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SYNTHESIS AND PRELIMINARY EVALUATION OF NOVEL 1, 5-BENZOTHIAZEPINE DERIVATIVES AS ANTI-LUNG CANCER AGENTS

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ABSTRACT

A series of novel 1, 5-benzothiazepine derivatives having a biologically active thiazepine moiety was synthesized by the condensation of substituted chalcones with 2-aminothiophenol using conventional as well as non-conventional methods. The structures of the newly synthesized compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis. All the newly synthesized compounds were evaluated for their *in vitro* anticancer activity against human lung cancer cell line (A549) using Adriamycin as a reference drug.

Keywords: 1,5-benzothiazepine derivatives, anticancer activity, chalcone, 2- aminothiophenol

INTRODUCTION

Heterocyclic compounds containing nitrogen and sulphur such as benzothiazepines and benzodiazepines have received considerable attention in recent years. Benzothiazepines have been claimed of various therapeutic activities like antimicrobial [1], anti-HIV [2], anti-cytotoxic [3], anti inflammatory [4] and anti cancer [5]. As a result, due to their wide range of biological and synthetic applications, several conventional [6] as well as non-conventional [7] green chemistry approaches have been developed for the synthesis of 1,5-benzothiazepine derivatives. The non-conventional protocol offers several advantages such as simple procedure, fast reaction rate, mild reaction condition and improved yields as compared to conventional methods. As cancer represents one of the most severe health problems worldwide and the development of new anti cancer drugs and more efficient strategies are the areas of utmost importance [8]. The major type of solid human tumours (breast, lung) which represent most cancer cases today, are multi casual in nature [9]. In continuation of our earlier endeavour on MORE (microwave induced organic reaction enhancement) chemistry [10-13] for the synthesis of bioactive compounds using solid phase conditions, we herein describe the synthesis of some novel 1, 5-benzothiazepine derivatives by conventional and non-conventional solid support conditions and their anti-lung cancer activity.

MATERIALS AND METHODS

General: All the chemicals were of AR grade and were obtained from Sigma–Aldrich (U.S.A) and Merck (Germany). All melting points were determined in open capillaries on Veego digital programmable melting point apparatus VMP-PM (Veego instrument corporation, Mumbai, India) and are uncorrected. Microwave assisted reactions were carried out with microwave synthesizer model CATA-R (Catalyst Systems, Pune, India), operating at 700W, generating 2450 MHz frequency. The purity of the compounds was routinely checked by thin layer chromatography (TLC) with Silica Gel-G (Merck, Germany).The instruments used for spectroscopic data are; IR-FTIR spectrophotometer (Brucker, Germany), ^{1}H NMR and ^{13}C NMR (DMSO-d_6) on 500 MHz FT-NMR spectrometer (Bruker, Germany) AV III and elemental analysis was carried out on a Carlo Erba (Italy) 1108 analyzer and were within the ± 0.4 % of the theoretical values. Column chromatography was performed on silica gel (Merck, Germany, 0.250-0.125 mm).

Syntheses : *2,4-dibromo-6-[2-(substituted phenyl)-2, 3-dihydro-1, 5-benzothiazepin-4-yl] benzene-1, 3-diol* ***(3a-j).*** *General procedure*

Solution phase conventional method.-
Compound **1** (0.01 mol) was dissolved in 50 mL of ethanol. To this, 2-aminothiophenol **2** (0.01 mol) was added and resulting reaction mixture was heated for 3 hours at 65-70 °C. Then the mixture was acidified by using 5-6 drops of glacial acetic acid and heating was continued for next 3-4 hours. After cooling, the content was poured into crushed ice, filtered and purified by recrystallization from methanol to afford compounds **3a-j**.

Solid phase non-conventional method.-
Compound **1** (0.01 mol) was dissolved in 1mL of DMF. To this, 2-aminothiophenol **2** (0.01 mol) and basic alumina (4g) was added. The resulting mixture was uniformly mixed with glass rod to make slurry and then air dried to remove the solvent. The absorbed material was irradiating inside the microwave for an appropriate time. After the completion of reaction, (monitored by TLC) the reaction mixture was cooled at room temperature and the product was extracted with methanol (2 x 20 mL). Removal of the solvent and subsequent recrystallization with methanol resulted compounds **3a-j**.

In vitro anticancer screening: Human tumour lung cancer cell line (A549) was obtained from the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, India, was used in this study and Adriamycin as a reference drug. The cytotoxic activity of the newly synthesized compounds was measured using the sulforhodamine B stain (SRB) assay method [14, 15]. The cell lines were grown in full (RPMI 1640) medium containing 10% fetal bovine serum and 2 mmol L^{-1} L-glutamine. For the present screening experiment, cells were inoculated into 96 well microtiter plates at 90 µL plating densities. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 hours prior to addition of experimental drugs to allow attachment of cells to the plate wall. After 24 hours, one plate with cell line was fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population at the time of drug addition (T_0). Experimental drugs were dissolved 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 was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing experimental drugs at a concentration of 10^{-3}. Additional three, 10-fold serial dilutions were made to provide a total of four drug concentrations plus control wells (cell line with the solvent without the drug). Aliquots of 10 µL of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µL of medium, resulting in the required final drug concentrations.

Different concentrations of the compounds under test (0.1, 1, 10 and 100 µ mol L^{-1}) were added to the cell monolayer and triplicate wells were prepared for each individual concentration. Monolayer cells were incubated at standard conditions for 48hours and assay were terminated by the addition of cold Trichloroacetic acid. After 48hours cells were fixed, washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4% (w/v) in 1% acetic acid was added to each well and plates were incubated for 20 min at room temperature. After staining, unbounded dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. Absorbance was read on an ELISA plate reader at a wavelength of 540nm (690 nm reference wavelength).

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Using the six absorbance measurements [time zero (T_0), control growth(C), and test growth in the presence of drug at the four concentration levels (T_i), the percentage growth inhibition (GI) was calculated at each of the drug concentration levels. The Growth inhibition of 50 % (GI_{50}) is the drug concentration causing 50% inhibition in cell viability was calculated and compared with the reference drug adriamycin.

RESULTS AND DISCUSSION

Chemistry: In the present study the starting 3', 5'-dibromo-2',4'-dihydroxychalcones **1a-j** were prepared by reacting 3,5 –dibromo-2,4-dihydroxyacetophenone and variously substituted aromatic aldehydes in the presence of base by conventional Claisen-Schmidt condensation method by our laboratory team. A novel series of 1, 5-benzothiazepine derivatives **3a-j** were synthesized by the reaction of substituted chalcones **1** with 2-aminothiophenol **2** in good yields. Physicochemical data of title compounds are shown in Table 1. The structures of compounds were confirmed on the basis of FTIR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis shown in Table 2. The purpose of the present study was to synthesize novel *2, 4-dibromo-6-[2-(substituted phenyl)-2, 3-dihydro-1, 5-benzothiazepin-4-yl] benzene-1, 3-diol* derivatives

expected to have good anti-lung cancer activity (Scheme 1). Treatment of compound **1** with 2-aminothiophenol **2** result in the formation of *2,4-dibromo-6-[2-(substitutedphenyl)-2, 3-dihydro-1, 5-benzothiazepin-4-yl] benzene-1, 3-diol* derivatives. In the FTIR spectrum of the synthesized compounds **3a-j**, the disappearance of band at 1680-1710 cm^{-1} due to the carbonyl group of chalcones, and the appearance of band at 1606-1629 (C=N), 1571-1581 (C=C), 627-671 (C-S) and 3289-3298 (C-H stretching) cm^{-1}, ^1H NMR spectrum revealed the presence of a doublet at δ 3.22- 3.52 ppm corresponding to $-CH_2$ group and a triplet at δ 4.98- 5.26 ppm corresponding to –CH group and ^{13}C NMR spectrum revealed the presence of $-CH_2$ group (52.29-57.93), -CH group (48.63-53.2) ppm, respectively. Mass spectra showed accurate $(M+H)^+$ peak for the synthesized compounds **3a-j** and elemental analysis data were within ±0.4% difference between calculated and the found values. All these analysis confirm the formation of 1, 5-benzothiazepine derivatives. In the present environment conscious scenario, the usage of microwave energy to accelerate the organic reactions, improved syntheses of the 1, 5-benzothiazepines were carried out using basic alumina as an inorganic solid support. The yields and reaction time of the conventional and non-conventional microwave assisted methods are compared in Table 3.

In vitro anti lung cancer activity: Newly synthesized compounds were evaluated for their *in vitro* anti lung cancer activity against human lung cancer cell line (A549) using Adriamycin as a reference drug. The relationship between percentage control growth and molar drug concentration was plotted to obtain the control growth of the lung cancer cell line (A549). The response parameter calculated was the GI_{50} value, which corresponds to the concentration required for a 50 % inhibition of cell viability. Table 4 shows the *in vitro* anticancer activity of the synthesized compounds compared to the reference drug.

CONCLUSIONS

In summary, we have developed a new series of 1,5-benzothiazepine derivatives. Our method has many advantages over existing conventional method, including high yield, simple work-up, shorter reaction span, no side reactions no critical purification method. This procedure represents a convenient, economic and environmentally friendly process for the synthesis of 1,5-benzothiazepines. It was observed from the results obtained that most of the tested compounds showed some anti-lung cancer activity. These preliminary results give an idea about the possible importance of the thiazepine moiety in the compounds acting as anti-lung cancer agents and give an encouraging framework in this field that may lead to the discovery of potent anticancer agent.

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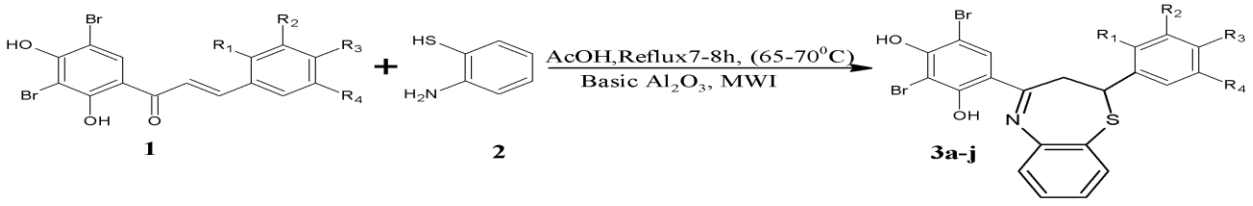

Entry	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j
R_1	H	Me	H	H	H	H	OH	H	H	
R_2	Cl	H	H	OH	OMe	OMe	H	OMe	NO_2	FURYL
R_3	H	H	OH	OMe	OMe	OMe	H	OH	H	
R_4	H	H	H	H	H	OMe	Br	Br	H	

Scheme-1: Synthesis of 1, 5-benzothiazepine derivatives 3a-j

Table 1: Physical and analytical data of newly synthesized compounds

Compd.	M.p. (°C)[a]	Yield (%)	Mol. formula (M_r)	Analysis calc. / found (%) C	H	N
3a	84-85	81	$C_{21}H_{14}O_2Br_2NSCl$ (539.30)	46.72 46.97	2.60 2.37	2.59 2.89
3b	74-75	83	$C_{22}H_{17}O_2Br_2NS$ (518.80)	50.89 50.55	3.28 3.61	2.70 2.98
3c	95-96	80	$C_{21}H_{15}O_3Br_2NS$ (520.80)	48.39 48.09	2.88 2.48	2.69 2.94
3d	87-88	84	$C_{22}H_{17}O_4Br_2NS$ (550.80)	47.93 47.89	3.09 3.12	2.54 2.50
3e	109-110	81	$C_{23}H_{19}O_4Br_2NS$ (654.80)	48.55 48.83	3.52 3.40	2.10 2.50
3f	81-82	85	$C_{24}H_{21}O_5Br_2NS$ (594.80)	48.42 48.10	3.53 3.20	2.35 2.67
3g	78-79	87	$C_{21}H_{14}O_3Br_3NS$ (599.70)	42.02 41.36	2.33 2.03	2.33 2.71
3h	89-90	83	$C_{22}H_{16}O_4Br_3NS$ (629.70)	41.92 41.52	2.54 2.16	2.22 2.55
3i	80-81	86	$C_{21}H_{14}O_4Br_2N_2S$ (549.80)	43.83 43.94	2.55 2.32	5.09 5.47
3j	84-85	89	$C_{19}H_{13}O_3Br_2NS$ (494.80)	46.08 46.31	2.62 2.35	2.83 2.44

[a]*Recrystallization solvent was methanol*

Table 2: Spectral data of newly synthesized compounds

Compd.	FTIR (ν, cm^{-1})	^{1}H NMR (DMSO-d_6) (δ, ppm)	^{13}C NMR (DMSO-d_6) (δ, ppm)	$(M+H)^+$
3a	3372 (Ar-OH), 3289 (aliphatic-CH), 3059-3008 (Ar - CH), 1629 (C=N), 1575 (C=C), 862 (C-Br), 753 (C-Cl), 666 (C-S)	9.5-8.4 (2H, brs, Ar-OH), 7.86 (1H, s, Ar-H), 7.45-7.15 (8H, m, Ar-H), 4.98 (1H, t, -CH, *J* = 12.5 Hz), 3.22 (2H, d, $-CH_2$, *J* = 12.5 Hz)	169.50, 158.53, 157.32, 154.91, 140.44, 139.51, 133.57, 131.49, 129.93, 129.11, 128.07, 127.85, 126.50, 125.21, 124.62,116.94, 107.80, 94.5, 52.29, 48.63, 40.76	537.27
3b	3371 (Ar-OH), 3289 (aliphatic-CH), 3008- 3059 (Ar-CH), 2913 (CH_3), 1606 (C=N), 1581 (C=C), 862 (C-Br), 627 (C-S)	9.5-8.52 (2H, brs, Ar-OH), 7.76 (1H, s, Ar-H), 7.22-6.96 (8H, m, Ar-H), 5.21 (1H, t, -CH, *J* =12.3 Hz), 3.38 (2H, d, $-CH_2$, *J* =12.3 Hz), 2.63 (3H, s, $-CH_3$)	165.93, 161.93, 159.06, 157.53, 141.03, 139.52, 138.13, 131.65, 129.79, 129.32, 126.89, 125.23, 124.87, 117.43, 116.16, 107.91, 96.93, 95.42, 57.93, 55.84, 51.57, 21.18	516.78
3c	3373 (Ar-OH), 3291 (aliphatic-CH), 3060- 3009 (Ar- CH), 1606 (C=N), 1581 (C=C), 862 (C-Br), 665 (C-S)	9.75 (1H, s, Ar-OH), 9.15-8.24 (2H, brs, Ar-OH), 7.86 (1H, s, Ar-H), 7.76-7.15 (8H, m, Ar-H), 4.9 (1H, t, -CH, *J* =12.5 Hz), 3.35 (2H, d, $-CH_2$, *J* = 12.5 Hz)	169.01, 166.32, 162.52, 158.59, 157.42, 150.57, 140.62, 140.54, 131.49, 129.62, 129.21, 128.57, 126.50, 115.42, 111.31, 108.81, 94.60, 55.63, 54.20, 50.37, 40.65	518.82
3d	3373 (Ar-OH), 3291 (aliphatic-CH), 3060- 3009 (Ar-CH), 2848 (OCH_3), 1606 (C=N), 1581 (C=C), 862 (C-Br), 665 (C-S)	9.96 (1H, s, Ar-OH), 9.0-8.32 (2H, brs, Ar-OH), 7.82 (1H, s, Ar-H), 7.26-7.16 (7H, m, Ar-H), 5.26 (1H, t, -CH, *J* = 12.5 Hz), 3.87 (3H, s, $-OCH_3$), 3.37 (2H, d, $-CH_2$, *J* = 12.5 Hz)	163.53, 159.77, 156.67, 150.13, 145.00, 141.03, 131.93, 129.62, 128.09, 125.95,125.12 117.98, 115.37, 113.77 111.95, 108.51, 97.07, 56.47, 55.98, 50.98, 41.24, 37.02	548.77

3e	3377 (Ar-OH), 3298 (aliphatic-CH), 3061-3018 (Ar-CH), 2830 (OCH_3), 1611 (C=N), 1581 (C=C), 862 (C-Br), 671 (C-S)	9.05-8.34 (2H, brs, Ar-OH), 7.76 (1H, s, Ar-H), 7.41-7.19 (7H, m, Ar-H), 5.16 (1H, t, -CH, *J* = 12.3 Hz), 3.60 (2 x 3H, s, -OCH_3), 3.32 (2H, d, -CH_2, *J* = 12.3 Hz)	166.57, 163.07, 159.14, 159.96, 158.02, 139.93, 131.85, 129.78, 128.69, 128.64, 127.78, 125.23, 115.73, 113.74, 107.97, 98.72, 96.23, 58.03, 56.08, 50.92, 42.23, 35.40, 30.62	562.82
3f	3377 (Ar-OH), 3298 (aliphatic-CH), 3061-3018 (Ar-CH), 2830 (OCH_3), 1611 (C=N), 1581 (C=C), 862 (C-Br), 631 (C-S)	9.5-8.24 (2H, brs, Ar-OH), 7.76 (1H, s, Ar-H), 7.41-7.58 (6H, m, Ar-H), 5.06 (1H, t, -CH, *J* = 12.5 Hz), 3.88 (3 x 3H, s, -OCH_3), 3.32 (2H, d, -CH_2, *J* = 12.5 Hz)	168.91, 163.07, 159.26, 159.14, 158.42, 139.93, 136.15, 135.91, 133.43, 128.69, 128.45, 128.02, 127.78, 117.06, 115.73, 114.74, 107.97, 105.71, 95.23, 59.03, 56.08, 55.52, 54.18, 51.35	592.77
3g	3377 (Ar-OH), 3298 (aliphatic-CH), 3061-3018 (Ar-CH), 1611 (C=N), 1575 (C=C), 862 (C-Br), 671 (C-S)	9.92 (1H, s, Ar-OH), 9.35-8.31 (2H, brs, Ar-OH), 7.82 (1H, s, Ar-H), 7.67-6.99 (7H, m, Ar-H), 5.18 (1H, t, -CH, *J* =12.5 Hz), 3.52 (2H, d, -CH_2, *J* =12.5 Hz)	168.62, 164.03, 159.37, 157.47, 153.61, 141.03, 137.43, 135.85, 131.43, 130.96, 130.13, 129.62, 128.69, 126.93, 121.45, 117.98, 108.51, 97.07, 56.47, 55.98, 50.98	597.72
3h	3373 (Ar-OH), 3291 (aliphatic-CH), 3060-3009 (Ar-CH), 2848 (OCH_3), 1606 (C=N), 1571 (C=C), 862 (C-Br), 665 (C-S)	9.91 (1H, s, Ar-OH), 9.27-8.11 (2H, brs, Ar-OH), 7.82 (1H, s, Ar-H), 7.26-7.16 (6H, m, Ar-H), 5.26 (1H, t, -CH, *J*=12.4 Hz), 3.87 (3H, s, -OCH_3), 3.37 (2H, d, -CH_2, *J*=12.4 Hz)	166.43, 163.56, 160.25, 156.67, 150.13, 145.00, 142.13, 131.93, 129.62, 128.09, 122.35, 117.98, 116.37, 113.27, 111.95, 108.51, 98.17, 56.48, 55.98, 50.23, 42.32, 36.42	627.67
3i	3372 (Ar-OH), 3289 (aliphatic-CH), 3059-3008 (Ar-CH), 1606 (C=N), 1575 (C=C), 1515 (Asy Ar-NO_2), 1335 (Sym Ar-NO_2), 862 (C-Br), 666 (C-S)	9.73-8.47 (2H, brs, Ar-OH), 7.82 (1H, s, Ar-H), 7.59-6.85 (8H, m, Ar-H), 4.98 (1H, t, -CH, *J* = 12.5 Hz), 3.25 (2H, d, -CH_2, *J* = 125 Hz)	169.93, 158.56, 157.79, 154.30,146.49, 139.57, 136.56, 132.42, 129.53, 128.77, 127.61, 125.98, 119.27, 117.98, 116.85, 108.98, 96.32, 54.47, 51.52, 40.65, 37.10	547.82
3j	3372 (O-H stretching), 3289 (aliphatic-CH), 3059-3008 (Ar-CH), 1606 (C=N), 1575 (C=C), 862 (C-Br), 666 (C-S)	9.43-8.77 (2H, brs, Ar-OH), 7.79 (1H, s, Ar-H), 7.49-7.23 (7H, m, Ar-H), 5.14 (1H, t, -CH, *J*=12.5 Hz), 3.44 (2H, d, -CH_2, *J*=12.5 Hz)	160.77, 156.29, 154.10, 151.18, 148.72, 143.16, 141.97, 138.36, 131.97, 129.63, 127.41, 117.82, 112.34, 111.31, 109.63, 95.32, 56.73, 43.02, 36.20	492.77

Table 3: Comparison of reaction time and yield of synthesized 1, 5-benzothiazepine derivatives under MW and classical method

Entry	**Reaction time**		**Yield (%)**	
	MW (min)	**Classical (h)**	**MW**	**Classical**
3a	7	7.7	81	66
3b	6	6.6	83	68
3c	5	7	80	63
3d	7	8	84	68
3e	8	7.5	81	65

3f	7	6.5	85	71
3g	6	7.5	87	69
3h	7	8	83	70
3i	6	7.5	86	70
3j	7	6.5	89	68

MW-microwave

TABLE 4: *In vitro* anticancer screening of the synthesized compounds against human lung cell line (A549)

Compd.	Compound concentration (mol L^{-1})				GI_{50} (μ mol L^{-1})
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
	Control growth (%)[a]				
3a	100. 00±0.00	100.00±0.00	95.50±3.66	4.77±4.55	54.6
3b	100. 00±0.00	99.26±1.30	97.33±3.80	16.14±5.40	61.8
3c	100. 00±0.00	100.00±0.00	69.00±7.81	-13.70±11.00	41.4
3d	100. 00±0.00	100.00±0.00	100.00±0.00	7.43±0.90	56.9
3e	100. 00±0.00	100.0±0.00	99.67±0.59	10.43±4.38	58.6
3f	100.00±0.00	100.00±0.00	99.43±0.99	2.30±2.44	54.1
3g	99.60±.69	98.43±2.70	85.00±16.58	2.57±6.27	51.1
3h	100.00±0.00	100.00±0.00	87.63±21.44	24.27±64.92	66.1
3i	100.00±0.00	100.00±0.00	98.70±1.12	3.80±4.00	54.7
3j	100.00±0.00	100.00±0.00	100.00±0.00	-21.10±4.92	44.6
ADR	88.23±2.80	48.67±3.31	12.73±4.62	1.00±0.96	6.6

[a]*Mean ± SEM, n=3*
ADR=Adriamycin

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