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Solid-phase synthesis and biological evaluation of a parallel library of 2,3-dihydro-1,5-benzothiazepines

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ABSTRACT

Solid-phase synthesis of a parallel library of 3′-hydroxy-2,3-dihydrobenzothiazepines has been carried out through [4+3] annulation of α,β-unsaturated ketones with aminothiophenol, using Wang resin as solid support. The synthesized compounds were evaluated for their potential as antibacterial, tumor inhibitors as well as acetyl- and butyrylcholinesterase inhibitors. None of the compounds showed any significant antibacterial activity. However, quite a few compounds showed significant potential as crown gall tumor inhibitors. These results reflect a strong exploratory potential in search of new benzothiazepines as source of anticancer agents. The results of the inhibition of cholinesterase revealed that benzothiazepines have a greater potential as butyrylcholinesterase inhibitors as compared to acetylcholinesterase. Moreover, the substitution of hydroxy group at C-3 in ring A led to increased activity when compared to unsubstituted- and 2′-OH substituted benzothiazepines.

1. Introduction

For more than a century heterocycles have constituted one of the largest areas of research in organic synthesis.[1] Over the last two decades, synthesis of N and S containing heterocyclic compounds especially benzothiazepines retained the interest of researchers due to the unique structural properties and broad spectrum of biological activities of these compounds.[2]

Benzothiazepines have their role in the treatment of cardiovascular disorders,[3] as Ca^{2+} channel blockers,[4] inhibitors of HIV-I integrase,[5] antibiotics,[6] muscle relaxants,[7] and cytotoxic agents.[8] They are also known to have antimicrobial[9–11] and antihypertensive activities[12] besides being used in the treatment of diabetes.[13] Keeping in view this broad spectrum of biological activity associated with these compounds, we have reported earlier the synthesis of some 2,3-dihydrobenzothiazepines with unsubstituted ring A and 2′-OH substitution on ring A. These compounds were evaluated against a number of bioassays, which revealed that the compounds have potential as enzyme inhibitors such as acetyl- and butyryl cholinesterase, urease and α-glucosidase, and quite a few compounds were found to be promising DPPH-free radical scavengers.[14] Molecular docking studies on the compounds that were identified as good inhibitors of cholinesterase and urease inhibitors were also carried out to investigate the ligand-enzyme interaction.[15,16] 2,3-Dihydro-1,5-benzothiazepines were synthesized by using the most widely accepted strategy that involved a [4+3] annulation of α,β-unsaturated ketones (chalcones) with *o*-aminothiophenol (*o*-ATP) under acidic conditions.[17] In continuation to these studies, synthesis of a parallel library of 2,3-dihydro-1,5-benzothiazepines with a 3′-hydroxy substitution on ring A precursors has been carried out with the objective of making a comparison with the compounds previously synthesized.

As observed earlier, classical or solution-phase synthesis of these compounds involves various problems, related to their yield and purity which in turn is affected by the substitution on both rings A and B of chalcones. The decomposition of *o*-ATP with the passage of time is another factor for low yield and less purity of the desired products. In the recent years solid-phase synthesis (SPS) has emerged as a powerful synthetic tool that not only ensures greater purity but also results in improved yield mainly due to a decrease in side products.[10] Easier work up, less time consumption, and easy purification are some extra advantages of SPS that has attracted the attention of researchers towards the use of this technique for the synthesis of these heterocyclic compounds. Wang resin has usually been employed when an OH group is available to anchor the solid support. It is an acid-labile *p*-alkoxybenzylalcohol resin and its applications to the synthesis of various types of compounds via benzyl-ether linkage under Mitsunobu conditions[18] are well known. Bio-evaluation of the synthesized benzothiazepines involved their screening as antibacterial, potato disk tumor inhibitors and as cholinesterase inhibitors.

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2. Results and discussion

A parallel library of twenty-five 2,3-dihydro-1,5-benzothiazepines (**27–52**) has been synthesized using chalcones (**1–26**) as precursors which in turn were synthesized through Claisen-Schmidt condensation of 3-hydroxy acetophenone with 25 different aryl aldehydes.

The generation of chalcone intermediates in good yield and high purity was critical for the success of the synthesis of benzothiazepines as the use of impure intermediates was detrimental to 1,5-benzothiazepine synthesis.[10] Analytical results showed that the desired chalcones as well as 1,5-benzothiazepines were easily synthesized in solution phase in high yield and acceptable purity as long as the aromatic aldehyde did not contain any electron-donating group on ring B by reducing the electrophilicity of the carbonyl and alkenic groups in chalcones toward nucleophilic attack by *o*-ATP.

Furthermore, the reactions with heteroaryl-substituted chalcones seem to undergo ring opening or complex decomposition reactions thereby reducing the yield and purity of the products. Moreover, attempt to catalyze these reactions by piperidine was also not successful. Therefore, there existed a dire need to look for an alternative synthetic technique. The problem of low yield in the case of hydroxy-substituted aldehydes and ketones was circumvented by protecting the hydroxy group by tetrahydropyran; however, the purity of the products could not be improved. Although the protecting group strategy led to high yield at the expense of an additional synthetic step, the problem of purity remained the same.

Therefore, solid-phase synthetic (SPS) strategy was chosen for the synthesis of 2,3-dihydro-1,5-benzothiazepines using 3′-hydroxy chalcones as precursors. The linking of 3-hydroxy group to Wang resin has been carried out under Mitsunobu conditions[18] by shaking acetophenone dissolved in *N*-methylmorpholine (NMM) with Wang resin in the presence of triphenylphosphine (TPP) and diisopropylazodicarboxylate (DIAD) in reaction mixture for 48 h. The resin-bound acetophenone was then reacted with different aryl- and heteroaryl aldehydes under Claisen-Schmidt condensations which led to resin-bound chalcones as shown in Scheme 1. Without getting involved in the isolation and purification of chalcones, they were treated directly with *o*-ATP in DMF under inert atmosphere, which led to resin-bound dihydrobenzothiazepines. The cleavage of the resin was done through 50% TFA in CH_2Cl_2 which resulted in free 2,3-dihydrobenzothiazepines (**27–52**). Table 1 shows a comparison of the yield and purity of some selected compounds which were synthesized both in solution and solid phase where a significant difference in yield was observed.

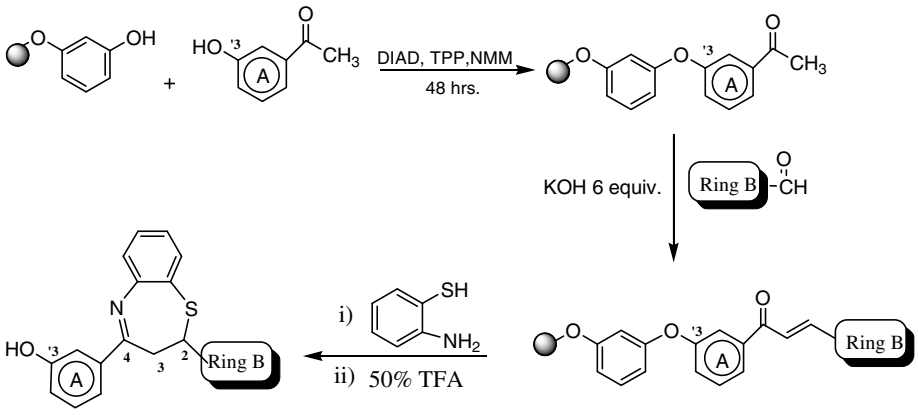
3. Biological screening

The synthesized compounds were tested for the following biological activities.

Table 1
Comparison of yield and purity through solution- and solid-phase synthesis of some selected benzothiazepines

Compound	R	Yield (%)		Purity[a] (%)
		Solution phase	Solid phase	
29	3-Cl C_6H_4	65	80	85
30	4-Cl C_6H_4	80	87	83
33	4-F C_6H_4	81	93	91
35	3-OH C_6H_4	51	70	75
39	4-OMe C_6H_4	47	58	80
42	3-NO_2 C_6H_4	78	83	>90
43	4-NO_2 C_6H_4	83	90	~90

[a] Solid phase.



Ring B
1, 27 = C_6H_5
2, 28 = 2''-Cl C_6H_4
3, 29 = 3''-Cl C_6H_4
4, 30 = 4''-Cl C_6H_4
5, 31 = 2''-F C_6H_4
6, 32 = 3''-F C_6H_4
7, 33 = 4''-F C_6H_4
8, 34 = 4''-Br C_6H_4
9, 35 = 3''-OH C_6H_4
10, 36 = 4''-OH C_6H_4
11, 37 = 2''-OMe C_6H_4
12, 38 = 3''-OMe C_6H_4
13, 39 = 4''-OMe C_6H_4

Ring B
14, 40 = 3'',4''-$OMe_2C_6H_3$
15, 41 = 4''-OH-3''-OMe C_6H_3
16, 42 = 3''-$NO_2C_6H_4$
17, 43 = 4''-NO_2 C_6H_4
18, 44 = 4''-MeC_6H_4
19, 45 = 4''-NMe_2 C_6H_4
20, 46 = Pyridin-2''-yl
21, 47 = Pyridin-3''-yl
22, 48 = Pyridin-4''-yl
23, 49 = Thien-2''-yl
24, 50 = 2''-Thienyl-5''-Me
25, 51 = 2''-Thienyl-5''-Br
26, 52 = Pyrrol-2''-yl

Scheme 1. Solid-phase synthesis of 2,3-dihydro-1,5-benzothiazepines (**27–52**).

3.1. Antibacterial assay

All synthesized compounds have been tested for their potential as antibacterial against five bacterial strains namely *Bordetella bronchiseptica, Micrococcus leuteus, Escherichia coli, Enterobacter aerogenes*, and *Salmonella setubal* by using *Agar well diffusion* method. However, none of the tested compounds showed any significant antibacterial activity against any bacterial strain.

The benzothiazepines **29**, **41**, **43**, and **45** showed weak activity against a few bacterial strains as shown in Table 2.

3.2. Potato disk tumor inhibition assay (PDT)

The efforts made in minimizing the screening process to hasten the pace of drug discovery are the development of bench top assays which shortlist potential candidates that could be targeted for more advanced screening. One of these bench top assays is the crown gall tumor assay also known as potato disk tumor bioassay as developed by McLaughlin. It is acceptable as a preliminary general screen for antineoplastic activity of different compounds and plant extracts regardless of the mode of inhibitory action on tumor growth. Crown gall is a neoplastic plant disease caused by *Agrobacterium tumefaciens.*[19] In this bioassay the ability of the test compounds to inhibit the tumors induced by *A. tumefaciens* in model systems such as potato tuber disks is evaluated. The rationale for employing this bioassay rests on the fact that the tumorogenic mechanism induced by *A. tumefaciens* in plants is in many ways similar to that of animals.[20] The use of this bioassay has resulted in many shortlists of test compounds with anticancer activity and has helped with the discovery of the novel anticancer compounds. The synthesized compounds have been tested for their potato disk tumor inhibition activity. The inhibition was studied at 1000, 100, and 10 ppm concentrations. These results along with IC_{50} values (McLaughlin and Rogers, 1998) are shown in Table 3.

It is evident from the data in Table 3 that the OMe-substituted compounds (**37–40**) were found to be the most promising in the series with IC_{50} values varying from 3.28 to 8.23. Moreover, benzothiazepines substituted with a 4-pyridyl and thiophene ring (**48, 49, and 51**) were also detected to be strong inhibitors of crown gall tumor with IC_{50} values 6.4, 4.8, and 11.2, respectively. The substitution of a chlorine atom on ring B (**28–30**) also resulted in an increased tendency of tumor inhibition. These compounds could therefore be targeted for more advanced screening. Studies on potato disk bioassay were also extended to some chalcones and it was detected that they possessed better inhibitory potential than the corresponding benzothiazepines as shown in Table 4.

IC_{50} values of chalcones varied from 2.8–40.0 ppm and chalcones **11, 14**, and **19** were found to have significantly inhibited the growth of tumors.

Table 2
Antibacterial activity of 2,3-dihydro-1,5-benzothiazepines (zone of inhibition, mm)

Compound	R	1	2	3	4	5
27	C_6H_5	—	—	—	—	—
28	2-Cl C_6H_4	—	—	—	—	—
29	3-Cl C_6H_4	—	—	—	11.5	—
30	4-Cl C_6H_4	—	—	—	—	—
31	2-F C_6H_4	—	—	—	—	—
33	4-F C_6H_4	—	—	—	—	—
34	4-Br C_6H_4	—	—	—	—	—
35	3-OH C_6H_4	—	—	—	—	—
36	4-OH C_6H_4	—	—	—	—	—
37	2-OMe C_6H_4	—	—	—	—	—
39	4-OMe C_6H_4	—	—	—	—	—
40	3,4-OMe_2 C_6H_3	—	—	—	—	—
41	3-OMe, 4-OH C_6H_3	9.5	10.0	10.0	10.0	11.0
42	3-NO_2 C_6H_4	—	—	—	10.0	Nil
43	4-NO_2 C_6H_4	10.5	10.0	9.0	13.0	Nil
44	4-Me C_6H_4	—	—	—	—	—
45	4-NMe_2 C_6H_4	11.0	9.0	9.0	10.0	11.0
47	3-Pyridyl	—	—	—	—	—
49	2-Thienyl	—	—	—	—	—
Standard	Roxithromycin	14.0	13.0	12.0	15.0	12.0

(1) E. coli, (2) B. bronchiseptica, (3) S. setubal, (4) M. leuteus, (5) E. aerogenes.

Table 3
Results of PDT bioassay conducted on 2,3-dihydro-1,5-benzothiazepines

Compound	R	% inhibition (ppm)			IC_{50}
		1000	100	10	
27	C_6H_5	100	97.8	72.3	5.41
28	2-Cl C_6H_4	95.7	93.6	74.4	6.94
29	3-Cl C_6H_4	93.6	80.8	68.0	14.01
30	4-Cl C_6H_4	74.4	68.0	51.93	9.0
31	2-F C_6H_4	91.4	40.4	31.9	295.5
33	4-F C_6H_4	40.4	34.0	25.5	1232
34	4-Br C_6H_4	85.1	63.8	21.2	61.5
35	3-OH C_6H_4	100	48.9	36.1	179.3
36	4-OH C_6H_4	100	48.9	27.6	237.9
37	2-OMe C_6H_4	98.5	87.1	81.42	7.06
38	3-OMe C_6H_4	100	84.2	67.142	3.28
39	4-OMe C_6H_4	100	78.7	57.4	4.69
40	3,4-OMe_2 C_6H_3	100	82.7	70.2	8.23
41	3-OMe, 4-OH C_6H_3	95.7	40.4	36.1	247.3
42	3-NO_2 C_6H_4	100	57.4	42.5	26.2
43	4-NO_2 C_6H_4	65.9	29.7	12.7	331.35
44	4-Me C_6H_4	87.2	72.3	65.9	30.7
45	4-NMe_2 C_6H_4	100	44.6	38.2	192.5
47	3-Pyridyl	51.0	38.2	29.7	926.2
48	4-Pyridyl	100	80.0	55.1	6.1
49	2-Thienyl	100	100	87.2	4.8
51	2-Thienyl-5-Br	92.8	91.4	80.3	11.2
	Vincristine[a]	—	—	—	0.003

[a] Concentrations at which standards is tested are 0.1, 0.01, 0.001, 0.000001, and 0.0000001.

Table 4
Results of PDT bioassay carried on some selected chalcones

Compound	R	% inhibition (ppm)			IC_{50}
		1000	100	10	
2	2-Cl C_6H_4	100	46.8	36.17	40
3	3-Cl C_6H_4	100	74.4	51.0	12.1
7	4-F C_6H_4	83.0	44.6	32.0	9.6
9	3-OH C_6H_4	100	100	57.4	12.0
11	2-OMe C_6H_4	100	100	76.5	3.1
14	3.4-OMe_2 C_6H_3	100	77.1	60.0	5.8
19	4-NMe_2 C_6H_4	100	88.5	75.5	2.8

3.3. Cholinesterase inhibition assay

It is an established fact that many life-saving dugs perform their function through enzyme inhibition pathways. The discovery of new enzyme inhibition has led to many interesting discoveries in drug development. We have reported earlier, a number of 2,3-dihydro and 2,3,4,5-tetrahydrobenzothiazepines as inhibitors of cholinesterase,[14] having potential to develop into drugs against Alzheimer's disease.[21] Continuing our on-going research for new inhibitors of cholinesterases, some of the synthesized benzothiazepines were screened for their potential as cholinesterase inhibitors and quite a few compounds were found to have valuable butyrylcholinesterase inhibitory characteristic as shown in Table 5. It is noteworthy that the same set of compounds did not show significant activity against acetylcholinesterase with the exception of compound **49**.

We have reported earlier the cholinesterase inhibitory potential of some benzothiazepines with ring A as unsubstituted and with a hydroxyl group at C-2 position.[14] However, these compounds were

Table 5
In vitro quantitative inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)

Compound	R	IC_{50} (μM)	
		AChE	BChE
27	Phenyl	40.0 ± 0.7	1421.0 ± 0.01
28	2-Cl C_6H_4	—	—
29	3-Cl C_6H_4	—	29.4 ± 1.6
30	4-Cl C_6H_4	18.8 ± 1.5	79.6 ± 6.8
31	2-F C_6H_4	70.6 ± 1.5	—
35	3-OH C_6H_4	71.1 ± 2.2	4.7 ± 0.1
36	4-OH C_6H_4	—	—
37	2-OMe C_6H_4	—	4.65 ± 0.1
40	3,4-diMe C_6H_3	—	19.7 ± 1.7
41	3-OMe, 4-OH C_6H_3	—	—
42	3-NO_2 C_6H_4	—	—
43	4-NO_2 C_6H_4	—	—
44	4-Me C_6H_4	49.4 ± 0.03	75.2 ± 2.1
45	4-NMe_2 C_6H_4	—	34.4 ± 0.7
47	3-Pyridyl	—	—
49	2-Thienyl	5.9 ± 0.45	3.97 ± 0.02
Galanthamine	—	0.5 ± 0.01	8 ± 0.05

generally found to be inactive. It seems that substitution of a hydroxyl group at C-3 played an important role in increasing the inhibitory potential of these compounds against butyrylcholinesterase. Molecular docking studies are being carried out to have a better insight into their mechanism of inhibitory action.

It may be seen from Table 5 that of the screened benzothiazepines most of the compounds were found to be good inhibitors of butyrylcholinesterase with IC_{50} varying from 3.97 to 1421 ppm. With IC_{50} of 5.9 and 3.97 ppm for AChE and BChE, respectively, compound **49** was found to be the most active member of the library. This is the only compound that showed activity against both AChE and BChE. The other benzothiazepines **35** and **37** were found to inhibit BChE significantly with IC_{50} 4.70 and 4.65 ppm, respectively. Moreover, 3-OH-, and 2-OMe-substituted benzothiazepines also emerged as good inhibitors of butyrylcholinesterase. It is important to note that all three compounds can be regarded as better inhibitors of butyrylcholinesterase than the standard galanthamine (IC_{50} 8 ± 0.05). In contrast to butyrylcholinesterase inhibition, most of the compounds were found to be inactive against acetylcholinesterase.

4. Conclusion

Solid-phase synthesis of 2,3-dihydro-1,5-benzothiazepines led to an improved yield and better purity when compared to their synthesis in solution phase. Compounds were tested for their potential as antibacterial, cholinesterase inhibitors and potato disk tumor inhibitors. Most of the tested compounds were found to be good inhibitors of potato disk tumors and may be considered as promising candidates for developing into antitumor drugs. Benzothiazepines **35, 37** and **49** were found to be better butyrylcholinesterase inhibitors than the standard galanthamine. None of the compounds showed significant activity against any bacterial strain.

5. Experimental

^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer in d_6-acetone or d_6-DMSO using tetramethylsilane as internal reference. ^{1}H NMR spectra were recorded at 300 MHz while ^{13}C NMR spectra were recorded at 75 MHz. Mass spectra were measured on Agilent GC–MS gas chromatography mass spectrometer. Melting points were determined on a MEL-Temp apparatus and are un-corrected.

5.1. Solution-phase synthesis

A solution-phase synthesis of 2,3-dihydro-1,5-benzothiazepines was carried out using chalcones as precursors which in turn were synthesized through Claisen-Schmidt condensation of 3-hydroxy acetophenone with different substituted aryl and heteroaryl aldehydes.

5.1.1. General method for the synthesis of chalcones (1–26)

All chalcones of the parallel library were synthesized by the following literature procedure[22]: A 4.0-M solution of sodium hydroxide (30 ml, 4.8 mmol) was added to precooled rectified spirit (17 ml) in a round-bottomed flask. 3′-Hydroxyacetophenone (5 mmol) and substituted aldehyde (5 mmol) were added to the cold mixture with stirring. The reaction mixture was stirred for 3–5 h and monitored for its completion through TLC. When the reaction was found to be complete, the reaction mixture was kept at 0–4 °C overnight. The solid mass obtained, thereby, was cooled and neutralized with ice-cooled dilute hydrochloric acid. The precipitates separated were filtered and recrystallized from distilled ethanol.

5.1.2. General method for the synthesis of 2,3-dihydro-1,5-benzothiazepines (27–52)

To a solution of chalcone (1 g/20 mmol) in dry acidic methanol acidified by adding few drops of glacial acetic acid, was added *o*-aminothiophenol (2.15 ml, 20 mmol). The mixture was refluxed until a crystalline solid separated out. After cooling the solid product was collected and washed with diethyl ether and cold methanol. The crude solid was recrystallized from ethanol.

5.2. Solid-phase synthesis

Solid-phase synthesis of 2,3-dihydro-1,5-benzothiazepines involved the following steps.

5.2.1. Synthesis of resin-bound acetophenone

3-Hydroxy acetophenone (7.35 mmol) were dissolved in 10 ml of NMM followed by the addition of 0.66 g of TPP. After that 0.444 ml of DIAD was added dropwise for 15 min. The resulting yellow solution was stirred for 5 h at room temperature. The resin-bound acetophenone was then filtered and washed successively and extensively with CH_2Cl_2/THF (2:1), THF/H_2O (1:1), MeOH/THF (1:1), MeOH/CH_2Cl_2 (6 ml each) and dried under high vacuum overnight (12 h). The substitution was confirmed by cleavage with 50% TFA in CH_2Cl_2 (1 h at room temperature) 21.

5.2.2. Synthesis of resin-bound chalcones

The resin-bound acetophenone was added to a solution of aryl aldehydes (3 equiv) in dry THF (25 ml). A solution of KOH (6 equiv) in ethanol was added over a period of 15 min. The flask was shaken for 24 h at room temperature. The solution was removed by filtering the resin. The filtered resin-bound chalcone was washed successively with 30% aqueous AcOH, H_2O, THF, MeOH, THF, and CH_2Cl_2 (all by 2× 40 ml), followed by drying under high vacuum for 24 h.

5.2.3. Synthesis of resin-bound 2,3-dihydro-1,5-benzothiazepines

The resin-bound chalcone was suspended in a solution of *o*-aminothiophenol in DMF and was refluxed for about 18 h. A few drops of glacial acetic acid were added to the reaction mixture as catalyst. The resin was washed successively with MeOH, CH_2Cl_2, DMF, THF, diethyl ether, acetone, and ethyl acetate. It was then dried under vacuum overnight. The product was cleaved with 50% TFA in CH_2Cl_2 (1 h at room temperature). The cleaved product was directly submitted for GC–MS analysis.

5.2.3.1. 2-(Phenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (27). Yield 71%; mp 102 °C; ^{1}H NMR (DMSO-d_6): δ 8.60 (s, 1H, O*H*), 6.93–7.70 (m, 13H, aryl H), 4.16 (dd, $J_{2,3a}$ = 5.0 Hz, $J_{2,3b}$ = 12.0 Hz, 1H, H-2), 3.23 (dd, $J_{3a,3b}$ = 12.0 Hz, $J_{3a,2}$ = 5.0 Hz, 1H, H-3a), 2.53 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.0 Hz, 1H, H-3b); ^{13}C NMR: δ 160.0 (C-4), 113.0–157.9 (aryl C), 45.5 (C-2), 28.2 (C-3); EIMS (*m/z*, %): 330 [M$^{+\cdot}$, 6].

5.2.3.2. 2-(2″-Chlorophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (28). Yield 81%; mp 45–47 °C; ^{1}H NMR (DMSO-d_6): δ 10.05 (s, 1H, O*H*), 7.09–8.43 (m, 12H, aryl H), 4.89 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 9.0 Hz, 1H, H-2), 3.43 (dd, $J_{3b,3a}$ = 14.0 Hz, $J_{3b,2}$ = 9.0 Hz, 1H, H-3b), 3.36 (dd, $J_{3a,3b}$ = 14.0 Hz, $J_{3a,2}$ = 5.1 Hz, 1H, H-3a); ^{13}C NMR: δ 167.5 (C-4), 115.2–162.2 (aryl C), 55.9 (C-2), 27.2 (C-3); EIMS (*m/z*, %): 367 [M$^{+\cdot}$+2, 3], 366 [M$^{+\cdot}$+1, 1], 365 [M$^{+\cdot}$, 9].

5.2.3.3. 2-(3″-Chlorophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (29). Yield 80%; mp 100–101 °C; ^{1}H NMR (DMSO-d_6): δ 10.0 (s, 1H, O*H*), 7.10–8.03 (m, 12H, aryl H), 4.32 (dd, J_2, $J_{2,3a}$ = 5.0 Hz, J_{3b} = 12.9 Hz, 1H, H-2), 3.74 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.6 Hz, 1H, H-3b), 3.51 (dd, $J_{3a,3b}$ = 12.9 Hz, $J_{3a,2}$ = 5.0 Hz, 1H, H-3a); ^{13}C NMR: δ 165.0 (C-4), 118.0–158.7 (aryl C), 56.3 (C-2), 33.5 (C-3); EIMS (*m/z*, %): 367 [M$^{+\cdot}$+2, 2], 366 [M$^{+\cdot}$+1, 1], 365 [M$^{+\cdot}$, 6].

5.2.3.4. 2-(4″-Chlorophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (30). Yield 87%; mp 114 °C; ^{1}H NMR (DMSO-d_6): δ 10.05 (s, 1H, O*H*), 7.09–8.44 (m, 12H, aryl H), 5.0 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12.9 Hz, 1H, H-2), 3.53 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, H-3b), 3.39 (dd, $J_{3a,3b}$ = 12.9 Hz, $J_{3a,2}$ = 5.1 Hz, 1H, H-3a); ^{13}C NMR: δ 160.0 (C-4), 113.0–157.9 (aryl C), 45.5 (C-2), 28.2 (C-3); EIMS (*m/z*, %): 367 [M$^{+\cdot}$+2, 3], 366 [M$^{+\cdot}$+1, 1], 365 [M$^{+\cdot}$, 10].

5.2.3.5. 2-(2″-Fluorophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (31). Yield 87%; mp 68–70 °C; ^{1}H NMR (DMSO-d_6): δ 9.72 (s, 1H, O*H*), 6.96–7.59 (m, 12H, aryl H), 5.31 (dd, $J_{2,3b}$ = 12.6 Hz, $J_{2,3a}$ = 9.9 Hz, 1H, H-2), 3.36 (dd, $J_{3a,3b}$ = 12.6 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, H-3a), 2.87 (t, $J_{3b,2}$ = $J_{3b,3a}$ = 12.6 Hz, 1H, H-3b); ^{13}C NMR: δ 168.9 (C-4), 114.0–160.6 (aryl C), 53.3 (C-2), 35.3 (C-3); EIMS (*m/z*, %): 349 [M$^{+\cdot}$, 7].

5.2.3.6. 2-(3″-Fluorophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (32). Yield 73%; mp 73 °C; ^{1}H NMR (DMSO-d_6): δ 9.95 (s, 1H, O*H*), 7.90–6.59 (m, 12H, aryl H), 5.10 (dd, $J_{2,3a}$ = 9.9 Hz, $J_{2,3b}$ = 12.9 Hz, 1H, H-2), 3.27 (dd, $J_{3a,3b}$ = 12.9 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, H-3a), 2.66 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, H-3b); ^{13}C NMR: δ 162.0 (C-4), 113.0–164.7 (aryl C), 51.4 (C-2), 32.8 (C-3); EIMS (*m/z*, %): 349 [M$^{+\cdot}$, 7].

5.2.3.7. 2-(4″-Fluorophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (33). Yield 93%; mp 79–80 °C; ^{1}H NMR (DMSO-d_6): δ 8.60 (s, 1H, O*H*), 7.06–8.19 (m, 12H, aryl H), 5.27 (dd, $J_{2,3b}$ = 12.9 Hz, $J_{2,3a}$ = 5.0 Hz, 1H, H-2), 3.50 (dd, $J_{3a,3b}$ = 12.9 Hz, $J_{3a,2}$ = 5.0, 1H, H-3a), 2.97 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, H-3b); ^{13}C NMR: δ 168.3 (C-4), 113.9–163.3 (aryl C), 59.5 (C-2), 37.3 (C-3); EIMS (*m/z*, %): 349 [M$^{+\cdot}$, 8].

5.2.3.8. 2-(4″-Bromophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (34). Yield 82%; mp 198 °C; ^{1}H NMR (DMSO-d_6): δ 9.78 (s, 1H, O*H*), 7.02–7.48 (m, 12H, aryl H), 4.94 (dd, $J_{2,3a}$ = 10.0 Hz, $J_{2,3b}$ = 12.9 Hz, 1H, H-2), 3.25 (dd, $J_{3a,3b}$ = 12.9 Hz, $J_{3a,2}$ = 10.0 Hz, 1H, H-3a), 3.14 (t, $J_{3b,2}$ = $J_{3b,3a}$ = 12.9 Hz, 1H, H-3b); ^{13}C NMR: δ 165.0 (C-4), 118.2–158.4 (aryl C), 50.1 (C-2), 40.0 (C-3); EIMS (*m/z*, %): 411 [M$^{+\cdot}$+2, 9], 409 [M$^{+\cdot}$, 10].

5.2.3.9. 2-(3″-Hydroxyphenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (35). Yield 70%; mp 142 °C dec; ^{1}H NMR (DMSO-d_6): δ 8.89 and 8.63 (s, 2H, O*H*), 6.87–8.06 (m, 12H, aryl H), 3.85 (d, $J_{2,3b}$ = 6.3 Hz, 1H, H-2), 3.34 (unresolved, 1H, H-3a), 2.41 (d, $J_{3b,2}$ = 6.3 Hz, 1H, H-3b); ^{13}C NMR: δ 157.0 (C-4), 113.0–157.9 (aryl C), 45.5 (C-2), 28.2 (C-3); EIMS (*m/z*, %): 347 [M$^{+\cdot}$, 9].

5.2.3.10. 2-(4″-Hydroxyphenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (36). Yield 53%; mp 170–172 °C; ^{1}H NMR (DMSO-d_6): δ 8.71 and 8.49 (s, 2H, O*H*), 6.76–8.00 (m, 12H, aryl H), 3.58 (t, $J_{2,3a}$ = $J_{2,3b}$ = 7.2 Hz, 1H, H-2), 3.06 (unresolved, 1H, H-3a), 2.87 (unresolved, 1H, H-3b); ^{13}C NMR: δ 157.1 (C-4), 113.0–147.0 (aryl C), 40.2 (C-2), 28.0C-3; EIMS (*m/z*, %): 347 [M$^{+\cdot}$, 8].

5.2.3.11. 2-(2″-Methoxyphenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (37). Yield 61%; mp 62–64 °C; ^{1}H NMR (d_6-acetone): δ 8.11 (s, 1H, O*H*), 7.04–8.08 (m, 12H, aryl H), 3.58 (t, $J_{2,3b}$ = 6.9 Hz, 1H, H-2), 2.87 (unresolved, 1H, H-3a*), 2.50 (s, 3H, O-C*H*$_3$), 1.036 (t, $J_{3b,2}$ = 6.9 Hz, 1H, H-3b*); ^{13}C NMR: δ 162.7 (C-4), 114.0–158.3 (aryl C), 56.0 (O-*C*H$_3$), 43.0 (C-2), 40.2 (C-3); EIMS (*m/z*, %): 361[M$^{+\cdot}$, 5].

5.2.3.12. 2-(3″-Methoxyphenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiaze-pine (38). Yield 59%; mp 58 °C; ^{1}H NMR (d_6-acetone): δ 8.63 (s, 1H, O*H*), 6.77–7.31 (m, 12H, aryl H), 3.41 (d, $J_{2,3b}$ = 7.1 Hz, 1H, H-2), 3.0 (unresolved, 1H, H-3a), 2.76 (s, 3H, O-C*H*$_3$), 1.65 (d, $J_{3b,2}$ = 7.1 Hz, 1H, H-3b); ^{13}C NMR: δ 161.6 (C-4), 115.0–158.0 (aryl C), 57.0 (O-*C*H$_3$), 41.7 (C-2), 39.0 (C-3); EIMS (*m/z*, %): 361 [M$^{+\cdot}$, 8].

5.2.3.13. 2-(4″-Methoxyphenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (39). Yield 56%; mp 80 °C; ^{1}H NMR (d_6-acetone): δ 9.10 (s, 1H, O*H*), 6.23–7.09 (m, 12H, aryl H), 3.83 (s, 3H, O-C*H*$_3$), 3.30 (d, $J_{2,3b}$ = J = 7.0 Hz, 1H, H-2), 2.9 (unresolved, 1H, H-3a*), 1.0 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 6.9 Hz, 1H, H-3b*); ^{13}C NMR: δ 159.6 (C-4), 114.7–158.0 (aryl C), 59.0 (O-*C*H$_3$), 43.5 (C-2), 40.0 (C-3); EIMS (*m/z*, %): 361 [M$^{+\cdot}$, 10].

5.2.3.14. 2-(3″,4″-Dimethoxyphenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzo-thiazepine (40). Yield 61%; mp 96 °C; ^{1}H NMR (d_6-acetone): δ 9.7 (s, 1H, O*H*), 6.46–7.15 (m, 12H, aryl H), 3.80 and 3.77 (s, 3H, 3″, 4″O-C*H*$_3$), 3.06 (d, $J_{2,3b}$ = 6.6 Hz, 1H, H-2), 2.83 (unresolved, 1H, H-3a), 2.0 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 6.6 Hz, 1H, H-3b); ^{13}C NMR: δ 162.1 (C-4), 112.0–157.0 (aryl C), 61.0 and 59.0 ((3″,4″-O-*C*H$_3$), 49.5 (C-2), 39.7 (C-3); EIMS (*m/z*, %): 391 [M$^{+\cdot}$, 6].

5.2.3.15. 2-(4″-Hydroxy-3″-methoxyphenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (41). Yield 55%; mp 108 °C; ^{1}H NMR (DMSO-d_6): δ 8.1 and 8.08 (s, 2H, O*H*), 7.04–8.04 (m, 11H, aryl H), 3.83 (s, 3H,O-C*H*$_3$), 3.43 (d, $J_{2,3b}$ = J = 14.1 Hz, 1H, H-2), 2.50 (unresolved, 1H H-3a), 1.03 (d, $J_{3b,2}$ = 14.1 Hz, 1H, H-3b); ^{13}C NMR: δ 158.3 (C-4), 113.0–157.9 (aryl C), 55.9 (O-*C*H$_3$), 45.5 (C-2), 28.2 C-3); EIMS (*m/z*, %): 376 [M$^{+\cdot}$, 5].

5.2.3.16. 2-(3″-Nitrophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (42). Yield 83%; mp 82–84 °C; ^{1}H NMR (DMSO-d_6): δ 9.70 (s, 1H, O*H*), 6.96–8.22 (m, 12H, aryl H), 5.42 (dd, $J_{2,3a}$ = J = 9.0 Hz, $J_{2,3b}$ = 12.6 Hz, 1H, H-2), 3.38 (dd, $J_{3a,3b}$ = 12.9 Hz, $J_{3a,2}$ = 9.0 Hz, 1H, H-3a), 2.86 (t, $J_{3b,2}$ = $J_{3b,3a}$ = 12.9 Hz, 1H, H-3b); ^{13}C NMR: δ 169.0 (C-4), 114.1–158.1 (aryl C), 58.0 (C-2), 37.0 (C-3); EIMS (*m/z*, %): 376 [M$^{+\cdot}$, 7].

5.2.3.17. 2-(4″-Nitrophenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (43). Yield 90%; mp 73 °C; ^{1}H NMR (DMSO-

d_6): δ 9.70 (s, 1H, O*H*), 6.96–8.22 (m, 12H, aryl H), 5.42 (dd, $J_{2,3b}$ = 12.6 Hz, $J_{2,3a}$ = 4.8 Hz, 1H, H-2), 3.47 (dd, $J_{3a,3b}$ = 12.6 Hz, $J_{3a,2}$ = 4.8 Hz, 1H, H-3a), 3.10 (t, $J_{3b,2}$ = $J_{3b,3a}$ = 12.6 Hz, 1H, H-3b); ^{13}C NMR: δ 179.8 (C-4), 115.1–167.3 (aryl C), 51.7 (C-2), 24.7 (C-3); EIMS (*m/z*, %): 376 [$M^{+\cdot}$, 5].

5.2.3.18. 2-(4″-Methylphenyl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (44). Yield 71%; mp 97 °C; ^{1}H NMR (d_6-acetone): δ 9.70 (s, 1H, O*H*), 6.23–7.48 (m, 12H, aryl H), 4.94 (dd, $J_{2,3a}$ = 4.8 Hz, $J_{2,3b}$ = 12.6 Hz, 1H, H-2), 3.25 (dd, $J_{3a,3b}$ = 12.6 Hz, $J_{3a,2}$ = 4.8 Hz, 1H, H-3a), 3.04 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.6 Hz, 1H, H-3b), 2.35 (s, 3H, C*H*$_3$); EIMS (*m/z*, %): 345 [$M^{+\cdot}$, 8].

5.2.3.19. 2-(4″-N,N-Dimethylamino)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (45). Yield 69%; mp 100 °C; ^{1}H NMR (DMSO-d_6): δ 8.45 (s, 1H, O*H*), 6.77–7.49 (m, 12H, aryl H), 4.96 (dd, $J_{2,3a}$ = J = 5.1 Hz, $J_{2,3b}$ = 12.0 Hz, 1H, H-2), 3.83 (s, 6H, N-(C*H*$_3$)$_2$), 3.26 (dd, $J_{3a,3b}$ = 12 Hz, $J_{3a,2}$ = 5.1 Hz, 1H, H-3a), 3.04 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12 Hz, 1H, H-3b); ^{13}C NMR: δ 167.0 (C-4), 115.2–162.2 (aryl C), 55.9 (C-2), 40.7 (N (*C*H$_3$)$_2$), 27.2 (C-3); EIMS (*m/z*, %): 376 [$M^{+\cdot}$+2, 1], 374 [$M^{+\cdot}$, 10].

5.2.3.20. 2-(Pyridin-2″-yl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (46). Yield 51%; mp 110–114 °C; ^{1}H NMR (DMSO-d_6): δ 9.52 (s, 1H, O*H*), 6.40–8.61 (m, 12H, aryl H), 4.91 (dd, $J_{2,3a}$ = 12.3 Hz, $J_{2,3b}$ = 14.4 Hz, 1H, H-2), 3.44 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 12.30 Hz, 1H, H-3a), 1.05 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 14.4 Hz, 1H, H-3b); ^{13}C NMR: δ 162.3 (C-4), 115.0–156.1 (aryl C), 50.2 (C-2), 37.9 (C-3); EIMS (*m/z*, %): 332 [$M^{+\cdot}$, 1].

5.2.3.21. 2-(Pyridin-3″-yl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (47). Yield 50%; mp 88–91 °C; ^{1}H NMR (DMSO-d_6): δ 8.62 (s, 1H,O*H*), 6.42–8.30 (m, 12H, aryl H), 4.38 (dd, $J_{2,3a}$ = 9.9 Hz, $J_{2,3b}$ = J = 12.9 Hz, 1H, H-2), 3.37 (dd, $J_{3a,3b}$ = 12.9 Hz, $J_{3a,2}$ = 9.9 Hz, 1H), 1.07 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, H-3b); ^{13}C NMR: δ 161.7 (C-4), 115.0–159.1 (aryl C), 49.1 (C-2), 30.1 (C-3); EIMS (*m/z*, %): 332 [$M^{+\cdot}$, 1].

5.2.3.22. 2-(Pyridin-4″-yl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (48). Yield. 48%; mp 119 °C dec; ^{1}H NMR (DMSO-d_6): δ 8.60 (s, 1H, O*H*), 7.65–7.91 (m, 12H, aryl H), 4.67 (dd, $J_{2,3a}$ = 10.0 Hz, $J_{2,3b}$ = J = 14.0 Hz, 1H, H-2), 3.42 (dd, $J_{3a,3b}$ = 14.0, $J_{3a,2}$ = 10.0 Hz, 1H, H-3a), 2.50 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 14.0 Hz, 1H, H-3b); ^{13}C NMR: δ 160.0 (C-4), 113.0–157.9 (aryl C), 45.5 (C-2), 28.2 (C-3); EIMS (*m/z*, %): 332 [$M^{+\cdot}$, 1].

5.2.3.23. 2-(Thien-2″-yl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (49). Yield 70%; mp 55 °C; ^{1}H NMR (DMSO-d_6): δ 8.41 (s, 1H, O*H*), 6.96–7.70 (m, 11H, aryl H), 5.50 (dd, $J_{2,3a}$ = 12.6 Hz, $J_{2,3b}$ = J = 13.2 Hz, 1H, H-2), 3.53 (dd, $J_{3a,3b}$ = 13.2 Hz, $J_{3a,2}$ = 12.6 Hz, 1H, H-3a), 2.90 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 13.2 Hz, 1H, H-3b); ^{13}C NMR: δ 168.2 (C-4), 113.9–157.7 (aryl C), 55.7 (C-2), 38.0 (C-3); EIMS (*m/z*, %): 339 [$M^{+\cdot}$+2, 12], 338 [$M^{+\cdot}$+1, 3], 337 [$M^{+\cdot}$, 9].

5.2.3.24. 2-(5″-Methylthien-2″-yl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (50). Yield 45%; mp 94–96 °C; ^{1}H NMR (DMSO-d_6): δ 8.30 (s, 1H, O*H*), 6.53–7.26 (m, 10H, aryl H), 4.61 (dd, $J_{2,3a}$ = 6.5 Hz, $J_{2,3b}$ = 12.3 Hz, 1H, H-2), 3.20 (dd, $J_{3a,3b}$ = 12.3 Hz, $J_{3a,2}$ = 6.5 Hz, 1H, H-3a), 2.78 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.3 Hz, 1H, H-3b), 1.91 (s, 3H, C*H*$_3$); ^{13}C NMR: δ 161.8 (C-4), 118.0–158.1 (aryl C), 50.6 (C-2), 38.9 (C-3), 16.0 (*C*H$_3$); EIMS (*m/z*, %): 353 [$M^{+\cdot}$+2, 10], 352 [$M^{+\cdot}$+1, 1], 351 [$M^{+\cdot}$, 9].

5.2.3.25. 2-(5″-Bromothien-2″-yl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (51). Yield 49%; mp 76 °C dec; ^{1}H NMR (DMSO-d_6): δ 9.35 (s, 1H, O*H*), 6.33–7.29 (m, 10H, aryl H), 5.07 (dd, $J_{2,3a}$ = 12.6 Hz, $J_{2,3b}$ = 13.2 Hz, 1H, H-2), 4.10 (dd, $J_{3a,3b}$ = 13.2 Hz, $J_{3a,2}$ = 12.6 Hz, 1H, H-3a), 3.39 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 13.2 Hz, 1H, H-3b); ^{13}C NMR: δ 163.0 (C-4), 109.0–158.1 (aryl C), 53.8 (C-2), 40.0 (C-3); EIMS (*m/z*, %): 416 [$M^{+\cdot}$+2, 7], 415 [$M^{+\cdot}$+1, 1], 414 [$M^{+\cdot}$, 8].

5.2.3.26. 2-(Pyrrol-2″-yl)-4-(3′-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine (52). Yield 37%; mp 133 °C dec; ^{1}H NMR (DMSO-d_6): δ 8.13 (s, 1H, O*H*), 6.96–7.70 (m, 11H, aryl H), 7.26 (br s, 1H, N*H*), 4.20 (dd, $J_{2,3a}$ = 6.3 Hz, $J_{2,3b}$ = 12.0 Hz, 1H, H-2), 3.41 (dd, $J_{3a,3b}$ = 12.0 Hz, $J_{3a,2}$ = 6.3 Hz, 1H, H-3a), 3.00 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.0 Hz, 1H, H-3b); ^{13}C NMR: δ 160.3 (C-4), 109.0–131.2 (aryl C), 53.2 (C-2), 34.7 (C-3); EIMS (*m/z*, %): 321 [$M^{+\cdot}$+1, 1], 320 [$M^{+\cdot}$, 3].

5.3. Biological screening

The synthesized compounds were screened for their potential as antibacterials, potato disk tumor and as cholinesterase inhibitors.

5.3.1. Antibacterial assay

All the synthesized compounds were tested against six bacterial strains namely: *B. bronchiseptica* ATCC 4617, *M. leuteus* ATCC, *P. picketti* ATCC 49129, *E. coli* ATCC 1522, *E. aerogenes* ATCC 13048, and *S. setubal* ATCC 19196. *Agar Well diffusion method* was used for these studies. 0.75 ml of the broth culture containing approx. 10^6 colony-forming units (CFU/ml) of testing strain was added to 75 ml of nutrient agar medium at 45 °C, mixed well, and poured into a 14-cm sterile metallic Petri plate. The medium was allowed to solidify and 8-mm wells were dug with sterile metallic borer. One hundred microliters of the test sample (1 mg/ml in DMSO) was poured in respective wells. In each plate DMSO and standard antibacterial drug (Roxithromycin 1 mg/ml) served as negative and positive controls, respectively. Triplicate plates of each bacterial strain were prepared. The plates were incubated at 37 °C for 24 h. The antibacterial activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control, Roxithromycin. All tested compounds showed non-significant antibacterial activities.

5.3.2. Potato disk tumor inhibition

The assay was performed according to a standard procedure[23], using a 48-h-old bacterial culture of the AT-10 strain of *Agrobacterium tumefaciens*. Inoculums with three concentrations of each test sample (10, 100, and 1000 ppm) containing bacterial culture was prepared. Red-skinned potatoes were surface-sterilized in 0.1% $HgCl_2$ solution. A bore of 8-mm diameter was used to create potato cylinders, which were cut into 5-mm disks. Autoclaved agar solution (1.5%) (Petri plates) was allowed to solidify. The potato disks were placed on agar, and inoculum (50 μl) was poured on each disk. The plates were sealed with *parafilm* to avoid contamination and moisture loss. The plates were then incubated at 28 °C for 21 days, the potato disks were stained with *Lugol's* solution (10% KI/5% I_2), and tumors were counted under a dissecting microscope. All the tests were performed in replicate, and the percent tumor inhibition was calculated by dividing the average number of tumors per sample by the average numbers of tumors of a control, and then multiplied by a factor of 100. IC_{50} values were calculated by the following literature procedure:

$$\%\ \text{inhibition} = 100 - \frac{\text{Average number of tumors of sample}}{\text{Average number of tumors of control}} \times 100$$

5.3.3. Cholinesterase inhibition

The synthesized 2,3-dihydro-1,5-benzothiazepines were screened for enzyme inhibition activity against acetylcholinester-

ase (AChE) and butyrylcholine esterase. IC_{50} values were calculated to show the efficiency of inhibition by these compounds. Acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates to assay acetylcholinesterase and butyrylcholinesterase, respectively. Sodium phosphate buffer (pH 8.0) (150 μl, 100 mM), 5,5′-dithio-bis(2-nitrobenzoic acid) DNTB (10 μl), test compound solution (10 μl), and acetylcholinesterase (or butyrylcholinesterase) solution (20 μl), were mixed and incubated for 15 min at 25 °C. The reaction was then initiated by the addition of 10 μl acetylthiocholine (or butyrylthiocholine) (10 μl), respectively. The hydrolysis of acetylthiocholine and butyrylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine released by the enzymatic hydrolysis of acetylthiocholine and butyrylthiocholine, respectively, at 412 nm. All test compounds and the control were dissolved in EtOH. All the reactions were performed in triplicate in a 96-well micro-plate in *SpectraMax 340* (Molecular Devices, USA). The percentage (%) inhibition was calculated as follows:

$(E–S)/E \times 100$, where E is activity of enzyme without test compound and S, the IC_{50} value were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

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