Research Article

Synthesis and Biological Activities of 5-(1, 3-benzthiazol-2-ylamino)-4-phenyl-2, 4-dihydro-3*H*-1, 2, 4-triazole-3-thione and its Derivatives

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ABSTRACT

The present study was aimed to synthesize a series of novel 5-(1,3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione and to evaluate their *in-vitro* anti-inflammatory activity and in vitro on panel of 60 different human tumor cell lines derived from nine neoplastic cancer types at NCI. The compounds were evaluated using inhibition of bovine serum albumin denaturation method have shown significant *in-vitro* anti-inflammatory activity. The findings of present study clearly demonstrate that chloro functional group possess inhibition of bovine serum albumin denaturation capacity and has *in-vitro* anti-inflammatory activity. However methoxy and dimethyl derivatives show mild to moderate *in-vitro* anti-inflammatory activity. 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 at NCI. The compound 7a, 7b, 7f shows moderate anticancer activity. Structural assignments are based on spectroscopic data (FT-IR, ^{1}H NMR, MASS spectra).

Keywords: 2-aminobenzothiazoles, 1,2,4-triazole-3-thione, anticancer activity, national cancer Institute.

INTRODUCTION

Nowadays, health is one of the most important domains which we human beings have focused on in our society. However, tumor is the biggest killer of our lives, so there has been steadily increasing research in the field of anticancer therapy over recent years. 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. Unwanted side effects of antitumor drugs could be overcome with agents capable of discriminating tumor cells from normal proliferative cells and the resistance is minimized using combined modality approach with different complementary mechanism of action.[1-3] Substituted benzothiazole derivatives have diverse chemical reactivity and broad spectrum of biological activity such as antitumor, antimicrobial, antitubercular, antimalaria, anticonvulsant, anthelmintic, analgesic and anti-inflammatory activity.[4-5]

Malleshapa N. Noolvi reported synthesis of novel derivatives of benzothiazoles. From the stand point of biological activity, fused hetero aromatic systems are often of much greater interest than the constituent monocyclic compounds. Benzothiazole type compounds have attracted considerable attention to anticancer research and several attempts were made for modifying the benzothiazole nucleus to improve their antitumor activities. They are widely found in bioorganic and medicinal chemistry with application in drug discovery.[6] Devmurari V.P. reported the synthesis of substituted 2-aminobenzthiazole and evaluated for its anticancer activity from that we can conclude that compound with more than one halogen atom showed cytotoxicity towards cancer cell.[7] Catriona G. Mortimer reported synthesis on new 2-phenylbenzothiazoles has been synthesized on the basis of the discovery of the potent and selective in vitro antitumor properties of 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole.[8] Sanjay K Yadav reported synthesis of benzothiazole derivatives and evaluated for anti-inflammatory activity. They concluded that the two moieties i.e. 2-substituted benzothiazole and 2-chloroacetamido-6-substituted independently are showing anti-inflammatory activity.[9] P. Venkatesh and S.N. Pandeya carried out the synthesis, characterisation and anti-inflammatory activity of some 2-amino benzothiazole derivatives.[10]

On the other hand, 1,2,4-triazoles are associated with diverse pharmacological activities such as analgesic, antiasthmatic, diuretic, antihypertensive, anticholinergic, antibacterial, antifungal and anti-inflammatory activity. The derivatization of triazole ring is based on the phenomenon of bioisosterism in which replacement of oxygen of oxadiazole nucleus with nitrogen triazole analogue. Out of the two triazoles 1, 2, 4- triazole has wide variety of activity. Triazole moiety is an important and frequent insecticide, agrochemical structure feature of many biological active compound as cytocrome p450 enzyme inhibitors and peptide analog inhibitor.[11] Md. Hasan Morshed reported the synthesis and antineoplastic activity of 1,2,4 triazole derivatives. The toxic effects of the compound as the host were not very high and the animals recovered gradually towards normal within a few days after treatment. The results suggested that the compound is capable to exhibit significant antitumor property with little adverse affects on the hematological profiles of the host.[12]

Taking in to consideration of literature review, it was clear that very little literatures were available for substituted 5-(1, 3-benzothiazol-2-ylamino)-2,4-dihydro-3*H*-1,2,4-triazole. So we had planned to synthesise titled compound & to screen for anticancer, anti-inflammatory activities.

MATERIALS AND METHODS

Materials

Melting points were determined in open capillaries. Reactions were monitored by thin layer chromatography using silica gel-G as adsorbent using benzene as mobile phase. IR spectra (KBr pellet) were recorded on Bruker α FT-IR spectrometer.^{1}H NMR spectrum (DMSO-d_6) were taken on NMR Bruker Avance II 400 MHz spectrometer from Punjab University, Chandigarh. Physicochemical parameters of synthesized compounds are depicted in table no.1

Synthesis procedure

1) **Synthesis of *N*-phenylhydrazinecarbothioamide**.

Synthesis was done by as per reported method. [13-14]

2) **Synthesis of substituted ethyl 1,3-benzothiazol-2-ylcarbamate** .

Synthesis was done by as per reported method.[15]

3) Synthesis of N-1,3-benzthiazol-2-yl - 2(phenylcarbamothioyl)hyddazinecarboxamide.

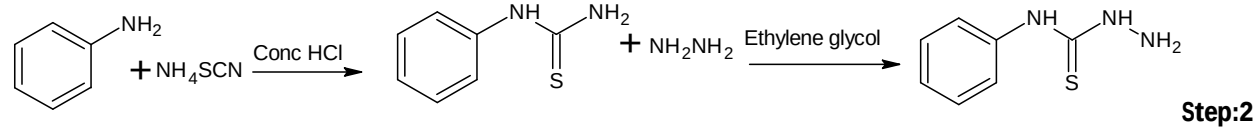
The solution of substituted **ethyl 1,3-benzothiazol-2-ylcarbamate** (0.01mol) and **N-1,3-benzthiazol-2-yl - 2(phenylcarbamothioyl)hyddazinecarboxamide** (0.01 mol) in ethanol (25 ml) refluxed independently for 4 hr. The residue was concentrated, cooled and poured over crushed ice to the precipitate which was filtered, wash with water.[16]

4) Synthesis of 5-(1,3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione

A mixture of substituted N-1,3-benzthiazol-2-yl - 2(phenylcarbamothioyl) hyddazine carboxamide (0.001) and 30 ml of 2 % aq. sodium hydroxide solution was refluxed independently for 6 hr. After completion of reaction mixture was filtered and filtrate was neutralized with conc. HCl drop wise till pH was adjusted to 7. The mixture was kept aside for few minutes and filter. Recrystallized from ethanol.[17]

SCEHME:

Step:1



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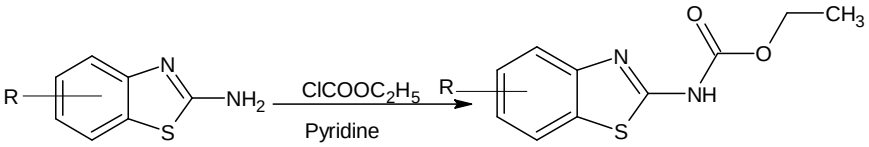

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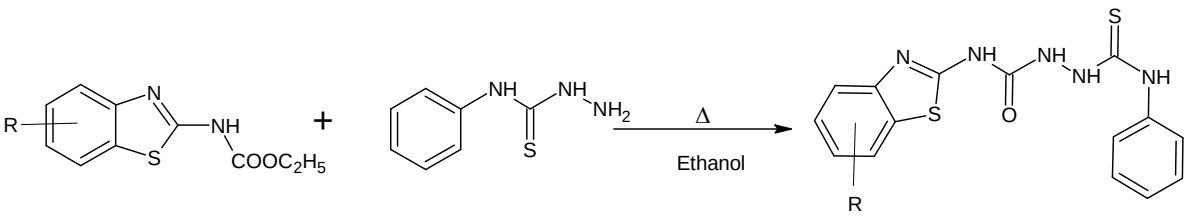

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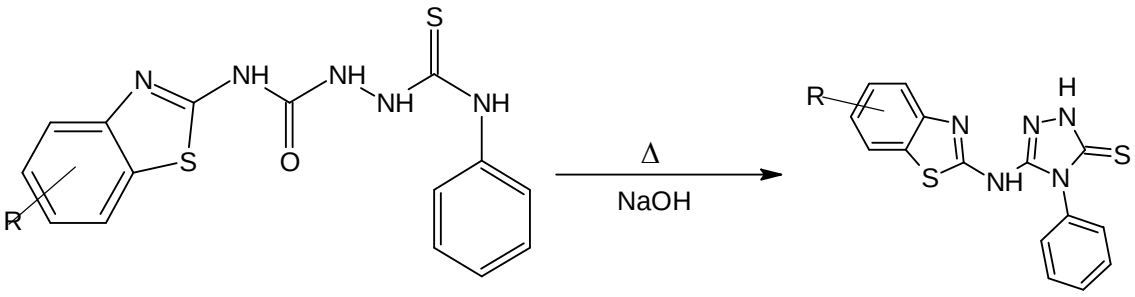


Table 1: Physicochemical parameters of all synthesized compounds

Comp. Id.	R	Mol. Formula	Mol. Wt	m.p.°C	*Rf Value	%Yield
7a	4Cl	$C_{15}H_{10}ClN_5OS_2$	359.85	232-234	0.73	55
7b	5Cl	$C_{15}H_{10}ClN_5OS_2$	359.85	244-246	0.67	53.83
7c	6Cl	$C_{15}H_{10}ClN_5OS_2$	359.85	240-242	0.68	55.23
7d	$4OCH_3$	$C_{16}H_{13}N_5OS_2$	355.43	262-264	0.70	51.11
7e	$5OCH_3$	$C_{16}H_{13}N_5OS_2$	355.43	232-234	0.62	52.87
7f	$6OCH_3$	$C_{16}H_{13}N_5OS_2$	355.43	220-222	0.66	52.42
7g	$6NO_2$	$C_{15}H_{12}N_6O_3S2$	388.85	180-182	0.64	60.11
7h	$6Br_2$	$C_{15}H_{10}N_5S_2Br$	404.30	192-194	0.72	58.69
7i	7-Cl 6-F	$C_{15}H_9ClFN_5S_2$	377.86	226-228	0.76	49.15
7j	4-7 CH_3	$C_{21}H_{20}ClFN_4O_2S$	446.92	238-240	0.65	45.28

*Mobile phase-benzene

Table 2: Anticancer screening data of tested compounds

NSC code (Comp No)	60 cell line assay in 1-dose 10^{-5} M concentration					
	Most sensitive cell lines	Mean growth %	Range	Delta	% Growth	% GI
D-771857 (7a)	Leukemia SR	99.16	59.23	32.90	66.36	33.64
D-773070 (7b)	Renal cancer UO-31	101.71	57.76	29.26	72.45	17.99
D-773069 (7h)	Prostate Cancer PC-3	102.30	58.75	23.75	78.55	27.55

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

5-(4-Chloro-1, 3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7a)

FT-IR(KBr cm^{-1}): 3430 (N-H str.); 3025 (C-H str); 1620 (C=N str); 1540 (C=C str); 1266 (C-S str); 730 (C-Cl str), ^{1}H NMR (DMSO d_6): (δ, ppm): δ 4.56 (s, 1H, NH); 7.12-8.32 (m, 8H, Ar-H); δ7.12 (s, 1H, NH), M^+-359, RA-72%; M^{+2}-361; RA-25.12%; % Anal calculated; C-50.06, H-2.80; N- 19.46, Found: C -49.90; H -2.75; N-19.53.

5-(5-chloro-1, 3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7b)

FT-IR(KBr cm^{-1}): 3413 (N-H str); 3032 (C-H str); 1718 (C=O str); 1601 (C=N str); 1530 (C=C str); 1277(C-S str); 733(C-Cl str), ^{1}H NMR (DMSO d_6) : 4.12 (s,1H,NH), 6.89 (s,1H,NH), 7.05-8.30 (m,8H,ArH), M^+-359; RA-70%; M^{+2}-361; RA-21.62%; % Anal. Calculated: C-50.06; H-2.80; N- 19.46; Found: C 50.13; H-2.88; N- 19.39.

5-(6-Chloro-1, 3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7c)

FT-IR(KBr cm^{-1}) : 3445 (N-H str); 3031 (C-H str); 1655 (C=N str); 1533 (C=C str); 1250 (C-S str); 740(C-Cl str), ^{1}H NMR (DMSO d_6): 4.02 (s, 1H, NH); 6.78 (s, 1H, NH); 7.02-8.24 (m, 8H, Ar H); M^+-359; RA-71%;M^{+2}-361; RA-20.55%, %Anal. Calculated: C-50.06; H-2.80; N- 19.46, Found: C -50.01; H-2.85; N- 19.40.

5-(4-Methoxy-1, 3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7d)

FT-IR (KBr cm^{-1}) : 3355 (N-H str) ; 2987(C-H str) ; 1585 (C=N str) ; 1543(C=C str) ; 1270 (C-S str) ; 1092 (C-0-C str), ^{1}H NMR (DMSO d_6): 3.49 (s, 1H, OCH_3); 4.11 (s, 1H, NH); 6.98 (s, 1H, NH); (7.12-8.51 (m, 8H, Ar H), M^+-355; RA – 65%; % Anal. Calculated C- 54.07, H- 3.69, N- 19.70, Found: C-54.14 , H-3.66 ,N-19.68.

5-(5-Methoxy-1, 3-benzthiazol-2-ylamino)-4-phenyl-2, 4-dihydro-3*H*-1, 2, 4-triazole-3-thione (7e)

FT-IR (KBr cm^{-1}): 3440 (N-H-str) ;3095 (C-H-str); 1590 (C=N-str) ; 1524 (C=C-str); 1272 (C-S-str) ; 1099(C-O-C-Str), ^{1}H NMR (DMSO d_6): 3.73 (s, 1H, OCH_3) ; 4.35(s, 1H, NH_1) ; 6.26(s, 1H, NH); 6.92-8.41 (m, 8H, Ar-H), M^+-355; RA – 67%; % Anal. Calculated: C- 54.07; H- 3.69; N- 19.70, Found: C -54.06; H- 3.56; N- 19.54.

5-(6-Methoxy-1, 3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7f)

FT-IR(KBr cm^{-1}): 3385 (N-H str); 2943 (C-H str); 1675 (C=N str); 1519 (C=C str); 1288 (C-S str);1092 (C-O-C Str), ^{1}H NMR (DMSO d_6): 3.73 (s, 1H, OCH_3); 4.35 (s, 1H, NH_1); 6.26 (s, 1H, NH); 6.92-8.41 (m, 8H, Ar-H), M^+-355; RA–2%; % Anal.Calculated: C-54.07; H-3.69; N-19.70, Found: C-54. 06; H 3.56; N 19.54.

5-(6-Nitro-1, 3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7g)

FT-IR(KBr cm^{-1}): 3424 (N-H str); 3022(C-H str); 1630(C=N str) ;1529 (-NO_2str); 1520 (C=C str); 1264 (C-S str) , ^{1}H NMR (DMSO d_6) : 4.32 (s, 1H, NH); 6.69 (s, 1H, NH); 7.05-8.30 (m, 8H, ArH) , M^+-370; RA –72%; % Anal. Calculated: C-48.64; H-2.72; N- 22.69; Found: C-48.74; H-2.68; N- 22.59.

5-(6-Bromo-1, 3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7h)

FT-IR(KBr cm^{-1}) : 3414 (N-H str); 2979 (C-H str); 1602 (C=N str), 1530 (C=C str), 1265 (C-S str), ^{1}H NMR (DMSO d_6) : 4.02 (s, 1H, NH); 6.89 (s, 1H, NH); 7.05-8.30 (m, 8H, ArH), M^+- 404.30; RA –67%; % Anal. Calculated: C-44.56; H-2.89; N- 17.32; Found: C 44.54; H-2.83; N- 17.39.

5-(7-chloro-6-fluro-1,3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7i)

FT-IR(KBr cm^{-1}): 3389 (N-H str); 2973 (C-H str); 1632 (C=N str); 1539 (C=C str); 1263 (C-S,str) 1100 (C-F str), ^{1}H NMR (DMSO d_6): 4.18 (s, 1H, NH); 6.73 (s, 1H, NH); 7.05-8.30 (m, 7H, ArH), M^+-377.84; RA–70%; % Anal. Calculated: C-47.68; H-2.40; N- 18.53; Found: C 47.54; H-2.43; N- 18.49.

5-(4-7-dimethyl-1,3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (7j)

FT-IR(KBr cm^{-1}) : 3390 (N-H str); 2954 (C-H str); 1614 (C=N str), 1509 (C=C str), 1272 (C-S str), ^{1}H NMR (DMSO d_6): 2.35 (s, 6H, CH_3); 4.18 (s, 1H, NH); 6.73 (s, 1H, NH); 7.05-8.30 (m,7H,ArH), M^+-353.456; RA – 69%. % Anal. Calculated: C-57.77; H-4.28; N- 19.81; Found: C 57.64; H-4.33; N- 19.89.

Anticancer activity

The compounds (7a, 7b, and 7h) were screened for preliminary anticancer assay by National Cancer Institute (NCI), Bethesda, Maryland, USA in an in *vitro* 60 human tumor cell panel derived from nine neoplastic cancer types.[18]

***In vitro* anti-inflammatory activity**

The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique. The standard drug and test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at 27° ±1°C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ° ±1°C in water bath for 10 min. After cooling the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SCHIMATZU 1800). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The diclofenac sodium was used as standard drug.

% of inhibition = 100 x ((Vc/Vt)-1)

Where, Vt and Vc are mean absorbance value of test group and control group.[19-21]

Table 3: *In vitro* anti-inflammatory activity

Name of compound	Absorbance value (mean±SE)	Inhibition of denaturation (in %)
Control	0.089±0.009	--
7a	0.162±0.001	82.02
7b	0.153± 0.003	71.91
7c	0.158± 0.0013	77.52
7d	0.112+ 0.002	25.84
7e	0.126±0.001	41.57
7f	0.121± 0.003	35.95
7g	0.137± 0.004	53.93
7h	0.159±0.002	78.64
7i	0.118±0.0015	32.58
7j	0.132± 0.0021	48.48
Standard (Diclofenac sodium)	0.173±0.001	94.38

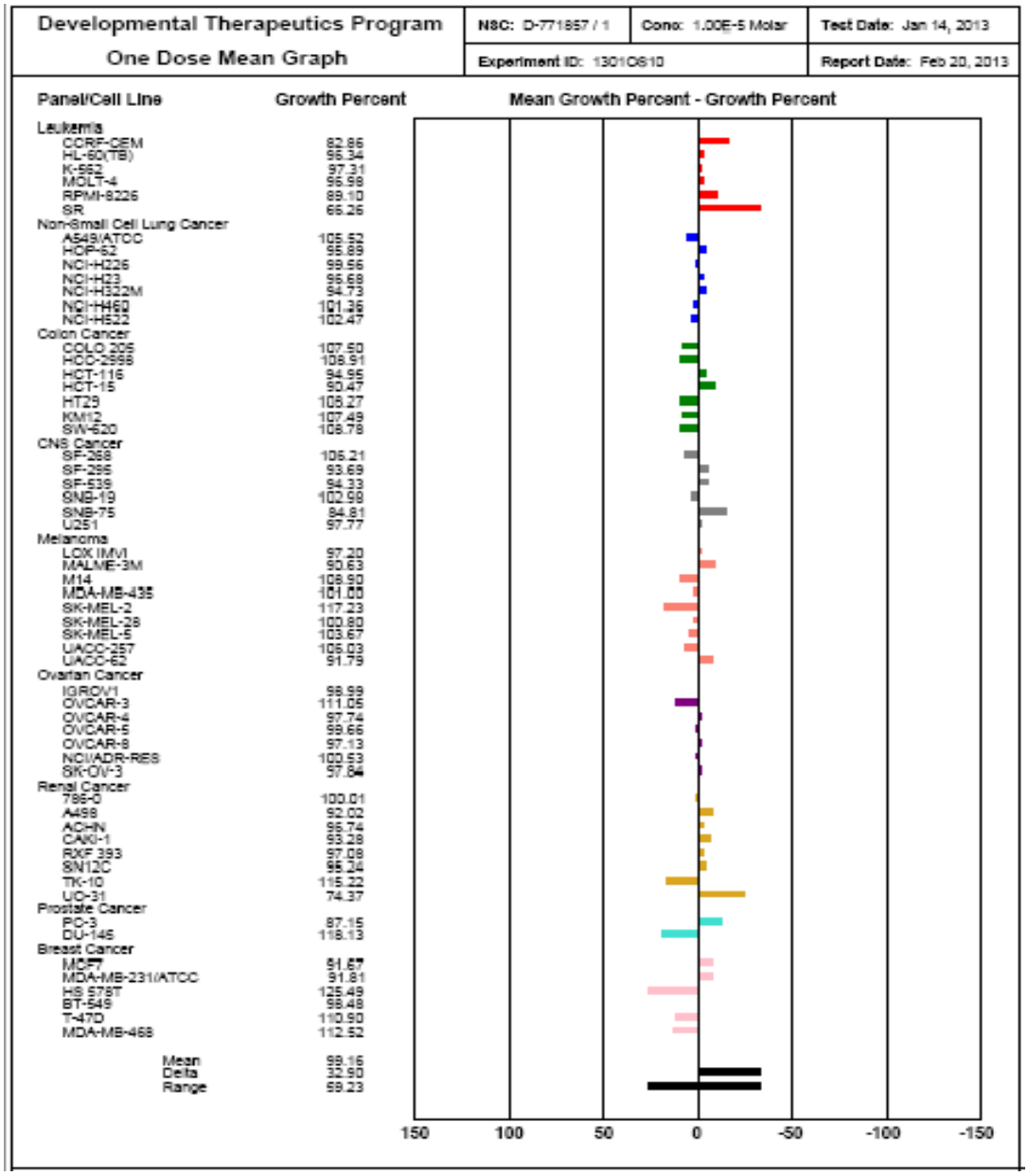
RESULTS AND DISCUSSION

The purpose of the present work was to synthesize a series of desired title compounds 5 -(1,3-benzthiazol-2-ylamino)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione(7a-7j) by reacting with hydrazinecarboxamide and ethyl 1-3 benzthiazole-2-yl carbamate. Furthermore, the procedure used commercially available reagents, giving the desired compounds in moderate yields (45–60%). The versatility of this methodology makes it suitable for library synthesis in drug discovery efforts.

The compounds were evaluated at single concentration of 10^{-5} M towards the panel of approximately 60 cancer cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostrate and breast cancers. Preliminary anticancer assay was performed according to the US NCI protocol.

The tested compounds showed a broad spectrum of growth inhibitory activity against human tumor cells, as well as some distinctive patterns of selectivity Fig.1. Compound (7a) was found to have good growth inhibitor activity against Leukemia (SR) with a growth % of most sensitive cell line to be 64.93, whilst least active over other cell lines. The mean growth % for compound (7a) was observed 99.16 and fall in a range of 59.23. Compounds (7g) and (7h) showed selectivity on renal cancer (UO-31) with a growth % of most sensitive cell line to be 78.91, 74.74 and respectively. Anticancer screening data of tested compounds are depicted in table no.2.

Figure 1: One dose mean graph of compound (NSC:D-771857/1)



All the newly synthesized compounds 7a-7j was tested for *in vitro* anti-inflammatory activity as compared to the standard Diclofenac sodium. Amongst all the tested compounds 7a, 7b,7c found with most potent activity i.e 82.02%, 71.91%, and 77.52 respectively and 7h having 78.64% inhibition of denaturation. Anti-inflammatory screening data of tested compounds are depicted in table no.3.

CONCLUSION

In the present work ten compounds were tested and three of them displayed antitumor activity on renal cancer, lung cancer cell lines. Compound **7a, 7b, 7c** was found to potent anti-inflammatory activity and all are showing good anti-inflammatory activity. Compound (**7a**) was found to be active with selective influence on non small cell lung cancer cell lines, especially on Leukemia SR with a % growth inhibition of 33.64. The obtained results prove the necessity for further investigations to clarify the features underlying the antitumor potential of tested compounds.

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