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SYNTHESIS, CHARACTERIZATION AND ANTI BACTERIAL AND CYTOTOXIC STUDIES OF NOVEL 1,5 BENZOTHIAZEPINES FROM CHALCONES OF 1-(2,4-DIFLUOROPHENYL) ETHANONE PRECURSOR

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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''-methylphenyl)-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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INTRODUCTION

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 separates out. After cooling, the solid product was collected and washed with diethylether 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-(3-nitro-4-methylphenyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{11})

IR (KBr) (cm^{-1}) : 1642 (C=N), 1548 (N=O, asymmetric), 1510 (C=C), 1380 (C-N), 1338 (N=O, symmetric), 927 (C-F) and 668 (C-S).^{1}H-NMR ($CDCl_3$) ppm : 4.16 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.23 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, C_3-H-3a), 2.53 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 2.50 (3H, s, Ar-CH_3), 7.30 (1H, s, Ar-H), 6.70 (3H, m, Ar-H), 7.45-8.78 (6, Ar-H)

2, 3-Dihydro-2-(3, 4, 5-trimethoxyphenyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{12})

IR (KBr) (cm^{-1}) : 1648 (C=N), 1505 (C=C), 1365 (C-N), 1225 (-O-CH_3), 923 (C-F) and 678 (C-S) ^{1}H-NMR ($CDCl_3$) ppm :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)

2, 3-Dihydro-2-(3, 4-methelenedioxyphenyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{13})

IR (KBr) (cm^{-1}) : 1592 (C=N), 1502 (C=C), 1370 (C-N), 1232 (-O-CH_2-O-), 921 (C-F) and 689 (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, $J_{3a,2}$ = 9.1 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)

2, 3-Dihydro-2-(5-bromofuran-2-yl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{14})

IR (KBr) (cm^{-1}): 1602 (C=N), 1505 (C=C), 1340 (C-N), 664 (C-S), 933 (C-F) and 790 (C-Br), ^{1}H-NMR ($CDCl_3$) ppm :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)

2, 3-Dihydro-2-(4-dimethylaminophenyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{15})

IR (KBr) (cm^{-1}) : 1608 (C=N), 1509 (C=C), 1390 (C-N), 1175 (-N-$(CH_3)_2$), 933 (C-F) and 679 (C-S), ^{1}H-NMR ($CDCl_3$) ppm :4.96 (dd, $J_{2,3a}$ = 5.3 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.83 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.2 Hz, 1H, C_3-H-3a), 3.26 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 3.20 (6H, s, N-$(CH_3)_2$, 7.20 (1H, s,Ar-H), 7.45 (3H, m, Ar-H), 6.70-8.20 (7H, Ar-H)

2, 3-Dihydro-2-(3-methoxy-4-hydroxyphenyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{16})

IR (KBr) (cm^{-1}) : 3540 (O-H), 1598 (C=N), 1502 (C=C), 1378 (C-N), 1234 (-O-CH_3) 913 (C-F), and 688 (C-S) ^{1}H-NMR ($CDCl_3$) ppm :3.43 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 2.50 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.4 Hz, 1H, C_3-H-3a), 1.03 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.20 (1H, s,Ar-H), 6.85 (3H, m, Ar-H), 7.15-7.90 (6H, Ar-H), 6.95 (1H, s, Ar-OH), 3.80 (3H, s, Ar-O-CH_3)

2, 3-Dihydro-2-(2-pyridinyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{17})

IR (KBr) (cm^{-1}) : 1602 (C=N), 1510 (C=C), 1390 (C-N), 924 (C-F) and 677 (C-S), ^{1}H-NMR ($CDCl_3$) ppm : 4.91 (dd, $J_{2,3a}$ = 5.3 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.44 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.4 Hz, 1H, C_3-H-3a), 1.05 (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), 7.10-8.15 (7H, Ar-H)

2, 3-Dihydro-2-(3-pyridinyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{18})

IR (KBr) (cm^{-1}) : 1599 (C=N), 1506 (C=C), 1382 (C-N), 927 (C-F) and 698 (C-S); ^{1}H-NMR ($CDCl_3$) ppm : 4.38 (dd, $J_{2,3a}$ = 5.3 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.37 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.8 Hz, 1H, C_3-H-3a), 1.07 (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), 6.75-8.90 (7H, Ar-H)

2, 3-Dihydro-2-(4-pyridinyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{19})

IR (KBr) (cm^{-1}) : 1606 (C=N), 1508 (C=C), 1388 (C-N), 933 (C-F) and 654 (C-S);^{1}H-NMR ($CDCl_3$) ppm : 4.67 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, C_2-H), 3.42 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.8 Hz, 1H, C_3-H-3a), 2.50 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.20 (1H, s,Ar-H), 7.50 (3H, m, Ar-H), 6.95-8.68 (7H, Ar-H)

2, 3-Dihydro-2-(2-thienyl)-4-(2, 4-difluorophenyl)-1, 5-benzothiazepine (BP_{20})

IR (KBr) (cm^{-1}) : 1605 (C=N), 1503 (C=C), 1386 (C-N), 928 (C-F) and 644 (C-S);^{1}H-NMR ($CDCl_3$) ppm : 5.50 (dd, $J_{2,3a}$ = 5.3 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), 2.90 (t, $J_{3b,3a} = J_{3b,2}$ = 12.9 Hz, 1H, C_3-H-3b), 7.20 (1H, s,Ar-H), 7.34 (3H, m, Ar-H), 6.60-7.80 (6H, Ar-H)

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 tropicalis.*

The results are shown in tables 2 in the case of antibacterial activity and table 3 in the case of antifungal activity.

RESULTS AND DISCUSSION

Antibacterial activity

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_1, $BP_3$and BP_4 3"-nitro-4"methylphenyl, 3",4"-methelenedioxyphenyl and 5"-bromofuran-2"-ylmoiety was found to be the most potent against B. subtilis, *E. coli* and *P. vulgaris* having a MIC value

of 64 µg/mL in each case. The chalcones, BP_{16}, BP_{17} shows MIC value of 128 µg/mL against *B.subtilis* and *E. coli* respectively. Some of the chalcones) showed a MIC of 256 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, BP_3, $BP_4$to be the most potent with a MIC value of 16 µg/mL against A. Nigerin the case of against *C.tropicalis* compounds BP_1, BP_3, BP_4 shows MIC of 32 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_1 having 3''-nitro-4''methylphenyl moiety shows IC_{50} value at 36 µg/mL, $BP_6$with 3''-methoxy-4''-hydroxyphenyl moiety shows IC_{50} value at 64µg/mL. Among the compounds tested for cytotoxicity on MCF-7 cell lines, the compounds BP_1 having 3''-nitro-4''methylphenyl moiety shows IC_{50} value at 28 µg/mL, BP_6 with 3''-methoxy-4''-hydroxyphenyl moiety shows IC_{50} value at 67 µg/mL. Among the compounds tested for cytotoxicity on DU-145 cell lines, the compounds BP_1 having 3''-nitro-4''methylphenyl moiety shows IC_{50} value at 16 µg/mL, BP_6 with 3''-methoxy-4''-hydroxyphenyl moiety shows IC_{50} value at 62 µg/mL. .The other compounds also showed activity but at a higher IC_{50} values.

Scheme- 12

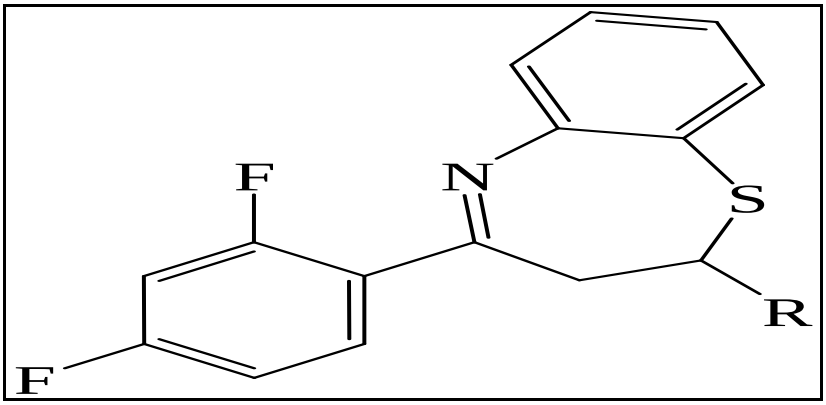
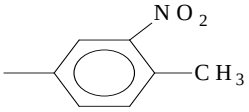
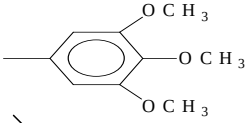
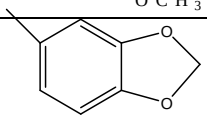
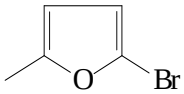
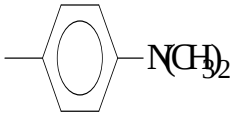
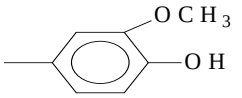
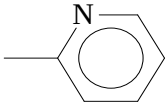
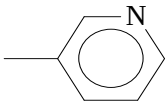
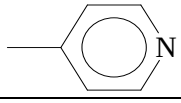
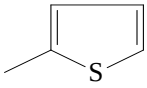


Table No.1: Physical characterization data of benzothiazepines (BP_1-BP_6)

S.No	Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (°C)	Yield %
1	BP_1		$C_{22}H_{16}F_2N_2O_2S$	410	176-179	94
2	BP_2		$C_{24}H_{21}F_2NO_3S$	441	148-151	85
3	BP_3		$C_{22}H_{15}F_2NO_2S$	395	156-157	74
4	BP_4		$C_{19}H_{12}BrF_2NOS$	420	132-135	79
5	BP_5		$C_{23}H_{20}F_2N_2S$	394	114-117	88
6	BP_6		$C_{22}H_{17}F_2NO_2S$	397	151-154	86
7	BP_7		$C_{20}H_{14}F_2N_2S$	352	111-114	78
8	BP_8		$C_{20}H_{14}F_2N_2S$	352	120-121	82
9	BP_9		$C_{20}H_{14}F_2N_2S$	352	110-101	92
10	BP_{10}		$C_{19}H_{13}F_2NS_2$	357	146-149	86

(Expressed as MIC in µg/mL)

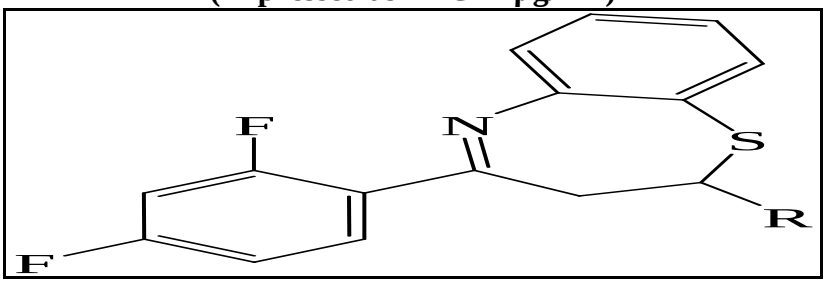


Table No.2: Antibacterial activity of synthesised compounds ($BP_1$to BP_{10})

S.No	Compound	R	*B.subtilis*	*S.aureus*	*E.coli*	*P.vulgaris*
1	B_1	3"-nitro-4"-methylphenyl	64	64	64	64
2	B_2	3",4",5"-trimethoxypheny	256	256	256	256
3	B_3	3",4"-methylendioxyphenyl	64	64	64	64
4	B_4	5"-bromofuran-2"-yl	64	64	64	128
5	B_5	4"-dimethylaminophenyl	128	128	128	128
6	B_6	3"-methoxy-4"-hydroxyphenyl	128	128	128	128
7	B_7	2"-pyridinyl	128	128	128	128
8	B_8	3"-pyridinyl	256	256	256	256
9	B_9	4"-pyridinyl	256	512	512	256
10	B_{10}	2"-thienyl	256	256	256	256
11	Standard (Ampicillin)		< 1	< 1	< 1	< 1

(Expressed as MIC in µg/mL)

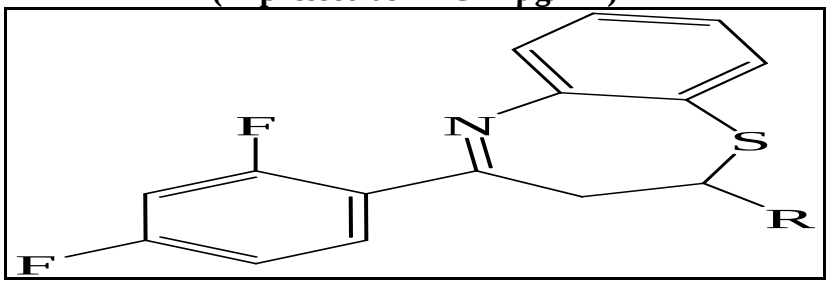


Table No.3: Antifungal activity of synthesised compounds ($BP_1$to BP_{10})

S.No	Compound	R	*Aspergillus niger*	*Candida tropicalis*
1	B_1	3"-nitro-4"-methylphenyl	16	16
2	B_2	3",4",5"-trimethoxypheny	128	128
3	B_3	3",4"-methylendioxyphenyl	16	32
4	B_4	5"-bromofuran-2"-yl	16	32
5	B_5	4"-dimethylaminophenyl	32	64
6	B_6	3"-methoxy-4"-hydroxyphenyl	32	64
7	B_7	2"-pyridinyl	32	64
8	B_8	3"-pyridinyl	32	64
9	B_9	4"-pyridinyl	256	128
10	B_{10}	2"-thienyl	32	64
11	Standard Fluconazole		< 2	< 2

(IC_{50} values in µg/mL)

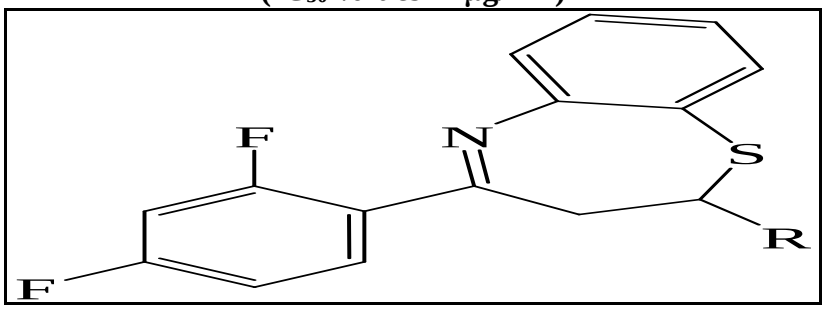
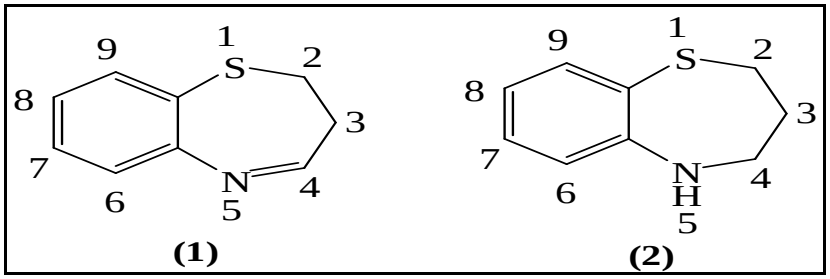


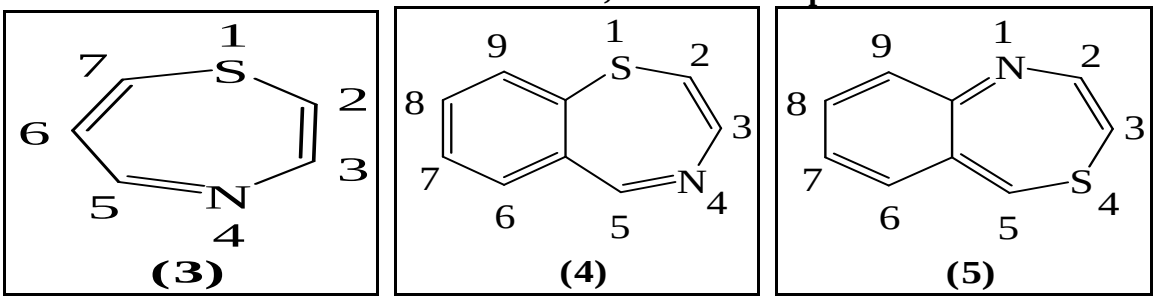
Table No.4: Cytotoxicity of the new chalcones (BP_1 to BP_{10})

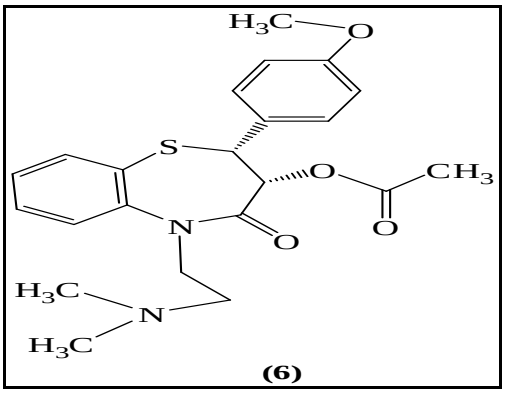
S.No	Compound	R	Cell line		
			HT-29	MCF-7	DU-145
1	B_1	3"-nitro-4"-methylphenyl	36 ± 2	28 ± 1	16 ± 2
2	B_2	3",4",5"-trimethoxypheny	148 ± 2	188 ± 2	105 ± 2
3	B_3	3",4"-methylendioxyphenyl	NA	NA	NA
4	B_4	5"-bromofuran-2"-yl	123 ± 2	129 ± 2	92 ± 2
5	B_5	4"-dimethylaminophenyl	155 ± 1	NA	110 ± 2
6	B_6	3"-methoxy-4"-hydroxyphenyl	NA	NA	NA
7	B_7	2"-pyridinyl	64 ± 2	67 ± 1	62 ± 2
8	B_8	3"-pyridinyl	195 ± 2	NA	155 ± 1
9	B_9	4"-pyridinyl	115 ± 2	106 ± 2	75 ± 2
10	B_{10}	2"-thienyl	132 ± 2	168 ± 1	98 ± 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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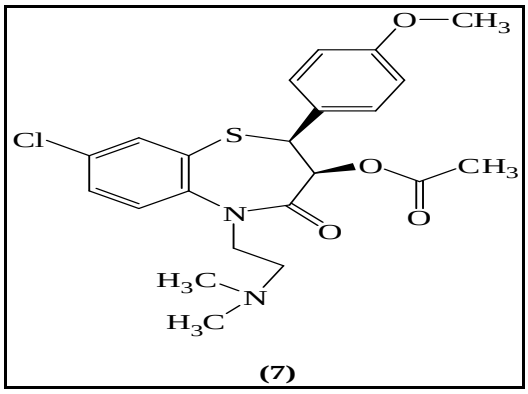

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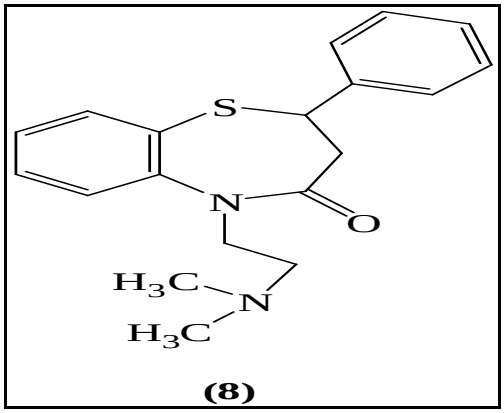

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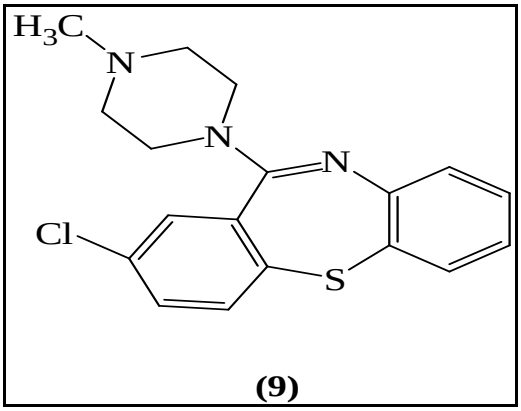

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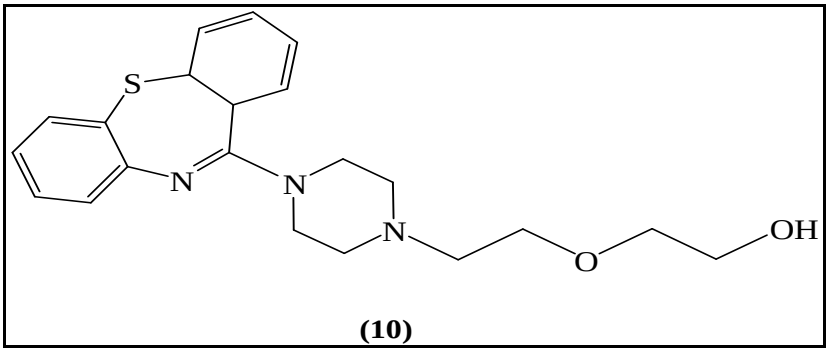
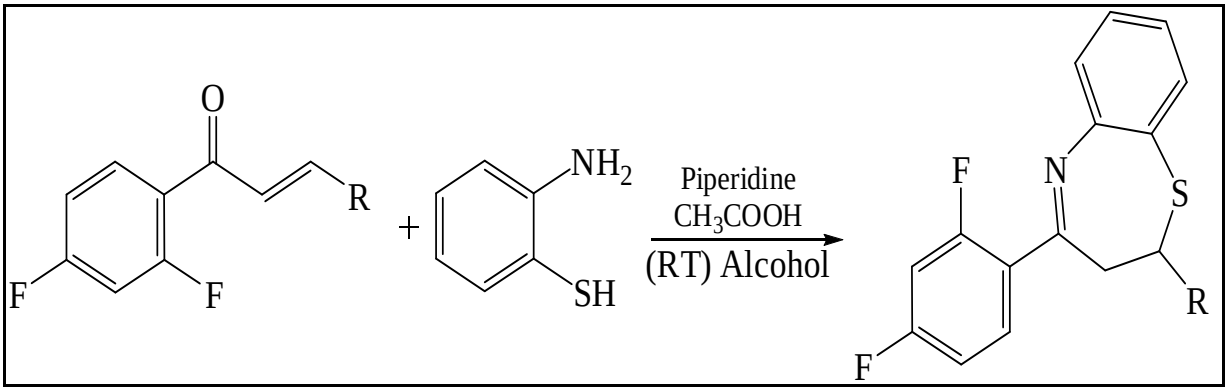


(10)

Chemical Reaction



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

CONCLUSION

In all synthesized benzothiazepines B_1,B_3 shows potent anti-bacterial activity, B_1,B_3shows potent anti-fungal activity, BP_1,BP_6,BP_8 shows potent activity against HT-29 (colon cancer), MCF-7 (breast cancer) 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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