₀Cl₂N₄S; C, 54.71; H, 2.69; N, 15.05; S, 8.55; Calculated: C, 54.70; H, 2.70; N, 15.01; S, 8.59

(1j) (4R) 2-amino-4-(4-bromophenyl)-7-chloro-8-fluoro-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile.

m.p. 142-144 (°C) IR :(KBr/cm⁻¹); 3381 (-NH str); 3183 (Ar-C-H); 2227 (CN); 1678 (C=N); ¹H NMR DMSO-d₆ δ (ppm) 7.0(m, 8H-Ar-H), 5.8 (s, 1H), 3.23 (s, 2H -NH₂), 2.1 (1H-Py-H); Mass (TOF MS-ES) m/z: 335.70 (M⁺); RA (10 %); % Anal. Found For C₁₇H₉BrClFN₄S; C, 46.86; H, 2.07; N, 12.88; S, 7.35; Calculated: C, 46.86; H, 2.08; N, 12.86; S, 7.36

(1k) (4R) 2-amino-8-bromo-4-(4-chlorophenyl)-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carbonitrile.

m.p. 158-160 (°C) IR :(KBr/cm⁻¹); 3387(-NH str); 3035 (Ar-C-H); 2227 (CN); 1627 (C=N); 687 (C-Cl); ¹H NMR DMSO-d₆ δ (ppm) 7.5(m, 8H-Ar-H), 5.7 (s, 1H), 3.64 (s, 2H -NH₂), 2.0 (1H-Py-H); Mass (TOF MS-ES) m/z: 417.71 (M⁺); RA (10 %); % Anal. Found For C, 48.91; H, 2.43; N, 13.42; S, 7.62; Calculated: C, 48.88; H, 2.41; N, 13.41; S, 7.68

Pharmacological Screening**Anti-cancer activity**

The compounds were submitted to National Cancer Institute (NCI), Bethesda, Maryland, USA and 4 compounds were selected and screened for preliminary



anticancer assay by NCI, in an *in vitro* 60 human tumor cell lines panel, derived from nine neoplastic cancer types.

Methodology of In-vitro cancer screening

Four compounds were selected to *in vitro* preliminary screening for anticancer activity. The screening was carried by two stage process. In first stage, all compounds were evaluated against the 60 cell lines at a single dose of 10 μ M. The result of anticancer activity from the single dose screen is reported as a mean graph. For second stage screening compounds which exhibit significant growth inhibition are selected by NCI for further anticancer activity evaluation against the 60 cell panel at five concentration levels. In graph, referred as 'mean graph', the mean of all cell lines results is considered as zero. This 'mean graph' is developed by plotting positive and negative values generated from a set of GI₅₀ values. The positive and negative values are plotted along a vertical line that represents the mean response of all the cell lines in the panel to the test agent. Positive values project to the right of the vertical line and represent cellular sensitivities to the test agent that exceed the mean. Negative values project to the left of the vertical line and represent cell line sensitivities to the test agent

that are less than the average value. Compounds with cell lines appearing on positive side of mean graph exhibit high growth inhibition of cancer cells of that particular cancer. The results of *in vitro* anticancer screening are summarized in below.

Selected Compounds for Screening Preliminary Anticancer Assay

Out of eleven, following four compounds were selected and screened for preliminary anticancer assay by NCI, in an *in vitro* 60 human tumor cell lines panel.

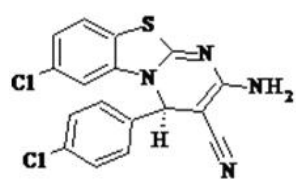
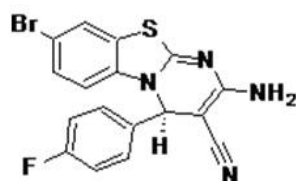
(1c) (4*R*) 2-amino-8-bromo-4-(4-fluorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile (NSC 771853)

(1i) (4*R*) 2-amino-6-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-Carbonitrile (NSC 771852)

(1g) (4*R*) 2-amino-7-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carboNitrile (NSC-773066)

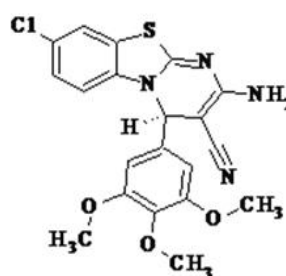
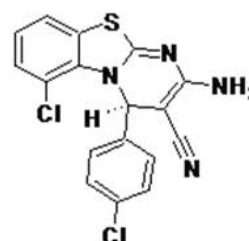
(1h) (4*R*) 2-amino-8-chloro-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile (NSC-773067)

(1c)



(1g)

(1i)



(1h)

SAR Study

Presence of hydrophobic moieties in molecule is conducive for cytotoxic activity of benzothiazole derivatives against cancer cell lines. The type of substituent attached at either position 6- or 5- is a controlling factor governing the total observed pharmacological properties. From the literature survey it was found that the amino, hydroxyl, and chloro group containing benzothiazole shows better anticancer activity.⁸ from the results of screening of cell lines it was observed that Chloro substituted amino benzothiazole were found to have encouraging sensitivity to cancer cell lines compared to other substituted benzothiazole. But

the p-OCH₃ derivative (1h) exhibited higher anticancer activity than the p-Cl, p-F derivatives.

For example compound(4*R*)-2-amino-8-chloro-4-(3,4,5-trimethoxyphenyl)-4*H*-Pyrimido[2,1-*b*][1,3]Benzothiazole-3-carbonitrile (1h) showing 52.55 % percent growth inhibition of Non small cell lung cancer (NCI-H522) cell line. Compound (4*R*)-2-amino-7-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]Benzothiazole-3-carbonitrile (1g) showing 48.32 % percent growth inhibition of Non small cell lung cancer (NCI-H522) cell line.

Criterion for submission and selection of compounds for testing in the NCI screens.

The compounds were submitted to NCI under the Developmental Therapeutic Program (DTP) which operates a tiered anticancer compound screening course for the benefit of the general research community with the goal of identifying novel chemical leads and biological mechanisms. Structures of the compounds were selected for screening based on their ability to add diversity to the NCI small molecule compound collection.

Process of NCI-60 DTP Human Tumor Cell Line Screen

The screening of the compounds operated with the *In Vitro* Cell Line Screening Project (IVCLSP) which is a dedicated service, providing direct support to the DTP anticancer drug discovery program. The process utilized 60 different human tumor cancers of the lung, colon, brain, ovary, breast, prostate and kidney which was aimed in showing selective growth inhibition or cell killing of particular tumor cell lines by specific compound. The screening begins with the evaluation of all selected compounds against these 60 cell lines at a single dose of 10⁻⁵ M.

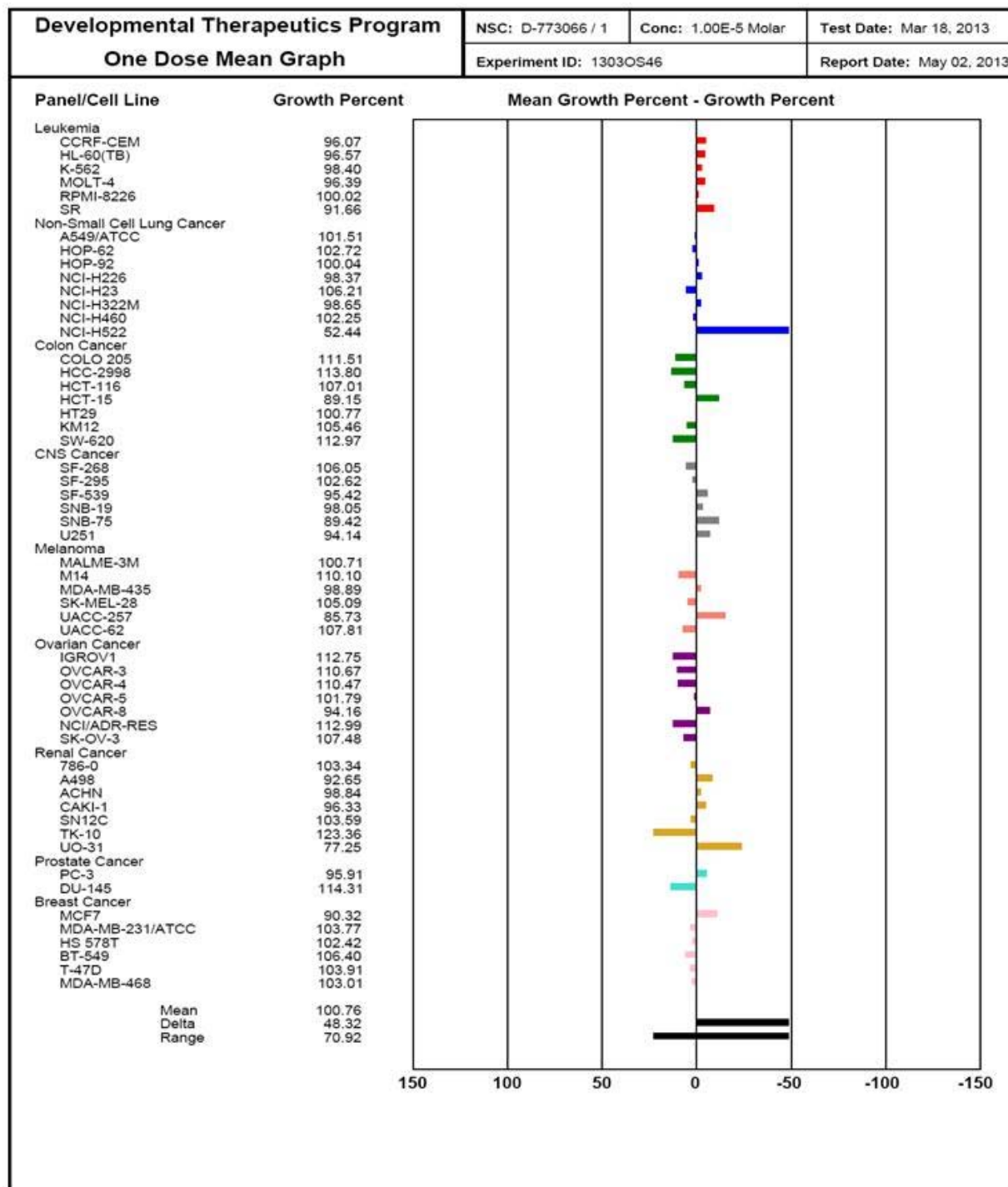


Figure 1: Selected NCI sixty cell screening data highlighting the potency of compound NSC: D-773066

Table 2: Anticancer Screening Data of Tested Compounds

| NSC Code. (Comp. Id) | Most sensitive cell lines | Mean growth % | Range | Delta | % Growth | % GI |
|-------------------------|--|---------------|-------|-------|----------|--------------|
| D-771852 (1i) | Renal cancer (UO-31) | 98.34 | 43.20 | 26.88 | 71.46 | 26.88 |
| | Non-Small Cell Lung Cancer (HOP-92) | 98.34 | 43.20 | 26.88 | 76.30 | 22.04 |
| D-771853 (1c) | Renal cancer (UO-31) | 98.55 | 60.47 | 32.72 | 65.83 | 32.72 |
| | CNS Cancer (SNB-75) | 98.55 | 60.47 | 32.72 | 83.75 | 14.80 |
| D-773066 (1g) | Non-Small Cell Lung Cancer (NCI-H522) | 100.76 | 70.92 | 48.32 | 52.44 | 48.32 |
| | Renal cancer (UO-31) | 100.76 | 70.92 | 48.32 | 77.25 | 23.51 |
| D-773067 (1h) | Non-Small Cell Lung Cancer (NCI-H522) | 101.40 | 71.52 | 52.55 | 48.85 | 52.55 |
| | Renal Cancer (UO-31) | 101.40 | 71.52 | 52.55 | 83.91 | 17.49 |

Range= Highest Growth Percent-Lowest Growth Percent; Delta= Mean Growth Percent-Lowest Growth Percent; % GI (Percent Growth Inhibition of most sensitive cell lines) = mean growth% -% Growth

RESULTS AND DISCUSSION

In the present study four newly substituted pyrimido[2,1-*b*][1,3]benzothiazole derivatives were submitted to NCI for in vitro human tumor cell line screening. The compounds were evaluated at single concentration of 10^{-5} M towards the panel of 60 cancer cell lines derived from nine different cancer types: leukemia, non small cell lung, colon, CNS, melanoma, Ovarian, Renal, prostate and breast cancer. The mean growth %, range of growth %, Delta, % growth and % GI relative to most sensitive cell lines is depicted in table 2. In general it was observed that the para substituted derivatives were more active than the other derivatives. This may be Because of the fact that the para substituted derivatives are better fitted into the receptor site. The structural modification resulted in the substantial improvement in activity. The position of the substituted group on the phenyl ring appeared to greatly influence the anticancer activity. The tested compounds showed growth inhibitory activity against mainly renal cancer, Non-Small Cell Lung Cancer and CNS cancer human tumor cells. Compounds (4*R*)-2-amino-8-chloro-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile(1h), (4*R*)-2-amino-7-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]Benzothiazole-3-carbonitrile (1g) showed significant anticancer activity against Non-Small Cell Lung Cancer showing %GI as 52.55,48.32 percents against NCI-H522 cell line respectively. Compound (4*R*)-2-amino-6-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-Carbonitrile(1i)also showing growth inhibition against Non-Small Cell Lung Cancer (HOP-92) cell line 22.04 %GI. The tested compounds also showed some distinctive potential pattern of selectivity. Compounds (4*R*)-2-amino-8-bromo-4-(4-fluorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile (1c),(4*R*)-2-amino-6-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3] benzothiazole-3-carbonitrile (1i), (4*R*)-2-amino-7-chloro-4-(4chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzoThiazole-3-carbonitrile

(1g)and(4*R*)-2-amino-8-chloro-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile (1h) have selectivity towards renal cancer showing % GI 32.72,26.88,23.51 and 17.49 against UO-31 cell line respectively.

CONCLUSION

The objective of this study was to synthesize and investigate the anticancer inhibition activity of substituted pyrimido benzothiazole derivatives with the hope of discovering new structure leads to serve as potential anticancer agents. We have reported a simple, economic and eco-friendly highly efficient one pot microwave irradiation synthetic strategy for exclusive synthesis of substituted pyrimido[2,1-*b*][1,3]benzothiazole derivatives using commercially available starting material. The newly synthesized compounds showed good inhibitory activity on different cell lines at a single concentration of 10µM. The results showed that a change in the substitution pattern in pyrimido[2,1-*b*][1,3]Benzothiazole derivatives may cause a marked effect on their anticancer activity. Compounds (4*R*)-2-amino-7-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile (1g) and (4*R*)-2-amino-8-chloro-4-(3,4,5-trimethoxyphenyl)-4*H* pyrimido [2,1-*b*][1,3]benzothiazole-3-carbonitrile (1h) showing potential selective growth inhibition against non small cell lung cancer (NCI-H522) Cell line. Also all selected compounds (1g), (1h), (1c) and (1i) showed distinctive potential pattern of selectivity against renal cancer (UO-31) Cell line.

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