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*Research Article*

# NEWER APPLICATIONS OF BENZOTHIAZEPINES AS POTENTIAL CYTOTOXIC AGENTS

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**ABSTRACT**

Benzothiazepines are synthesized by conventional synthesis method. The compounds have been screened for Cytotoxic activity. Tested compounds were prepared by the reactions between 1,3-diarylprop-2-enones with orthoamino thio phenol. All the products were tested for purity by TLC and charecterised by elemental analysis and different spectroscopic methods.

**Keywords**: 4-Chloroacetophenone, Benzothiazepine, 2-Aminothiophenol, piperidine.

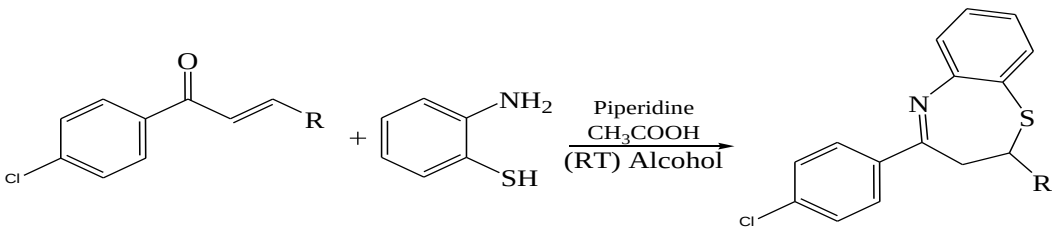
## INTRODUCTION

The benzothiazepines are important nitrogen- and sulfur-containing seven-membered heterocyclic compounds in drug research since they possess diverse bioactivities. Benzothiazepines are the most well-known representatives of benzologs of 1, 4-thiazepine and one of the three possible benzo-condensed derivatives, viz. 1,4-and 1,5-benzothiazepines.The benzothiazepine derivatives are of particular interest for lead discovery because they have been found active against different families of targets. The first molecule of benzothiazepine used clinically was diltiazem, followed by clentiazem, for their cardiovascular action. Therefore, the benzothiazepines are useful compounds in the drug research which has stimulated the invention of a wide range of synthetic methods for their preparation and chemical transformations.

## MATERIALS AND METHODS

### Procedure for Synthesis of 1, 5-Benzothiazepines

Chalcones of 4-Chloroacetophenone (1 mill mole) and O-Amino thiophenol (1 mill mole) was dissolved in 10 ml of boiling methanol the heat was removed and piperidine (2 drops) was added. After the mixture had cooled to room temperature the additional 10 ml of methanol was added and heated until the slurry was dissolved. Then add 1 ml of Glacial acetic acid and allow the mixture at 250C for overnight. The yellow color crystals benzothiazepine was separated out. This was recrystallized with methanol and filtered. The scheme and physical characterization data will be given below.




**Chalcone derivative** **2-Aminothiophenol** **Benzothiazepine derivative**

**Table 1: Physical characterization data of benzothiazepines ($BP_1$-$BP_{12}$)**

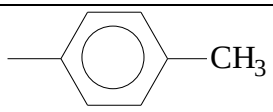
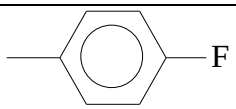
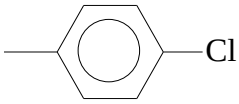
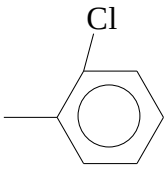
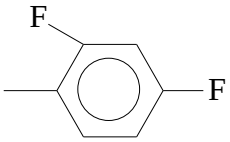
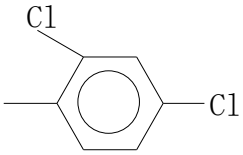
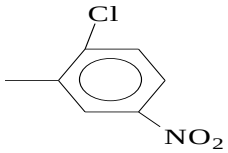
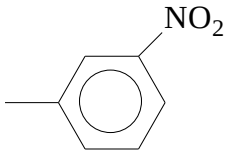
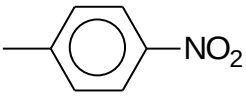
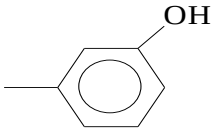
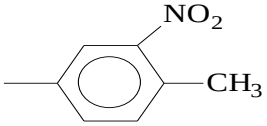
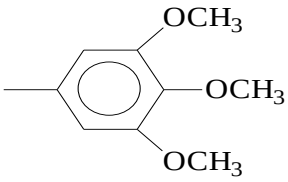
| Compound | R | Molecular Formula | Relative Molecular Mass (RMM) | Melting Point (°C) | Yield % |
|---|---|---|---|---|---|
| $BP_1$ |  | $C_{22}H_{18}ClNS$ | 363 | 140-143 | 89 |
| $BP_2$ |  | $C_{21}H_{15}ClFNS$ | 367 | 153-154 | 89 |
| $BP_3$ |  | $C_{21}H_{15}Cl_2NS$ | 383 | 143-145 | 93 |
| $BP_4$ |  | $C_{21}H_{15}Cl_2NS$ | 383 | 120-123 | 71 |
| $BP_5$ |  | $C_{21}H_{14}ClF_2NS$ | 385 | 138-141 | 75 |
| $BP_6$ |  | $C_{21}H_{14}Cl_3NS$ | 416 | 117-120 | 86 |
| $BP_7$ |  | $C_{21}H_{14}Cl_2N_2O_2S$ | 428 | 164-167 | 77 |
| $BP_8$ |  | $C_{21}H_{15}ClN_2O_2S$ | 394 | 142-145 | 82 |
| $BP_9$ |  | $C_{21}H_{15}ClN_2O_2S$ | 394 | 130-131 | 89 |
| $BP_{10}$ |  | $C_{21}H_{16}ClNOS$ | 365 | 226-229 | 84 |
| $BP_{11}$ |  | $C_{22}H_{17}ClN_2O_2S$ | 408 | 176-179 | 94 |
| $BP_{12}$ |  | $C_{24}H_{22}ClNO_3S$ | 439 | 148-151 | 85 |

**Table 2: IR spectral data (KBr disc) of benzothiazepines ($BP_1$-$BP_{12}$)**

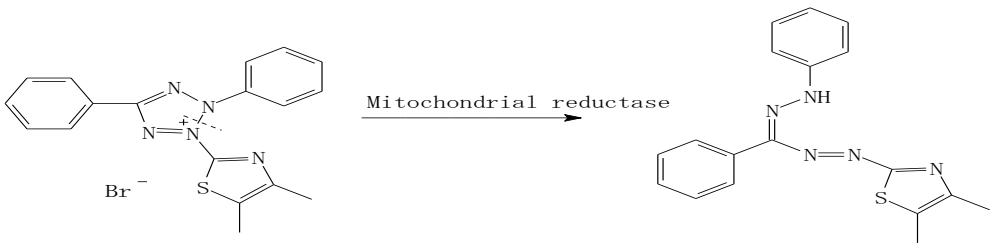
| Compound | Position of absorption band ($cm^{-1}$) |
|---|---|
| $BP_1$ | 1585 (C=N), 1505 (C=C), 1395 (C-N), 823 (C-Cl) and 654 (C-S). |
| $BP_2$ | 1625 (C=N), 1509 (C=C),1399 (C-N), 689 (C-S) and 831 (C-Cl) |
| $BP_3$ | 1595 (C=N ), 1502 (C=C), 1384 ( C-N), 778 (C-Cl), 821 (C-Cl) and 667 (C-S) |
| $BP_4$ | 1596 (C=N), 1510 (C=C), 1365 (C-N), 688 (C-S), 823 (C-Cl) and 805 (C-Cl) |
| $BP_5$ | 1612 ( C=N), 1501 ( C=C),1382 (C-N), 689 (C-S), 813 (C-Cl) and 944 (C-F) |
| $BP_6$ | 1593 ( C=N), 1502 ( C=C), 1382 ( C-N), 687 (C-S) and 925 (C-Cl) |
| $BP_7$ | 1588 (C=N), 1520 (N=O, asymmetric), 1505 ( C=C), 1382 ( C-N), 1340 (N=O, symmetric), 656 ( C-S) and 833 (C-Cl) |
| $BP_8$ | 1580 (C=N), 1522 (N=O, asymmetric), 1501 ( C=C), 1385 ( C-N), 1345 (N=O, symmetric), 824 (C-Cl) and 689 ( C-S) |
| $BP_9$ | 1586 (C=N), 1515 (N=O, asymmetric), 1506 ( C=C), 1380 ( C-N), 1338 (N=O, symmetric), 825 (C-Cl) and 713 ( C-S) |
| $BP_{10}$ | 1653 ( C=N), 1528 ( C-N), 1502 (C=C), 825 (C-Cl) and 694 (C-S) |
| $BP_{11}$ | 1642 ( C=N), 1548 (N=O, asymmetric), 1510 (C=C), 1380 (C-N), 1338 (N=O, symmetric), 827 (C-Cl) and 668 (C-S) |
| $BP_{12}$ | 1648 ( C=N), 1505 ( C=C), 1365 (C-N), 1225 (-O-$CH_3$), 823 (C-Cl) and 678 (C-S) |

**Table 3: $^1$H NMR spectral data of benzothiazepines ($BP_1$ – $BP_{10}$)**

| Compound | Chemical shift ( δ ) in ppm |
|---|---|
| **BP1** | 4.94 (dd, *J2,3a* = 5.1 Hz, *J2,3b* = 12 Hz, 1H, C2-H), 3.25 (dd, *J3a,3b* = 14.4 Hz, *J3a,2* = 9.9 Hz, 1H, C3-H-3a), 3.04 (t, *J3b,3a* = *J3b,2* = 12.9 Hz, 1H, C3-H-3b), 2.40 (3H, s, Ar-CH3), 7.22 (1H, s, Ar-H), 7.61 (3H, m, Ar-H), 7.20-8.10 (7H, Ar-H). |
| **BP2** | 5.27 (dd, *J2,3a* = 5.1 Hz, *J2,3b* = 12 Hz, 1H, C2-H), 3.50 (dd, *J3a,3b* = 14.4 Hz, *J3a,2* = 9.6 Hz, 1H, C3-H-3a), 2.97 (t, *J3b,3a* = *J3b,2* = 12.9 Hz, 1H, C3-H-3b), 7.05 (1H, s, Ar-H), 7.19 (3H, m, Ar-H), 7.20-8.09 (7H, Ar-H). |
| **BP3** | 5.0 (dd, *J2,3a* = 5.1 Hz, *J2,3b* = 12 Hz, 1H, C2-H), 3.53 (dd, *J3a,3b* = 14.4 Hz, *J3a,2* = 9.9 Hz, 1H, C3-H-3a), 3.39 (t, *J3b,3a* = *J3b,2* = 12.9 Hz, 1H, C3-H-3b), 7.25 (1H, s, Ar-H), 7.65 (3H, m, Ar-H), 7.22-8.08 (7H, Ar-H). |
| **BP4** | 4.89 (dd, *J2,3a* = 5.1 Hz, *J2,3b* = 12 Hz, 1H, C2-H), 3.43 (dd, *J3a,3b* = 14.4 Hz, *J3a,2* = 9.6 Hz, 1H, C3-H-3a), 3.36 (t, *J3b,3a* = *J3b,2* = 12.9 Hz, 1H, C3-H-3b), 7.12 (1H, s, Ar-H), 7.72 (3H, m, Ar-H), 6.95-7.60 (7H, Ar-H). |
| **BP5** | 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). |
| **BP6** | 5.10 (dd, *J2,3a* = 5.1 Hz, *J2,3b* = 12 Hz, 1H, C2-H), 3.27 (dd, *J3a,3b* = 14.4 Hz, *J3a,2* = 9.6 Hz, 1H, C3-H-3a), 2.66 (t, *J3b,3a* = *J3b,2* = 12.9 Hz, 1H, C3-H-3b), 7.15 (1H, s, Ar-H), 7.20 (3H, m, Ar-H), 7.05-7.95 (6H, Ar-H). |
| **BP7** | 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). |
| **BP8** | 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). |
| **BP9** | 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). |
| **BP10** | 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). |
| **BP11** | 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) |
| **BP12** | 3.06 (dd, *J2,3a* = 5.3 Hz, *J2,3b* = 12 Hz, 1H, C2-H), 2.83 (dd, *J3a,3b* = 14.4 Hz, *J3a,2* = 9.9 Hz, 1H, C3-H-3a), 2.0 (t, *J3b,3a* = *J3b,2* = 12.9 Hz, 1H, C3-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-OCH3), 3.88 (6H, s, 2XAr-OCH3) |

**CYTOTOXICITY STUDIES**

The *in vitro* cytotoxicity of the test compounds (**B1 to B12**) was evaluated by the MTT assay. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passess into the mitrochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. When the amount of dark purple formazan produced by the cells is treated with a agent compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced through the production of a dose-response curve.




**MTT** **Purple coloured complex**

**REDUCTION OF MTT**

**MATERIALS**

HT-29 (colon cancer), MCF-7 (breast cancer) and DU- 145 (prostate cancer) cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagels Medium), MEM (Minimum Essential Media Eagle), MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, EDTA were purchased from Sigma chemicals (St.Louis,MO). Fetal bovine serum (FBS) was purchased from Arrow Labs, 96 well flat bottom tissue culture plates were purchased from Tarson.

**METHOD**

**a) Maintainence of cell lines**

HT-29 and DU-145 cell lines were grown as adherent in DMEM media, whereas MCF-7 was grown in MEM media supplemented with 10% fetal bovine serum. The cultured was maintained in a humidified atmosphere with 5% CO2.

**b) Preparation of samples for cytotoxicity**

Stock solutions of test compounds (B1 to B25**)** were prepared (10 mg/mL) in DMSO and from them various dilutions were made with sterile water to get the final drug concentrations of 10, 50, 100 and 200 mg/mL.

**c) Cytotoxicity evaluation**

The cells were seeded in 96 well plates at a density of 1x104 (counted by Tryphan blue exclusion dye method) per well and were incubated for 24 h to recover. After incubation the medium was replaced with fresh media containing different dilutions of the test compounds. Then the plated were incubated for additional 48 h at 370C in DMEM/MEM with 10% FBS medium. Following incubation, the medium was removed and replaced with 90 µl of fresh DMEM without FBS. To the above wells, 10 µl of MTT reagent (5 mg/mL of stock solution in DMEM without FBS) was added and incubated at 370C for 3-4 h, there after the above media was replaced by adding 200 µl of DMSO to each well (to dissolve the blue formazan crystals) and incubated at 370C for 10 min. The absorbance at 570 nm was measured on a spectrophotometer.

Methotrexate was used as reference drug for comparison. Assay was performed in triplicate for three independent determinations. The cytotoxicity was expressed as IC50 (µg/mL) which is the concentration of the compound that inhibited proliferation rate of the tumor cells by 50% as compared to the control untreated cells. IC50 values were determined from the plot: % inhibition versus concentration.

**% inhibition at the given concentration =**

$$\frac{\text{1- (Absorbance average)}}{\text{(Control absorbance average)}} \times 100$$

IC50=Inv.log(50-c) / m; c and m derived from y=mx+c of plot of % inhibition Vs log C. The results of the compounds are shown in table 4.

**Table 4: Cytotoxicity of the new benzothiazepines ($BP_1$ to $BP_{11}$):($IC_{50}$ values in µg/mL)**

| Compound | R | Cell line | | |
|---|---|---|---|---|
| | | HT-29 | MCF-7 | DU-145 |
| $BP_1$ | 4"-methyl phenyl | 55 ± 2 | 62 ± 2 | 52 ± 1 |
| $BP_2$ | 4"-fluorophenyl | 42 ± 2 | 48 ± 1 | 62 ± 2 |
| $BP_3$ | 4"-chlorophenyl | 92 ± 2 | 78 ± 2 | 65 ± 2 |
| $BP_4$ | 2"-chlorophenyl | 105 ± 2 | 168 ± 1 | 122 ± 2 |
| $BP_5$ | 2",4"-difluorophenyl | 28 ± 1 | 42 ± 2 | 33 ± 2 |
| $BP_6$ | 2",4"-dichlorophenyl | 42 ± 2 | 67 ± 1 | 56 ± 2 |
| $BP_7$ | 2"-chloro-5"-nitrophenyl | 115 ± 2 | NA | NA |
| $BP_8$ | 3"-nitrophenyl | 180 ± 2 | NA | NA |
| $BP_9$ | 4"-nitrophenyl | 155 ± 1 | NA | 105 ± 2 |
| $BP_{10}$ | 3"-hydroxyphenyl | 148 ± 2 | 129 ± 2 | 155 ± 1 |
| $BP_{11}$ | 3"-nitro-4"methylphenyl | 64 ± 2 | 58 ± 1 | 46 ± 2 |
| $BP_{12}$ | 3",4",5"-trimethoxyphenyl | 132 ± 2 | NA | 93 ± 2 |
| **Methotrexate** | | 11 ± 1 | 9 ± 1 | 6 ± 1 |

**CONCLUSION**

All the synthesized benzothiazepines have been evaluated for their cytotoxicity against HT-29 (colon cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines. Methotrexate was used as the reference standard. The results clearly revealed that most of the 1,5-benzothiazepines possessed cytotoxic activity as evidenced by the IC50 values and is much higher than that of the chalcones indicating the positive contribution of benzothiazepine nucleus in enhancing the cytotoxic activity. Infact, a number of anticancer drugs being used currently possessed benzothiazepine nucleus as part of their structures.

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