ISSN 2515-8260 Volume 09, Issue 07, 2022

NOVEL 1, 5-BENZOTHIAZEPINES: SYNTHESIS, CHARACTERIZATION, AND CYTOTOXICITY STUDIES

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ABSTRACT:

Fifteen benzothiazepine compounds were synthesized and their cytotoxicity was studied because of the significance of the benzothiazepine pharmacophore. Benzothiazepines were synthesised by combined 0.01 mol of 1,3-substituted prop-2-en-1-one, 0.01 mol of 2-aminothiophenol, 1.25 ml of water, and a pinch of zinc acetate as a catalyst in a conical flask. The solvent free reaction mixture was heated in a microwave oven for 2-3 minutes at a temperature of 80-85^{O}C. Products were filtered, dried, and recrystallized in ethanol after being washed in water to remove the catalyst. The compounds' characterization was done using the IR, NMR and Mass spectroscopy. The in vivo cytotoxicity studies were performed using MTT assay method. Spectral data reveals the structure of the compound benzothiazepine. Of all the compounds tested against HT-29 cell lines, the compound BT-09 having a nitrophenyl moiety in its structure showed maximum activity with a IC_{50} value of 28 µg/mL. Among the compounds tested for cytotoxicity on MCF-7 cell lines, the compound

BT-09 showed maximum activity (IC_{50} 27 µg/mL). Among the compounds tested for cytotoxicity on DU-145 cell lines, the compounds, BT-09 and BT-11 showed maximum activity (IC_{50} 16 µg/mL). It was also observed that most of the compounds tested on these three cell lines showed maximum activity on prostate cancer cell lines (DU-145). The cytotoxic results showed that the compound BT-09 with a nitrophenyl moiety in its structure was the most effective against the HT-29 cell line, MCF-7 cell line, and DU-145 cell line, with an IC_{50} value of 28 g/mL, 27 g/mL, and 16 g/mL, respectively.

KEYWORDS:

Benzothiazepines, o-Amino thiophenol, Zinc acetate, Cytotoxicity, MTT assay

INTRODUCTION:

Synthetic medicinal chemists have access to a wide variety of privileged structures with diverse functional groups, and 1,5- benzothiazepines (BTZ) are an important part of one such privileged scaffold that has had a profound impact on the field. Diltiazem, Thiazesim, and Clentiazem are all examples of BTZs that are widely used to treat cardiovascular disorders today [1]. Since BTZ derivatives have been shown to be effective against a variety of target proteins, they have attracted a lot of interest as a potential new class of therapeutic leads [2]. The BTZ nucleus has been utilised as a cardiovascular modulator by serving as an antagonist on a variety of G-protein coupled receptors [3-4], including the antiarrhythmic (CCK) receptor [5-6], the angiotensin-converting enzyme and the angiotensin II receptor [7-8]. Recent reports have mentioned haemostatic effects, anti-cancer activity [9], spasmolytic activity [10], anti-ulcer activity, and a central nervous system depressant effect. Reportedly, stabilisation of the FKBP12 complex at the skeletal muscle channel-ryanodine receptor inhibits the growth receptor tyrosine kinase [11].

MATERIALS AND METHODS:

Materials:

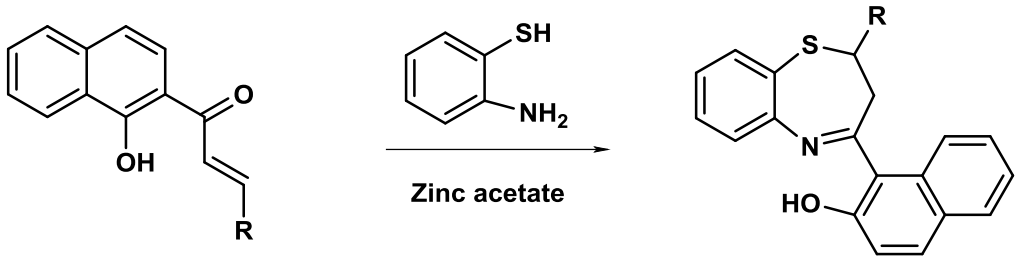
Analytical-grade organic solvents were used directly from the bottle, including methanol, ethanol, acetone, chloroform, and ethyl acetate. Some solvents came from local manufacturers, while others came from S.D. Fine Chem. Ltd in Mumbai, India. The synthesis employed only chemicals available from standard commercial sources. Silica gel-G (Merck grade) was used as the adsorbent in TLC, and the solvent systems used were hexane and ethyl acetate (50:50) for C1-C20 and hexane and ethyl acetate (70:30) for BT1-BT15; the plates were sprayed with 10% sulphuric acid and viewed under a UV lamp for analysis. A Carlo

Erba 1108 elemental analyzer was used for the tests. Elemental studies showed values for carbon, hydrogen, and nitrogen that were within 0.4% of the predicted range.

We procured the colon cancer (HT-29), breast cancer (MCF-7), and Prostate cancer (DU-145) cell lines from the National Centre for Cell Science (NCCS), Pune, India. Sigma chemicals were used to procure DMEM (Dulbeccos Modified Eagels Medium), MEM (Minimum Essential Media Eagle), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), Trypsin, EDTA (St.Louis, MO). Arrow Labs foetal bovine serum (FBS) and Tarson 96-well flat-bottom tissue culture plates were used for this experiment.

Methods:

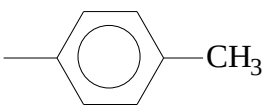
Synthesis of 2,3-dihydro-2-substituted-4(naphthalene-2-ol)-yl--1,5-benzothiazepines using potassium acetate as catalyst (BT-1 to BT-15)

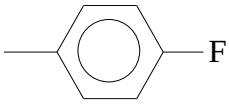
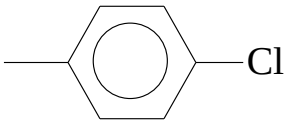
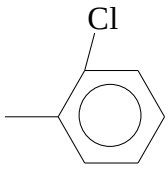
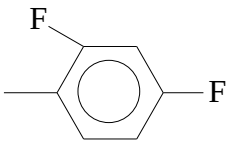
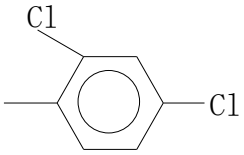
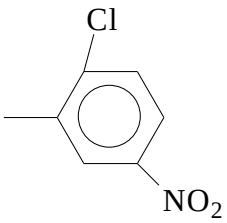
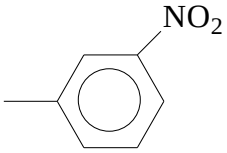
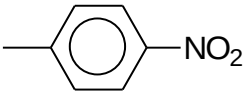


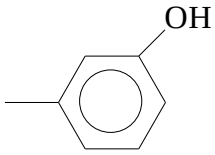
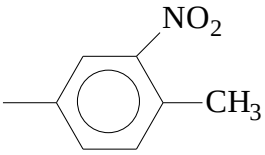
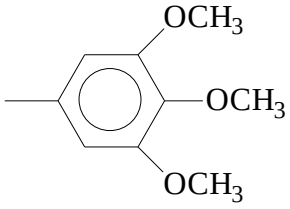
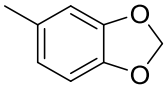
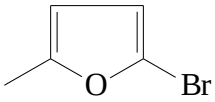
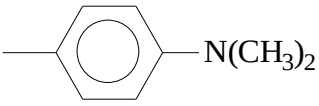
1-(1-Hydroxy-naphthalen-2-yl)-prop-2-ene-1-one 2,4-substituted-1,5-benzothiazepine

In a clean borosil beaker, we combined 0.01 mol of 1,3-substituted prop-2-en-1-one, 0.01 mol of 2-aminothiophenol, 1.25 ml of water, and a pinch of zinc acetate as a catalyst. The solvent-free reaction mixture was heated in a microwave oven for 2-3 minutes at a temperature of 80-85 degrees Celsius. After letting the reaction mixture cool to room temperature, cold water was poured and stirred vigorously to disperse the solids. Products were filtered, dried, and recrystallized in ethanol after being washed in water to remove the catalyst [12-13].

Table 1: Chemical Derivatives used for synthesis BT-1 to BT-15

Compound	R
BT-1	

BT-2	
BT-3	
BT-4	
BT-5	
BT-6	
BT-7	
BT-8	
BT-9	

BT-10	
BT-11	
BT-12	
BT-13	
BT-14	
BT-15	

Cytotoxic studies:

The MTT assay was used to measure the cytotoxicity of the test compounds (BT-01 to BT-15) in vitro. Mitochondrial succinate dehydrogenase activity is quantified in a colorimetric assay by monitoring the reduction of 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Within the mitochondria, the MTT is reduced to an insoluble, colored (dark purple) formazan product. Subsequently, the cells are solubilized with DMSO, and then released, solubilized formazan reagent is measured spectrophotometrically at 570 nm. Only metabolically active cells can reduce MTT, so the activity level is a proxy for cell viability. Dose-response curves can be generated to infer the efficacy of a given agent in causing cell death by comparing the amount of dark purple formazan produced by treated cells to the amount of formazan produced by untreated control cells

Maintenance of cell lines

Adherent cultures of HT-29 and DU-145 cell lines were maintained in DMEM, while MCF-7 cells were cultured in MEM media supplemented with 10% foetal bovine serum. Humidity and 5% carbon dioxide were used to maintain the culture.

Preparation of samples for cytotoxicity

We prepared 10 mg/mL DMSO stock solutions of each test compound (BT-01 to BT-15), then we diluted those solutions to final drug concentrations of 10, 50, 100, and 200 g/mL using sterile water.

Cytotoxicity evaluation

After 24 hours in the incubator, the cells were reseeded at a density of $1x10^4$ (counted using the Tryphan blue exclusion dye method) per well in 96-well plates. Fresh media containing various dilutions of the test compounds was added after incubation, and the previous medium was discarded. Afterward, they left the plates in a DMEM/MEM medium containing 10% FBS at 370 degrees for another 48 hours. After incubation, 90 µl of fresh DMEM devoid of FBS was added to the cells. Following incubation at 37^0C for 3-4 hours with 10 µl of MTT reagent (5 mg/mL of stock solution in DMEM without FBS), the above media was replaced by adding 200 µl of DMSO to each well (to dissolve the blue formazan crystals), and the wells were incubated for 10 minutes. The spectrophotometer reading for the absorbance at 570 nm was taken. The drug methotrexate was used as a standard for comparison purposes. The assay was repeated three times to ensure accuracy. The IC_{50} (ng/mL) value represents the concentration of the compound at which the proliferation rate of the tumour cells was inhibited by 50% compared to the untreated control cells and is a measure of the cytotoxicity. Values for the half-inhibitory concentration (IC_{50}) were calculated from the percentage inhibition versus concentration plot [14].

$$\% \text{ Inhibition at the given concentration} = 1 - \frac{(\text{Absorbance average})}{(\text{Control absorbance average})} \times 100$$

IC_{50}=Inv.log (50-c) / m;

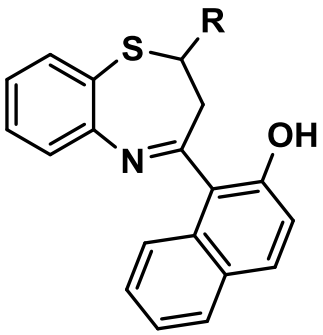
c and m derived from y = mx+c of plot of percentage inhibition Vs log C.

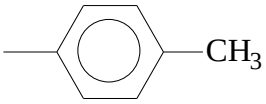
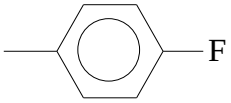
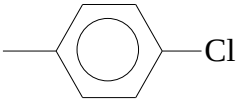
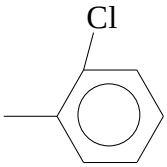
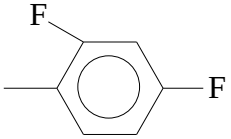
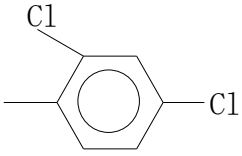
RESULTS AND DISCUSSION:

Results:

Physicochemical data, elemental analysis, spectral analysis and cytotoxicity evaluation results were given below.

Table 2: Physical data for BT-01 to BT-15



Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (^{o}C)	Yield %
BT-1		$C_{26}H_{21}NOS$	395.52	162-164	89
BT-2		$C_{25}H_{18}FNOS$	399.48	176-178	89
BT-3		$C_{25}H_{18}ClNOS$	415.93	160-162	93
BT-4		$C_{25}H_{18}ClNOS$	415.93	140-142	71
BT-5		$C_{25}H_{17}F_2NOS$	417.47	158-160	75
BT-6		$C_{25}H_{17}Cl_2NOS$	450.38	136-138	86

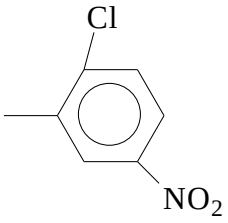
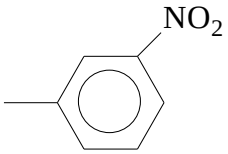
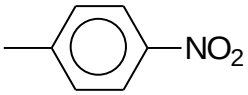
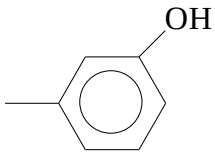
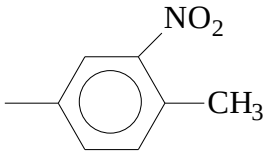
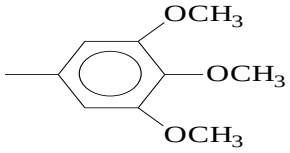
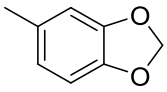
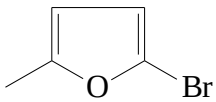
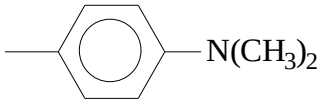
BT-7		$C_{25}H_{17}ClN_2O_3S$	460.93	184-186	77
BT-8		$C_{25}H_{18}N_2O_3S$	426.49	168-170	82
BT-9		$C_{25}H_{18}N_2O_3S$	426.49	150-152	89
BT-10		$C_{25}H_{19}NO_2S$	397.49	246-248	84
BT-11		$C_{26}H_{20}N_2O_3S$	440.51	196-198	94
BT-12		$C_{27}H_{24}N_2OS$	471.57	168-170	85
BT-13		$C_{26}H_{19}NO_3S$	425.5	176-178	74
BT-14		$C_{23}H_{16}BrNO_2S$	450.35	152-154	79
BT-15		$C_{27}H_{24}N_2OS$	424.56	134-136	88

Table 3: Elemental Analysis of BT-01 to BT-15

Compound	%Found							
	C	H	N	O	S	F	Cl	Br
BT-1	78.95	5.35	3.54	4.05	8.11	-	-	-
BT-2	75.16	4.54	3.51	4.61	8.03	4.76	-	-
BT-3	72.19	4.36	3.37	3.85	7.71	-	8.52	-
BT-4	72.19	4.36	3.37	3.85	7.71	-	8.52	-
BT-5	71.93	4.10	3.36	3.83	7.68	9.10	-	-
BT-6	66.67	3.80	3.11	3.55	7.12	-	15.74	-
BT-7	65.14	3.72	6.08	10.41	6.96	-	7.69	-
BT-8	70.40	4.25	6.57	11.25	7.52	-	-	-
BT-9	70.40	4.25	6.57	11.25	7.52	-	-	-
BT-10	75.54	4.82	3.52	8.05	8.07	-	-	-
BT-11	70.89	4.58	6.36	10.90	7.28	-	-	-
BT-12	71.32	5.34	2.97	13.57	6.80	-	-	-
BT-13	73.39	4.50	3.29	11.28	7.54	-	-	-
BT-14	61.34	3.58	3.11	7.11	7.12	-	-	17.74
BT-15	76.38	5.70	6.60	3.77	7.55	-	-	-

Spectral analysis:

1-(2,3-dihydro-2-p-tolylbenzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-01)

IR:1584 (C=N), 1506 (C=C), 1397 (C-N) and 655 (C-S), H^1NMR : 4.93 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.26 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.9 Hz, 1H, C3-H-3a), 3.05 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 2.41 (3H, s, Ar-CH3), 7.23 (1H, s, Ar-H), 7.62 (3H, m, Ar-H), 7.21-8.11 (7H, Ar-H).m/z value: 596.69

1-(2-(4-fluorophenyl)-2,3-dihydrobenzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-02)

IR: 1624 (C=N), 1510 (C=C),1394 (C-N), 687 (C-S) and 1245 (C-F): H^1NMR : 5.28 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.52 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.6 Hz, 1H, C3-H-3a), 2.98 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.07 (1H, s, Ar-H), 7.18 (3H, m, Ar-H), 7.20-8.09 (7H, Ar-H).m/z value: 399.10

1-(2-(4-chlorophenyl)-2,3-dihydrobenzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-03)

IR**:** 1596 (C=N), 1503 (C=C), 1385 (C-N), 779 (C-Cl) and 668 (C-S): H^1NMR: 5.1 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.54 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.9 Hz, 1H, C3-H-3a), 3.38 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.27 (1H, s, Ar-H), 7.67 (3H, m, Ar-H), 7.23-8.09 (7H, Ar-H): m/z value: 415.08

1-(2-(2-chlorophenyl)-2,3-dihydrobenzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-04)

IR: 1597 (C=N), 1511 (C=C), 1366 (C-N), 689 (C-S) and 806 (C-Cl) : H^1NMR : 4.88 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.44 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.6 Hz, 1H, C3-H-3a), 3.37 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.13 (1H, s, Ar-H), 7.73 (3H, m, Ar-H), 6.95-7.60 (7H, Ar-H).:m/z value: 415.02

1-(2-(2,4-difluorophenyl)-2,3-dihydrobenzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-05)

IR: 1612 (C=N), 1501 (C=C),1382 (C-N), 689 (C-S) and 944 (C-F): H^1 NMR: 5.31 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.36 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.9 Hz, 1H, C3-H-3a), 2.87 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.08 (1H, s, Ar-H), 7.30 (3H, m, Ar-H), 6.98-8.12 (6H, Ar-H):m/z value:447.58

1-(2-(2,4-dichlorophenyl)-2,3-dihydrobenzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-06)

IR: 1593 (C=N), 1502 (C=C), 1382 (C-N), 687 (C-S) and 805 (C-Cl): H^1 NMR : 5.10 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.27 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.6 Hz, 1H, C_3-H-3a), 2.66 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.15 (1H, s, Ar-H), 7.20 (3H, m, Ar-H), 7.05-7.95 (6H, Ar-H). m/z value:449.62

1-(2-(2-chloro-5-nitrophenyl)-2,3-dihydrobenzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-07)

IR: 1588 (C=N), 1520 (N=O, asymmetric), 1505 (C=C), 1382 (C-N), 1340 (N=O, symmetric), 656 (C-S), 933 (C-F) and 781 (C-Cl): H^1NMR : 4.32 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.74 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.9 Hz, 1H, C3-H-3a), 3.51 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.09 (1H, s, Ar-H), 7.12 (3H, m, Ar-H), 6.98-8.10 (6H, Ar-H).m/z value:458.19

1-(2,3-dihydro-2-(3-nitrophenyl) benzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-08)

IR: 1580 (C=N), 1522 (N=O, asymmetric), 1501 (C=C), 1385 (C-N), 1345 (N=O, symmetric) and 689 (C-S); H^1NMR : 5.42 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.38 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.6 Hz, 1H, C3-H-3a), 2.86 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.30 (1H, s, Ar-H), 7.80 (3H, m, Ar-H), 7.48-8.60 (7H, Ar-H).m/z value:425.06

1-(2,3-dihydro-2-(4-nitrophenyl) benzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-09)

IR: 1586 (C=N), 1515 (N=O, asymmetric), 1506 (C=C), 1380 (C-N), 1338 (N=O, symmetric) and 713 (C-S) H^1NMR: 5.42 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.47 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.7 Hz, 1H, C3-H-3a), 3.10 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.18 (1H, s, Ar-H), 7.25 (3H, m, Ar-H), 7.25-8.20 (7H, Ar-H). m/z value:425.19

1-(2,3-dihydro-2-(3-hydroxyphenyl) benzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-10)

IR: 1653 (C=N), 1528 (C-N), 1502 (C=C) and 694 (C-S): H^1NMR : 3.85 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.34 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.0 Hz, 1H, C3-H-3a), 2.41 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 7.25 (1H, s, Ar-H), 7.30 (3H, m, Ar-H), 7.15-7.80 (7H, Ar-H), 6.85 (1H, s, Ar-OH).m/z value: 396.25

1-(2,3-dihydro-2-(4-methyl-3-nitrophenyl) benzo[b][1,4] thiazepin-4-yl)naphthalen-2-ol (BT-11)

IR: 1642 (C=N), 1548 (N=O, asymmetric), 1510 (C=C), 1380 (C-N), 1338 (N=O, symmetric) and 668 (C-S) H^1NMR : 4.16 (dd, J2,3a = 5.1 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.23 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.9 Hz, 1H, C3-H-3a), 2.53 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 2.50 (3H, s, Ar-CH3), 7.30 (1H, s, Ar-H), 6.70 (3H, m, Ar-H), 7.45-8.78 (6, Ar-H) m/z value:439.59

1-(2,3-dihydro-2-(3,4,5-trimethoxyphenyl)benzo[b][1,4]thiazepin-4-yl)naphthalen-2-ol (BT-12)

IR: 1648 (C=N), 1505 (C=C), 1365 (C-N), 1225 (-O-CH3) and 678 (C-S):H^1NMR: 3.06 (dd, $J_{2,3a}$ = 5.3 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 2.83 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C_3-H-3a), 2.0 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.22 (1H, s,Ar-H), 6.60 (3H, m, Ar-H), 7.30-7.50 (5H, Ar-H), 3.70 (3H, s, Ar-OCH_3), 3.88 (6H, s, 2Ar-OCH_3) m/z value:479.25

1-(2-(benzo[d][1,3]dioxol-6-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)naphthalen-2-ol (BT-13)

IR: 1592 (C=N), 1502 (C=C), 1370 (C-N), 1232 (-O-CH_2-O-), and 689 (C-S)H^1NMR: 4.94 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.25 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.1 Hz, 1H, C_3-H-3a), 3.14 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.25 (1H, s,Ar-H), 7.40 (3H, m, Ar-H), 6.10 (2H, s, O-CH_2-O), 7.21-7.85 (6H, Ar-H) m/z value:425.05

1-(2-(5-bromofuran-2-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)naphthalen-2-ol (BT-14)

IR: 1602 (C=N), 1505 (C=C), 1340 (C-N), 664 (C-S) and 790 (C-Br) :H^1NMR: 5.07 (dd, $J_{2,3a}$ = 5.3 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 4.10 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.2 Hz, 1H, C_3-H-3a), 3.39 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.10 (1H, s,Ar-H), 6.80 (3H, m, Ar-H), 6.80-7.30 (5H, Ar-H): m/z value:450.05

1-(2-(4-(dimethylamino)phenyl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)naphthalen-2-ol (BT-15)

IR: H^1NMR: 4.96 (dd, J2,3a = 5.3 Hz, J2,3b = 12 Hz, 1H, C2-H), 3.83 (dd, J3a,3b = 14.4 Hz, J3a,2 = 9.2 Hz, 1H, C3-H-3a), 3.26 (t, J3b,3a = J3b,2 = 12.9 Hz, 1H, C3-H-3b), 3.20 (6H, s, N-(CH3)2, 7.20 (1H, s,Ar-H), 7.45 (3H, m, Ar-H), 6.70-8.20 (7H, Ar-H) m/z value:424.12" [18].

Cytotoxic Analysis:

Table 4: cytotoxic results for benzothiazepines (BT-01 to BT-15)

Compound	R	Cell line		
		HT-29	MCF-7	DU-145
BT-1	4"-methylphenyl	56 ± 2	62 ± 2	56 ± 2
BT-2	4"-fluorophenyl	148 ± 2	188 ± 2	105 ± 2
BT-3	4"-chlorophenyl	92 ± 2	74 ± 1	65 ± 2
BT-4	2"-chlorophenyl	42 ± 2	42 ± 2	33 ± 2
BT-5	2",4"-difluorophenyl	182 ± 1	NA	148 ± 1
BT-6	2",4-dichlorophenyl	42 ± 2	48 ± 1	46 ± 2
BT-7	2"-chloro-5"-nitrophenyl	180 ± 2	NA	122 ± 2
BT-8	3"-nitrophenyl	55 ± 2	58 ± 1	52 ± 1
BT-9	4"-nitrophenyl	28 ± 1	27 ± 1	16 ± 1
BT-10	3"-hydroxyphenyl	105 ± 2	78 ± 2	68 ± 2
BT-11	3"-nitro-4"-methylphenyl	36 ± 2	28 ± 1	16 ± 2
BT-12	3",4",5"-trimethoxypheny	NA	NA	NA
BT-13	3",4"-methylendioxyphenyl	123 ± 2	129 ± 2	92 ± 2
BT-14	5"-bromofuran-2"-yl	155 ± 1	NA	110 ± 2
BT-15	4"-dimethylaminophenyl	NA	NA	NA
Methotrexate		12 ± 1	9 ± 1	5 ± 1

The data (n=3) is displayed as a mean ± SD. IC50 > 200 g/mL = No Activity.

Discussion:

The HT-29 cell lines were most sensitive to the nitrophenyl moiety-containing compound BT-09 (IC_{50} 28 g/mL). Compounds with 3-methyl-4-phenyl moieties include BT-11 (IC_{50} 36 g/mL), chlorophenyl moieties comprise BT-04 and BT-06 (IC_{50} 42 g/mL), nitrophenyl moieties comprise BT-08 (IC_{50} 55 g/mL), and methylphenyl moieties comprise BT-01 (IC_{50} 56 g/mL). There was activity with the other compounds, but the IC_{50} values were higher. Maximum cytotoxicity (IC_{50} 27 g/mL) was observed for compound BT-09 among those tested against MCF-7 cell lines. Subsequently, the compounds BT-11 (IC_{50} 28 g/mL), BT-04 (IC_{50} 42 g/mL), and BT-06 (IC_{50} 48 g/mL) were tested. The cytotoxicity of all the other compounds was only detectable at elevated concentrations. Compounds BT-09 and BT-11 demonstrated the highest cytotoxic activity (IC_{50} 16 g/mL) in tests with DU-145 cell lines. A 33 g/mL IC_{50} was found for compound BT-04, followed by 46 g/mL for BT-06 with a dichlorophenyl moiety, 52 g/mL for BT-08, and 56 g/mL for BT-01. In addition, it was found that most of the compounds tested exhibited the highest activity against prostate cancer cell lines, out of the three cell lines used (DU-145).

CONCLUSION:

Based on the cytotoxic results, the compound BT-09 with a nitrophenyl moiety in its structure was the most active against HT-29 cell lines, MCF-7 cell lines, and DU-145 cell lines, with an IC_{50} value of 28 g/mL, 27 g/mL, and 16 g/mL respectively.

ACKNOWLEDGEMENT:

Authors are thankful to Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, for providing necessary facilities for this research work.

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