Research Article

Design, Microwave assisted Synthesis and Pharmacological activities of Substituted Mannich Base and its derivatives

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Accepted on: 12-05-2013; **Finalized on:** 31-07-2013.

ABSTRACT

The present study was aimed to developing multicomponent reactions in order to produce biologically active compounds has been accelerated and thus became one of the very important areas of research in organic and medicinal chemistry. Synthesize a series 2-[(1,3-benzothiazol-2-ylamino)methyl]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3a-3l) and evaluate their *in-vitro* cancer activity. The starting material (1a-1l) was prepared by the application of cyclization reaction. Synthesize a series of desired title compounds 2-[(1,3-benzothiazol-2-ylamino)methyl]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3a-3l), (1a-1l) by reacting with formaldehyde and pyrazolone. Synthesized compounds were having excellent % yields in a range of 75-85% under microwave irradiation. All the synthesized compounds were characterized by their physical and spectral data. *In-vitro* anti-cancer activity of synthesized compounds [3a-3l] was evaluated at National Cancer Institute, Maryland, USA against selected 60 Human Cancer cell lines, derived from various cancer types. Conventional cancer chemotherapy is seriously limited by tumor cells exhibiting multidrug resistance (MDR).The results showed that a change in the substitution pattern, substituted Mannich base derivatives may cause a marked effect on their selective anticancer activity. Compound (3i) showed significant anticancer activity and distinctive potential pattern of selectivity on Leukemia Cancer and Non Small cell lung Cancer and compounds 3h, 3g, 3e showed significant anticancer activity and distinctive potential pattern of selectivity on Renal Cancer.

Keywords: 2-Aminobenzothiazole, *In vitro*-anticancer activity, Mannich base, Pyrazole.

INTRODUCTION

Cancer incidence and mortality varies significantly between developed and developing countries. The top five most frequent cancers in less developed countries in males and females are cancers of the lung (12.4% of new cases), stomach (10% of new cases), breast (9.7% of new cases), liver (8.8% of all new cases) and colorectum (7.1% of all new cases).[1] The existing data demonstrates that there are some key differences that emerge in the pattern of cancer incidence. Conventional cancer chemotherapy is seriously limited by tumor cells exhibiting multidrug resistance (MDR), caused by the over expression of integral membrane transporters, such as P-gp and MDR-associated proteins (MRPs) which decrease the drug accumulation and cell death. The classic resistance to cytotoxic drugs has most often been linked to over expression of P-glycoprotein (P-gp). P-gp acts as an ATP-dependent multidrug efflux pump transporting a broad range of drugs across membrane out of cytosol, therefore P-gp reduces intracellular bioavailability and toxicity of these drugs.[2] Therefore, the discovery and development of new potent and selective anticancer drugs are of high importance in modern cancer research. An intense search for new chemical structures beneficial to designing antitumor drugs has been sparked. In recent years, developing multicomponent reactions in order to produce biologically active compounds has been accelerated and thus became one of the very important areas of research in organic and medicinal chemistry.[3] Several heterocyclic compounds containing pyrazolone moiety were found to be useful intermediates for medical drugs and play an important role among biologically active compounds.[4] Typical pyrazole derivatives are pyrazol-3-ones ('pyrazolones') whose syntheses, reactivity and numerous applications are well-documented. Pyrazolone derivatives were examined for growth inhibitory properties in several human cancer cells. Some of them are effective inhibitors of heptosyl transferase WaaC, others inhibit accumulation of the abnormal protease resistant form of prion protein (PrP-res).[5] Pyrazolone framework plays an essential role and represents an interesting template for combinatorial and medicinal chemistry.[6,7] Benzothiazole type compounds have attracted significant attention to anticancer research, and some attempts were made for modifying the benzothiazole nucleus to improve their antitumor activities. Among them imidazobenzothiazoles as well as polymerized benzothiazoles. Substituted benzothiazoles such as 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole has been shown to exhibit exquisitely potent (GI50 < 0.1 nM) and selective in vitro antitumor properties in human cancer cell lines (e.g., colon, non small cell lung and breast subpanels) of the National Cancer Institute (NCI) 60 human cancer cell line screen and also exhibited remarkable antitumor activity against malignant cell lines. 2-(4-aminophenyl)-benzothiazole and its analogs comprise a novel mechanistic class of antitumor agents, due to formation of reactive intermediates that can bind covalently to DNA.[7,8] Aminoalkylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and Modifications of biologically active compounds[8], Hence in continuation of our efforts on the design and

synthesis of novel anti-cancer agents and keeping in mind the medicinal importance of benzothiazole and pyrazole moieties we have synthesized and evaluated for in vitro anti-tumor activity at National Cancer Institute (NCI-USA) for mannich bases.[9-11]

MATERIALS AND METHODS

Synthesis of 2-aminobenzothiazole[8-11]

Substituted 2-aminobenzothiazole was synthesized as per the reported procedure.

Synthesis of Mannich Base[13-16]

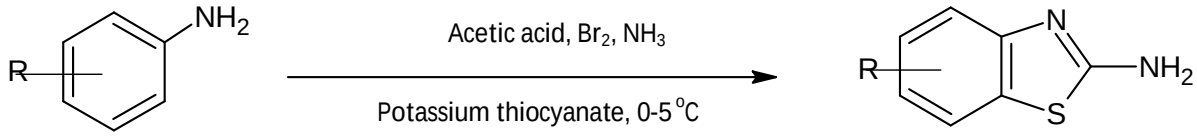
Corresponding equimolar solution of substituted 2-aminobenzothiazole derivatives in DMF and 3-methyl-5-pyrazolone in DMF were irradiated with microwave radiation at 560 W for 8 mins in presence of formaldehyde and concentrated HCl. The resultant mixture was added into crush ice or ice-cold water with vigorous stirring till precipitation occurs. Reaction completion was monitored with help of TLC. All synthesized compounds were recrystallized with DMF: ethanol as per reaction scheme.

Experimental protocols

All chemicals and solvents were supplied by Merck, Loba chem Chemical Limited. All the solvents were distilled and dried before use. The reactions were monitored with the help of thin layer chromatography using silica gel, The solvent system used to carry out the TLC is n-hexane: Toluene: formic acid (5:4:1). Melting points of the synthesized compounds were recorded by open capillaries and are uncorrected as in Table 1. IR spectrum (cm^{-1}) was acquired on a KBr on a bruker alpha T FTIR spectrometer.^{1}H-NMR d_6 DMSO spectra of the synthesized compounds were performed with Bruker Avance-II 400 NMR Spectrometer operating at 400 MHz in SAIF, Punjab University (Chandigarh). Chemical shifts were measured relative to internal standard TMS. Chemical shifts are reported in δ scale (ppm). Mass spectra of the synthesized compounds were recorded at TOF-ES in SAIF, Punjab University.

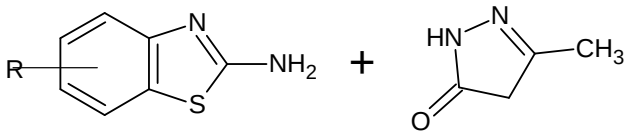
Reaction Schemes

I. Synthesis of substituted 2-Amino-benzothiazole



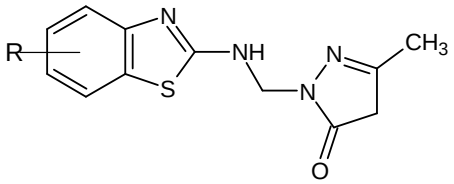
Substituted-Aniline — Substituted 2-Amino-benzothiazole

II. Synthesis of Mannich base



Substituted 2-Amino-benzothiazole

5-methyl-2,4-dihydro-3*H*-pyrazol-3-one



Mannich base

Spectral data

1) 2-{[(4-chloro-1,3-benzothiazol-2-yl)amino]methyl} 5-methyl-2,4-dihydro-3*H*-pyrazol-3-one; (3a);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); 721.85 (C-Cl); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 293 (M^+) RA (60%) 295 (M^{+2}) RA (25%) Elemental Analysis Calculated (found) C 48.90 % (48.72 %) H 3.76 % (3.78 %) N 19.01 % (19.07 %) O 5.43 % (5.49 %).

2) 2-{[(5-chloro-1,3-benzothiazol-2-yl)amino]methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one;(3b);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); 721.85 (C-Cl); ^{1}H NMR DMSO- d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 293 (M^+) RA (60%) 295 (M^{+2}) RA (30%) Elemental Analysis Calculated (found) C 48.90 % (48.72 %) H 3.76 % (3.78 %) N 19.01 % (19.07%) O 5.43 % (5.49 %).

Table 1: Physicochemical properties of the newly synthesized compounds

Sr.No	R	MW (g/mol)	M.P. (°C)	R_f value	% yield
3a	4-Cl	294.75	206-208	0.43	80.22
3b	5-Cl	294.75	210-212	0.44	82.54
3c	6-Cl	294.75	208-212	0.43	79.65
3d	4-CH_3	274.341	256-258	0.42	80.30
3e	4,7-CH_3	288.368	260-262	0.44	85.33
3 f	5-CH_3	274.341	256-258	0.43	80.55
3g	4-OCH_3	290.340	258-260	0.46	79.25
3h	5-OCH_3	290.340	258-260	0.44	82.25
3 i	6-OCH_3	290.340	262-264	0.47	86.32
3 j	6-Br	339.210	246-248	0.45	80.21
3k	6-NO_2	305.312	260-262	0.45	85.40
3 l	6-Cl,7-F	312.31	252-254	0.47	75.60

*Mobile phase n-hexane: Toluene: formic acid (5:4:1)

Table 2: Most sensitive cancer cell lines screening data of tested compounds

NSC Code	Mean Growth %	Range	Delta	Most sensitive cell lines	% Growth	% GI
773058 3g	102.37	45.21	26.06	**Non Small cell lung cancer**	76.45	25.92
				NCI-H522		
				Renal Cancer	76.31	26.06
				UO-31		
773059 3h	103.14	50.77	29.36	**Non Small cell lung cancer**	85.07	18.07
				NCI-H522		
				Renal Cancer	73.78	29.36
				UO-31		
773060 3i	80.34	124.75	65.36	**Leukemia**	14.98	**65.36**
				SR		
				CCRF-CCM	40	40.34
				Non Small cell lung cancer		
				A549/ATCC	49.69	30.65
				HOP-62	33.77	46.57
				NCI-H460	23.9	56.44
				NCI-H522	21.21	**59.13**
773061 3e	103.41	45.9	22.72	**Renal Cancer**	86.78	16.63
				UO-31		
				Prostate Cancer	80.69	22.72
				PC-3		
				Breast Cancer	84.5	18.91
				MCF7		

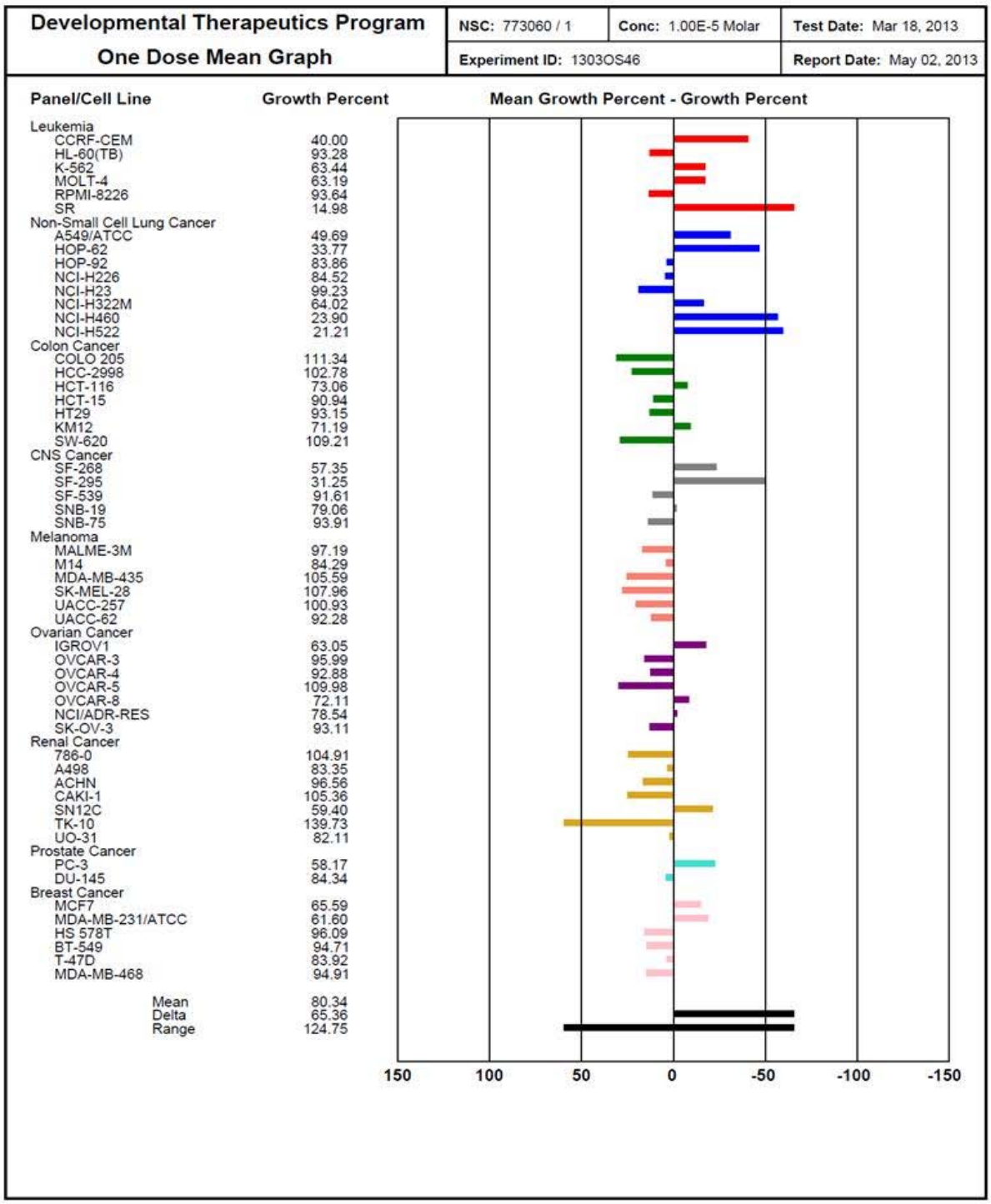


Figure 1: Selected NCI sixty cell screening data highlighting the potency of compound NSC: D-773060 **(3i)**

3. 2-{[(6-chloro-1,3-benzothiazol-2-yl)amino]methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one;(3c);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); 721.85 (C-Cl); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 293 (M^+) RA (60%) 295 (M^{+2}) RA (25%) Elemental Analysis Calculated (found) C 48.90 % (48.72%) H 3.76 % (3.78 %) N 19.01 %(19.07 %) O 5.43 % (5.49 %).

4. 5-methyl-2-{[(4-methyl-1,3-benzothiazol-2-yl)amino]methyl} -2,4-dihydro-3*H*-pyrazol-3-one;(3d);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); 721.85; ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 2.2(s 3H –CH_3), 5.1 (s 2H –CH_2), 2.5-2.6 (d 2H –CH_2), 2.0(s 3H –CH_3), Mass (TOF MS ES) m/z: 273 (M^+) RA (30%) Elemental Analysis Calculated (found) C 56.91 % (56.93 %) H 5.14 % (5.15 %) N 20.42 % (20.42 %) O 5.83 % (5.90%).

5.

5. 5-methyl-2-{[(4,7-dimethyl-1,3-benzothiazol-2-yl)amino]methyl}-2,4-dihydro-3*H*-pyrazol-3-one; (3e);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 2.2(s 3H –CH_3), 2.3(s 3H –CH_3), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 287 (M^+) RA(20%) Elemental Analysis Calculated (found) C 58.31 % (58.29 %) H 5.59 % (5.61 %) N 19.43 % (19.43 %) O 5.55 % (5.57 %)

6. 5-methyl-2-{[(6-methyl-1,3-benzothiazol-2-yl) amino]methyl}-2,4-dihydro-3*H*-pyrazol-3-one; (3f);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 2.2(s 3H –CH_3), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 273 (M^+) RA(40%) Elemental Analysis Calculated (found) C 56.91 % (56.93 %) H 5.14 % (5.15 %) N 20.42 % (20.42 %) O 5.83 %(5.90) %)

7. 2-{[(4-methoxy-1,3-benzothiazol-2-yl)amino] methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one ;(3g);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 3.3(s 3H –OCH_3), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 289 (M^+) RA (30%) Elemental Analysis Calculated (found) C 53.78% (53.75%) H 4.86 % (4.88 %) N19.30 % (19.30 %) O 11.02 % (11.05 %).

8. 2-{[(5-methoxy-1,3-benzothiazol-2-yl)amino] methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one;(3h);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 3.4(s 3H –OCH_3), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 289 (M^+) RA (60%) Elemental Analysis Calculated C 53.78% (53.75%) H 4.86 % (4.88 %) N19.30 % (19.30 %) O 11.02 % (11.05 %)

9. 2-{[(6-methoxy-1,3-benzothiazol-2-yl)amino] methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one;(3i);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 3.40(s 3H –OCH_3), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 289 (M^+) RA (20%) Elemental Analysis Calculated (found) C 53.78% (53.75%) H 4.86 % (4.88 %) N19.30 % (19.30 %) O 11.02 % (11.05 %)

10. 2-{[(6-bromo-1,3-benzothiazol-2-yl)amino]methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one; (3j);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); 802-810 (C-Br); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 338 (M^+) RA (40%) Elemental Analysis Calculated (found) C 53.78% (53.75%) H 4.86 % (4.88 %) N19.30 % (19.30 %) O 11.02 % (11.05 %)

11. 2-{[(6-nitro-1,3-benzothiazol-2-yl)amino]methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one; (3k);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); 1355-1320(-NO_2); ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 304 (M^+) RA (30%) Elemental Analysis Calculated (found) C 47.21 % (47.21%) H 3.36 % (3.65 %) N 22.94 % (22.94 %) O 15.72 % (15.70 %)

12. 2-[(6-chloro,7-fluro-1,3-benzothiazol-2-ylamino) methyl]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one; (3l);

3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67 (HC=N); 827.19-811.08 (C-N); 721.85 (C-Cl); 1150-1100 (C-F). ^{1}H NMR DMSO-d_6 δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 311 (M^+) RA (60%) 313 (M^{+1}) RA (30%) Elemental Analysis Calculated (found) C 46.08 % (46.08%) H17.91 % (17.91%) N 5.12 % (5.12 %) O 10.25 % (10.25 %).

Pharmacology [18-20]

NCI-60 DTP Human Tumor Cell Line Screen

Process

The operation of this screen utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The aim is to prioritize for further evaluation, synthetic compounds showing selective growth inhibition or cell killing of particular tumor cell lines. This screen is unique in that the complexity of a 60 cell line dose response produced by a given compound results in a biological response pattern which can be utilized in pattern recognition algorithms (COMPARE program). Using these algorithms, it is possible to assign a putative mechanism of action to a test compound, or to determine that the response pattern is unique and not similar to that of any of the standard prototype compounds included in

the NCI database. 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 µM. The output from the single dose screen is reported as a mean graph. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels.

Methodology of the *In Vitro* Cancer Screen

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 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_2, 95 % air and 100 % relative humidity for 24 hr prior to addition of experimental drugs. After 24 hr, 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 DMSO 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 thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml Gentamycin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 hr at 37°C, 5 % CO_2, 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 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes 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 µl) at 0.4 % (w/v) 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 mM 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 µl 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 % (GI50) 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 LC50 (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.

RESULTS AND DISCUSSION

The purpose of the present work was to synthesize and investigate the anticancer inhibition activity of selected substituted benzothiazolo-pyrazolone derivatives with the hope of discovering new structure leads to serve as potential anticancer agents. Furthermore, the procedure used commercially available reagents, giving the desired compounds in good to excellent yields (75-85%). The structure of newly synthesized compounds was confirmed by IR, ^{1}H NMR, Mass spectra and elemental analysis. Synthesized 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, 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.

The tested compounds showed growth inhibitory activity against mainly Non-Small Cell Lung Cancer, Leukemia Cancer, Renal Cancer, and Breast Cancer, Prostate Cancer human tumor cells. The tested compounds showed some pattern of selectivity. At the commencement of this study in the screening, compound 2-{[(6-methoxy-1,3benzothiazol-2-yl)amino]methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**3i**) exhibited better anticancer activity and showed GI% **40.34, 65.36** against **Leukemia (**CCRF-CCM) **and** (SR) cell line respectively and GI% 46.57, 56.44**, 59.13** against **Non Small cell lung Cancer (**HOP-62), **(**NCI-H460), **(**NCI-H522) respectively as shown in Figure1. The position was modified with differently substituted aromatic amine such as 6-OCH_3, 5-OCH_3, 4–OCH_3, 4,7di-CH_3 amines. The promising nature of compounds may be attributed to the substitution at hydrophobic domain. These compounds have electron donating group at para position (6 position) of hydrophobic aryl ring. 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. Enhancement in activity indicates that this modification is step up toward synthesis of a pharmacophore. The position of the substituted group on the phenyl ring appeared to greatly influence the anticancer activity; the 6-OCH3 derivative exhibited higher anticancer activity than the derivatives 4-OCH_3, 5–OCH_3, 4,7di-CH_3 as **3g, 3h, and 3e,** on other hand 2-{[(5-methoxy-1,3-benzothiazol-2-yl)amino]methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3h) showed selectivity on **Renal Cancer** (UO-31) GI% **29.36.** 2-{[(4-methoxy-1,3-benzothiazol-2-yl)amino]methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3g) showed selectivity on **Renal Cancer** (UO-31) GI% **26.06**. 2-{[(4,7-dimethyl-1,3-benzothiazol-2-yl)amino]methyl}-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3e) showed selectivity against **Renal Cancer (**UO-31) and **Prostate Cancer** (PC-3) with GI% 16.63 **and 22.72** respectively.

CONCLUSION

The objectives was to synthesize a series of desired title compounds 2-[(1,3-benzothiazol-2-ylamino)methyl]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3a-3l), (1a-1l) by reacting with formaldehyde and Pyrazolone. We have developed a simple, rapid, and efficient one-pot protocol for the preparation of the microwave-assisted reaction. The versatility of this methodology makes it suitable for library synthesis in drug discovery efforts. The newly synthesized compounds showed good inhibitory activity on different cell lines at a concentration of 10µM. On correlating the structures of the sample candidate with their biological activity, the results showed that a change in the substitution pattern substituted benzothiazolo-pyrazolone derivatives may cause a marked effect on their anticancer activity. Compound (3i) showed anticancer activity and selectivity on **Leukemia Cancer** and **Non Small cell lung Cancer** and (3h), (3g), (3e) showed significant anticancer activity and selectivity on **Renal Cancer**. Further structure-activity studies are also required to clearly elucidate the role of the substitution on the Substituted mannich base. The possible improvement of the anticancer properties of Substituted mannich base series through a QSAR study and mechanistic determination on activity of lead compound will be the focus of our further investigation.

Acknowledgement: We express sincere gratitude to Principal, Amrutvahini College of pharmacy, and Sangamner for providing laboratory facilities. Authors thanks to US National Cancer Institute (Harold Varmus, M.D. NCI, Bethesda) for screening anticancer activity of newly synthesized compounds using NCI 60 cell line screening. Director, SAIF Punjab University, Chandigarh for spectral analysis.

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Source of Support: Nil, **Conflict of Interest:** None.