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SYNTHESIS, CHARACTERIZATION AND ANTI BACTERIAL AND CYTOTOXIC STUDIES OF NOVEL 1, 5 BENZOTHIAZEPINES FROM CHALCONES OF 2, 4 DI FLUORO ACETOPHENONE

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ABSTRACT

1, 5 Benzothaizepines heterocyclic ring system having the diverse pharmacological activities. The present work focus on synthesis of novel Benzothaizepines molecules by condensation of 1- (2', 4'-difluorophenyl) -3- (4''-methyl phenyl) -2-propen-1-one derivatives and O-amino thiophenol in the presence Piperidine and glacial acetic acid. The structures of compounds were confirmed by spectral analysis using IR, 1HNMR and Mass analysis. The biological evolution of compounds was performed for anti-microbial activity by using serial dilution method and cytotoxicity studies by MTT assay method.

KEYWORDS

Chalcones, Antimicrobial Activity, Antifungal activity and Cytotoxicity.

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INTRODUCTON

The Benzothiazepines[1-6] (1 and 2) are important nitrogen and sulfur-containing seven-membered heterocyclic compounds in drug research since they possess diverse bioactivities[7-14]. 1, 5-Benzothiazepines are the most well-known representatives of benzologs of 1, 4-thiazepine (3) and one of the three possible benzo-condensed derivatives, viz. 1, 4- (4), 4, 1- (5) and 1, 5-benzothiazepines[15-18].

The 1, 5-benzothiazepine derivatives are of particular interest for lead discovery because they have been found active against different families of

targets[19-24]. The first molecule of 1, 5-benzothiazepine used clinically was diltiazem (6), followed by clentiazem (7), for their cardiovascular action. Some of the 1, 5-benzothiazepine derivatives were also used clinically for CNS disorders (8), clothiapine (9) and quetiapine (10). Therefore, the 1, 5-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[25-45].

The importance of the l, 5-benzothiazepine nucleus has been well established as illustrated by a large number of compounds which have been patented as chemotherapeutic agents[46]. A number of biological activities have been associated with it, such as antifeedant[47], coronary vasodilatory[48], tranquilizer[49], antidepressant[50], CNS stimulant[51], antihypertensive[52], calcium channel blocker[53], antiulcer[54], calcium antagonist[55], antimicrobial[56] and anticonvulsant agents[57]. l, 5-Benzothiazepine molecules have been found to be useful in mucosal blood flow, as antiulcer and gastric secretion inhibitor. Recently, anticancer activities[58], hemodynamic effects[59], and spasmolytic activities[60] have also been reported.

Keeping this broad spectrum of biological activities in mind, in the present investigation it has been considered worthwhile to synthesize Benzothiazepines from chalcones derivatives. The compounds were characterized by H^1 NMR and IR analysis. The compounds were tested for their antimicrobial activity by standard protocols.

EXPERIMENTAL WORK[61-62]

SCHEME OF SYNTHESIS

Synthesis of benzothiazepines from chalcones obtained from 2, 4-difluoroacetophenone (Scheme-12).

General procedure for the synthesis of benzothiazepines

To a solution of chalcone derivative in dry acidic methanol acidified by adding few drops of glacial acetic acid to it, 2-aminothiophenol was added. The mixture was then refluxed until a crystalline solid sepearates out. After cooling, the solid product was collected and washed with diethyl ether and cold methanol. The crude solid was recrystallized from ethanol.

Spectral data for synthesised 1, 5 Benzothiazepines: B_1-B_{10}

2, 3- Dihydro-2- (4-methylphenyl) -4- (2, 4-difluorophenyl) -1, 5- benzothiazepine (BP_1)

IR (KBr) (cm^{-1}): 1585 (C=N), 1505 (C=C), 1395 (C-N), 923 (C-F) and 654 (C-S)., ^{1}H-NMR ($CDCl_3$) ppm: 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, $J_{3a,2}$ = 9.9 Hz, 1H, C_3-H-3a), 3.04 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 2.40 (3H, s, Ar-CH_3), 7.22 (1H, s, Ar-H), 7.61 (3H, m, Ar-H), 7.20-8.10 (7H, Ar-H).

2, 3- Dihydro -2- (4-fluorophenyl) -4- 2, 4-difluorophenyl) -1, 5-benzothiazepine (BP_2)

IR (KBr) (cm^{-1}): 1625 (C=N), 1509 (C=C),1399 (C-N), 689 (C-S) and 931 (C-F) ^{1}H-NMR ($CDCl_3$) ppm: 5.27 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.50 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.6 Hz, 1H, C_3-H-3a), 2.97 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.05 (1H, s, Ar-H), 7.19 (3H, m, Ar-H), 7.20-8.09 (7H, Ar-H).

2, 3- Dihydro -2- (4-chlorophenyl) -4- (2, 4-difluorophenyl) -1, 5- benzothiazepine (BP_3)

IR (KBr) (cm^{-1}): 1595 (C=N), 1502 (C=C), 1384 (C-N), 778 (C-Cl), 921 (C-F) and 667 (C-S),^{1}H-NMR ($CDCl_3$) ppm: 5.0 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.53 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C_3-H-3a), 3.39 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.25 (1H, s, Ar-H), 7.65 (3H, m, Ar-H), 7.22-8.08 (7H, Ar-H).

2, 3- Dihydro -2- (2-chlorophenyl) -4- (2, 4-difluorophenyl) -1, 5- benzothiazepine (BP_4)

IR (KBr) (cm^{-1}): 1596 (C=N), 1510 (C=C), 1365 (C-N), 688 (C-S), 923 (C-F) and 805 (C-Cl) ^{1}H-NMR ($CDCl_3$) ppm: 4.89 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.43 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.6 Hz, 1H, C_3-H-3a), 3.36 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.12 (1H, s, Ar-H), 7.72 (3H, m, Ar-H), 6.95-7.60 (7H, Ar-H).

2, 3- Dihydro-2- (2, 4-difluorophenyl) -4- (2, 4-difluorophenyl) -1, 5-benzothiazepine (BP_5)

IR (KBr) (cm^{-1}): 1612 (C=N), 1501 (C=C),1382 (C-N), 689 (C-S), 913 (C-F) and 944 (C-F), ^{1}H-NMR ($CDCl_3$) ppm: 5.31 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.36 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ =

9.9 Hz, 1H, C_3-H-3a), 2.87 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.08 (1H, s, Ar-H), 7.30 (3H, m, Ar-H), 6.98-8.12 (6H, Ar-H).

2, 3- Dihydro -2- (2, 4-dichlorophenyl) -4- (2, 4-difluorophenyl) -1, 5- benzothiazepine (BP_6)

IR (KBr) (cm^{-1}): 1593 (C=N), 1502 (C=C), 1382 (C-N), 687 (C-S), 925 (C-F) and 805 (C-Cl)): ^{1}H-NMR ($CDCl_3$) ppm: 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).

2, 3- Dihydro -2- (2-chloro-5-nitrophenyl) -4- (2, 4-difluorophenyl) -1, 5-benzothiazepine (BP_7)

IR (KBr) (cm^{-1}): 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); ^{1}H-NMR ($CDCl_3$) ppm: 4.32 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.74 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C_3-H-3a), 3.51 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.09 (1H, s, Ar-H), 7.12 (3H, m, Ar-H), 6.98-8.10 (6H, Ar-H).

2, 3- Dihydro -2- (3-nitrophenyl) -4- (2, 4-difluorophenyl) -1, 5-benzothiazepine (BP_8)

IR (KBr) (cm^{-1}): 1580 (C=N), 1522 (N=O, asymmetric), 1501 (C=C), 1385 (C-N), 1345 (N=O, symmetric), 924 (C-F) and 689 (C-S); ^{1}H-NMR ($CDCl_3$) ppm: 5.42 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.38 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.6 Hz, 1H, C_3-H-3a), 2.86 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.30 (1H, s, Ar-H), 7.80 (3H, m, Ar-H), 7.48-8.60 (7H, Ar-H).

2, 3- Dihydro -2- (4-nitrophenyl) -4- (2, 4-difluorophenyl) -1, 5-benzothiazepine (BP_9)

IR (KBr) (cm^{-1}): 1586 (C=N), 1515 (N=O, asymmetric), 1506 (C=C), 1380 (C-N), 1338 (N=O, symmetric), 925 (C-F) and 713 (C-S),^{1}H-NMR ($CDCl_3$) ppm: 5.42 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.47 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.7 Hz, 1H, C_3-H-3a), 3.10 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.18 (1H, s, Ar-H), 7.25 (3H, m, Ar-H), 7.25-8.20 (7H, Ar-H).

2, 3- Dihydro -2- (3-hydroxyphenyl) -4- (2, 4-difluorophenyl) -1, 5-benzothiazepine (BP_{10})

IR (KBr) (cm^{-1}): 1653 (C=N), 1528 (C-N), 1502 (C=C), 925 (C-F) and 694 (C-S); ^{1}H-NMR ($CDCl_3$) ppm: 3.85 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.34 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.0 Hz, 1H, C_3-H-3a), 2.41 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-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).

Biological evolution 62

Antimicrobial Activity

Since the chalcones were reported to possess antimicrobial activity, the chalcones prepared during the present work were tested for antibacterial and antifungal activity.

Antibacterial activity

The antibacterial activity was tested by determining the minimum inhibitory concentration (MIC) for each compound using serial tube dilution technique. The following test organisms were used.

Gram positive bacteria

Staphylococcus aureus, Bacillus subtilis.

Gram negative bacteria

Escherichia coli, Proteus vulgaris.

Antifungal activity

The antifungal activity was tested by the same procedure as described in the antibacterial activity, except using Potato-Dextrose-Agar medium. These two organisms were used. *Aspergillus niger*, Candida tropical is The results are shown in tables 2 in the case of antibacterial activity and Table No.3 in the case of antifungal activity.

RESULTS AND DISCUSSION

Antibacterial activity[14]

From the above results it is evident that all the synthesized Benzothiazepines, showed antibacterial activity with different MIC values against the tested organisms, but not comparable with that of the standard. Among the compounds tested, BP_2 and BP_5 fluorophenyl and difluoro phenyl moiety was found to be the most potent against *B.subtilis, E.coli* and *P.vulgaris* having a MIC value of 256 µg/mL in each case. The chalcones, BP_1, BP_3, BP_4, BP_6, BP_7 and BP_{10} shows MIC value of 128 µg/mL against *E.coli, B.subtilis* and *E.coli* respectively. Some of the chalcones (BP_8, BP_9) showed a MIC of 64 µg/mL against both Gram-positive and Gram-negative bacteria. But most of them showed a MIC value in between 128-256 µg/mL.

Antifungal activity

Among the compounds tested for antifungal activity, compounds BP_1, BP2, BP_3,BP_5, BP_7 to be the most potent with a MIC value of 32 µg/mL against *A.niger* in the case of against *C.tropicalis* compounds BP_2,BP_4,BP_5,BP_6,B_{10} shows MIC of 64 µg/mL. The compounds with electron releasing groups show moderately the activity.

CYTOTOXICITY STUDIES[61]

The in vitro cyto toxicity of the test compounds (BP_1-BP_{10}) were performed based on MTT assay method onHT-29 (colon cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines. The cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. Methotrexate was used as reference drug for comparison. Assay was performed in triplicate for three independent determinations. The cytotoxicity was expressed as IC_{50} (µg/mL) which is the concentration of the compound that inhibited proliferation rate of the tumour cells by 50% as compared to the control untreated cells. IC_{50} values were determined from the plot: % inhibition versus concentration.

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

IC_{50} = Inv.log (50-c) / m; c and m derived from y=mx +c of plot of % inhibition Vs log C. The results were tabulated.

The prepared 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 compounds Possessed cytotoxic activity as evidenced by the IC 50 values. Of all the compounds tested against HT-29 cell lines, the compounds BP_4, $BP_5$with choloro phenyl, dichloro phenyl shows IC_{50} value at 42 µg/mL, BP_8 having nitro phenyl moiety (IC_{50} value 55µg/mL), BP_1 having methyl phenyl moiety (IC_{50} value 73 µg/mL). The other compounds also showed activity but at a higher IC_{50} values. Among the compounds tested for cytotoxicity on MCF-7 cell lines, the compounds BP_4, BP_5 with choloro phenyl, dichloro phenyl (IC_{50} value 38 µg/mL), BP_8 having nitro phenyl moiety (IC_{50} value 58 µg/mL), BP_1 having methyl phenyl moiety having IC_{50} value 88 µg/mL. The other compounds also showed activity but at a higher IC_{50} values. Among the compounds tested for cytotoxicity on DU-145 cell lines, the compounds BP_{10} with theinyl (IC_{50} value 18 µg/mL), BP_3 having bromo furanyl moiety (IC_{50} value 23 µg/mL), BP_5 having methoxy hydroxyl moiety (IC_{50} value 74 µg/mL). The other compounds also showed activity but at a higher IC_{50} values.

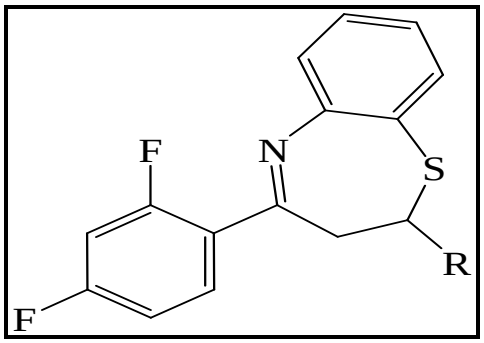
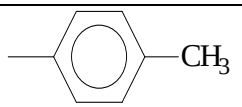
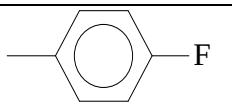
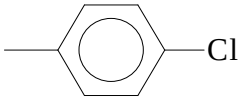
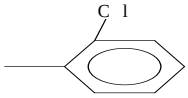
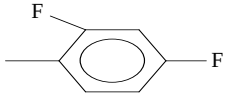
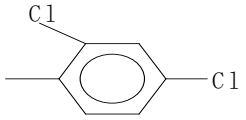
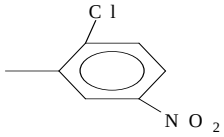
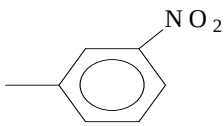
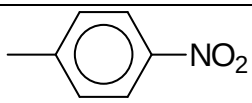
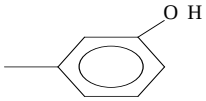


Table No.1: Physical characterization data of benzothiazepines (BP_1-BP_6) and Spectral Data

S.No	Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (°C)	Yield %
1	BP_1		$C_{22}H_{17}F_2NS$	365	140-143	89
2	BP_2		$C_{21}H_{14}F_3NS$	369	153-154	89
3	BP_3		$C_{21}H_{14}ClF_2NS$	385	143-145	93
4	BP_4		$C_{21}H_{14}ClF_2NS$	385	120-123	71
5	BP_5		$C_{21}H_{13}F_4NS$	387	138-141	75
6	BP_6		$C_{21}H_{13}Cl_2F_2NS$	420	117-120	86
7	BP_7		$C_{21}H_{13}ClF_2N_2O_2S$	430	164-167	77
8	BP_8		$C_{21}H_{14}F_2N_2O_2S$	396	142-145	82
9	BP_9		$C_{21}H_{14}F_2N_2O_2S$	396	130-131	89
10	BP_{10}		$C_{21}H_{15}F_2NOS$	367	226-229	84

(Expressed as MIC in µg/mL)

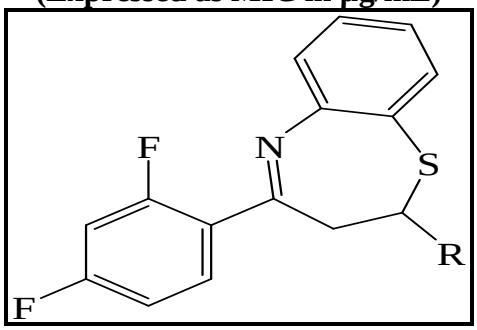


Table No.2: Antibacterial activity of synthesised compounds ($BP_1$to BP_{10})

S.No	Compound	R	*B.subtilis*	*S.aureus*	*E.coli*	*P.vulgaris*
1	**B_1**	4"-methyl phenyl	128	128	128	128
2	**B_2**	4"-fluorophenyl	256	128	128	128
3	**B_3**	4"-chlorophenyl	128	128	128	128
4	**B_4**	2"-chlorophenyl	128	128	128	128
5	**B_5**	2",4"-difluorophenyl	256	128	256	128
6	**B_6**	2",4-dichlorophenyl	128	128	128	128
7	**B_7**	2"-chloro-5"-nitro phenyl	128	64	64	128
8	**B_8**	3"-nitro phenyl	64	64	64	64
9	**B_9**	4"-nitro phenyl	64	64	32	64
10	**B_{10}**	3"-hydroxyphenyl	128	128	128	128
11	Standard (Ampicillin)	---	< 1	< 1	< 1	< 1

(Expressed as MIC in µg/mL)

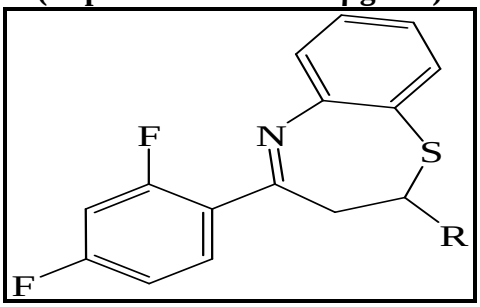


Table No.3: Antifungal activity of synthesised compounds ($BP_1$to BP_{10})

S.No	Compound	R	*Aspergillus niger*	*Candida tropicalis*
1	B_1	4"-methyl phenyl	32	32
2	B_2	4"-fluorophenyl	32	64
3	B_3	4"-chlorophenyl	32	34
4	B_4	2"-chlorophenyl	128	64
5	B_5	2",4"-difluorophenyl	32	64
6	B_6	2",4-dichlorophenyl	32	64
7	B_7	2"-chloro-5"-nitro phenyl	32	32
8	B_8	3"-nitro phenyl	16	32
9	B_9	4"-nitro phenyl	16	16
10	B_{10}	3"-hydroxyphenyl	64	64
11	Standard Fluconazole	---	< 2	< 2

(IC_{50} values in µg/mL)

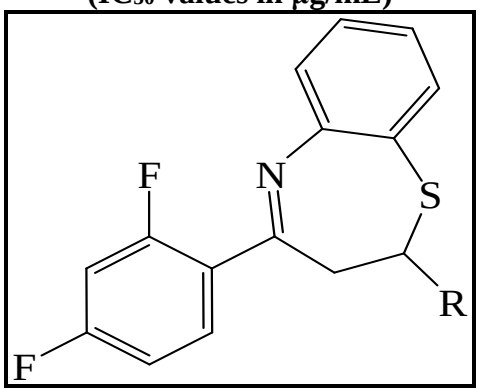
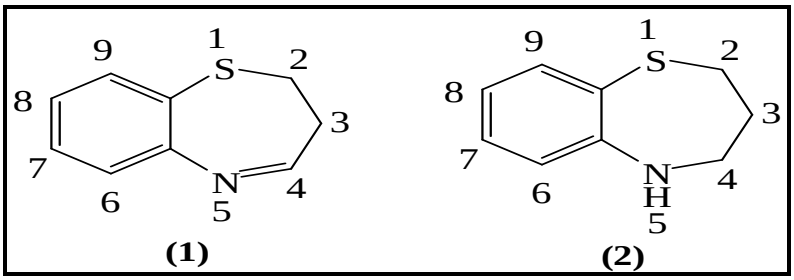


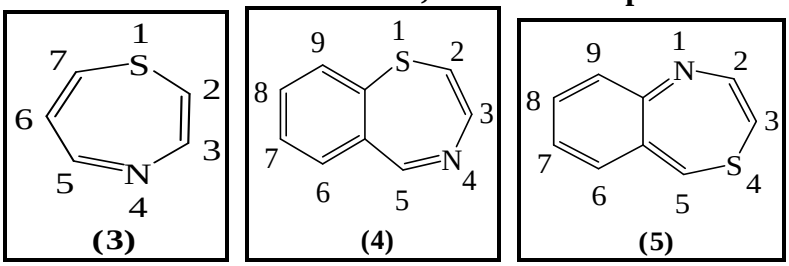
Table No.4: Cytotoxicity of the new chalcones (BP_1 to BP_{10})

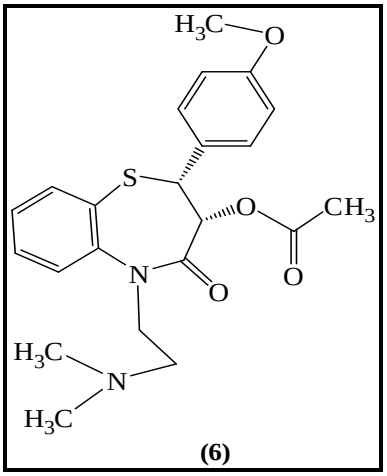
S.No	Compound	R	Cell line		
			HT-29	MCF-7	DU-145
1	B_1	4"-methyl phenyl	56 ± 2	62 ± 2	56 ± 2
2	B_2	4"-fluorophenyl	148 ± 2	188 ± 2	105 ± 2
3	B_3	4"-chlorophenyl	92 ± 2	74 ± 1	65 ± 2
4	B_4	2"-chlorophenyl	42 ± 2	42 ± 2	33 ± 2
5	B_5	2",4"-difluorophenyl	182 ± 1	NA	148 ± 1
6	B_6	2",4"-dichlorophenyl	42 ± 2	48 ± 1	46 ± 2
7	B_7	2"-chloro-5"-nitro phenyl	180 ± 2	NA	122 ± 2
8	B_8	3"-nitro phenyl	55 ± 2	58 ± 1	52 ± 1
9	B_9	4"-nitro phenyl	28 ± 1	27 ± 1	16 ± 1
10	B_{10}	3"-hydroxyphenyl	105 ± 2	78 ± 2	68 ± 2
11	Methotrexate		11 ± 1	9 ± 1	6 ± 1

Data presented as mean ± SD (n=3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1% DMSO. The control cells were treated with culture medium containing 0.1% DMSO. NA- No Activity (i.e IC_{50} > 200 µg/mL)



General structures of 1, 5-benzothiazepine





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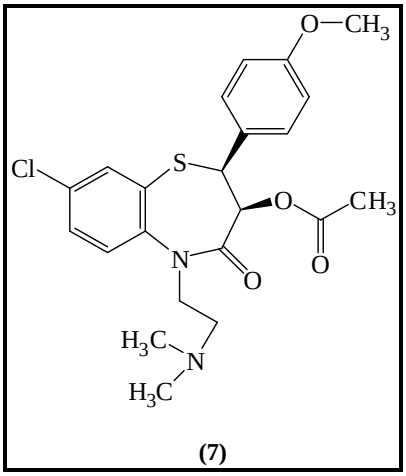

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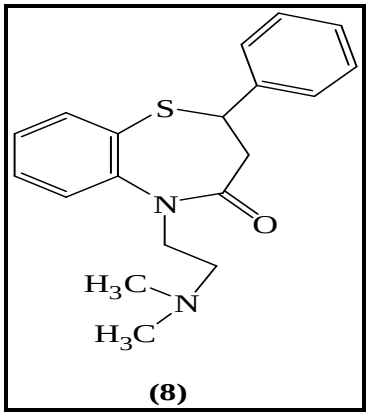

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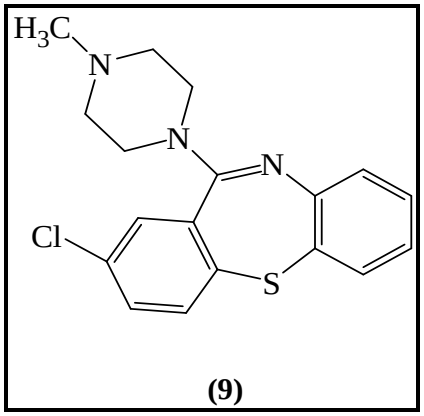

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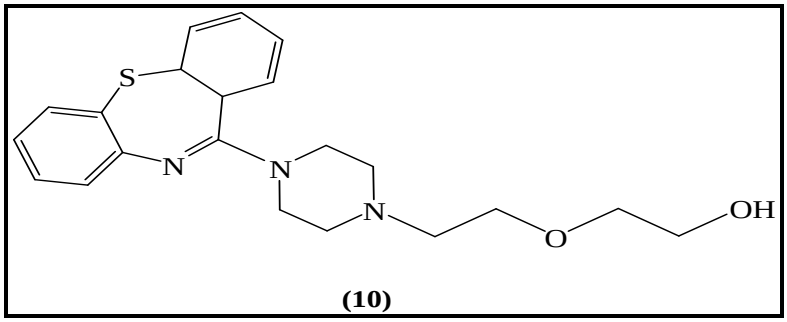
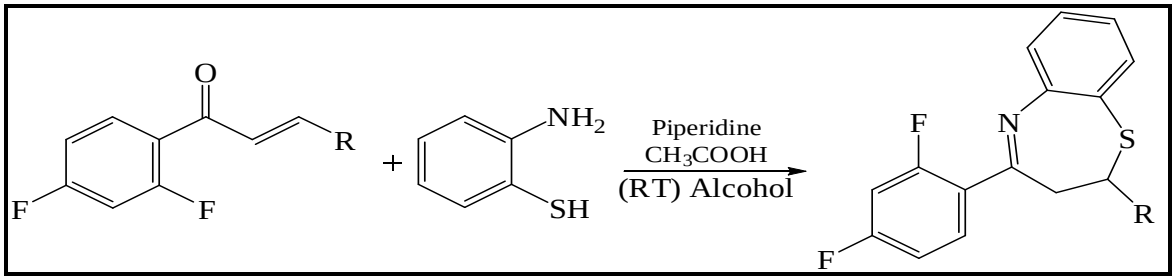


(10)

Scheme- 12

Chemical Reaction



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

CONCLUSION

In all synthesized Benzothiazepines B_2, B_5 shows potent anti-bacterial activity, BP_4 shows potent anti-fungal activity, BP_9, BP_6, BP_4 shows potent activity against HT-29 (colon cancer), BP_9, BP_6, BP_4 shows potent activity MCF-7 (breast cancer) and BP_9, BP_6, BP_4 shows potent activity DU-145 (prostate cancer) cell lines.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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