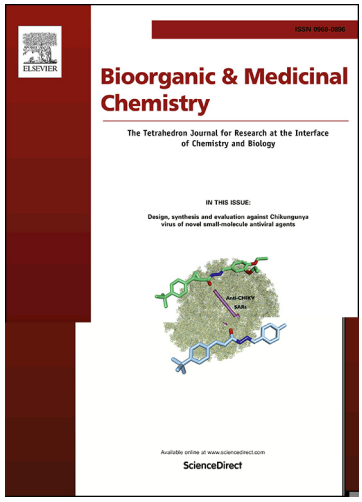
Accepted Manuscript



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PII: S0968-0896(17)32409-4
DOI: https://doi.org/10.1016/j.bmc.2018.02.049
Reference: BMC 14235

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 14 December 2017
Revised Date: 17 February 2018
Accepted Date: 27 February 2018

Please cite this article as: Mostofi, M., Mohammadi Ziarani, G., Foroumadi, A., Khoobi, M., Lashgari, N., Design, Synthesis and Biological Evaluation of Benzofuran appended Benzothiazepine Derivatives as Inhibitors of Butyrylcholinesterase and Antimicrobial Agents, *Bioorganic & Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.bmc.2018.02.049

Design, Synthesis and Biological Evaluation of Benzofuran appended Benzothiazepine Derivatives as Inhibitors of Butyrylcholinesterase and Antimicrobial Agents

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Abstract:

A series of bezofuran appended 1,5-benzothiazepine compounds **7a-v** was designed, synthesized and evaluated as cholinesterase inhibitors. The biological assay experiments showed that most of the compounds displayed a clearly selective inhibition for butyrylcholinesterase (BChE), while a weak or no effect towards acetylcholinesterase (AChE) was detected. All analogs exhibited varied BChE inhibitory activity with IC_{50} value ranging between 1.0 ± 0.01-72 ± 2.8 μM when compared with the standard donepezil (IC_{50}, 2.63±0.28 μM). Among the synthesized derivatives, compounds **7l**, **7m** and **7k** exhibited the highest BChE inhibition with IC_{50} values of 1.0, 1.0 and 1.8 μM, respectively. The results from a Lineweaver-Burk plot indicated a mixed-type inhibition for compound **7l** with BChE. In addition, docking studies confirmed the results obtained through *in*

vitro experiments and showed that most potent compounds bind to both the catalytic anionic site (CAS) and peripheral anionic site (PAS) of BChE active site. The synthesized compounds were also evaluated for their *in vitro* antibacterial and antifungal activities. The results indicated that the compounds possessed a broad spectrum of activity against the tested microorganisms and showed high activity against both gram positive and gram negative bacteria and fungi.

Keywords: Alzheimer's disease; Cholinesterase inhibitors; Specific butyrylcholinesterase inhibitor, Benzofuran; Benzothiazepine, Molecular docking

1. Introduction

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are two important types of cholinesterase species. They are mainly responsible for the hydrolysis of acetylcholine (ACh) into choline and acetic acid which is an essential process allowing for the control of the cholinergic transmission.[1] Low levels of ACh is a key pathological hallmark of Alzheimer's disease (AD).[2] AD is the leading cause of dementia and is characterized by a progressive decline in cognitive function, which typically begins with deterioration in memory.[3,4] In a healthy brain, ACh is predominantly (80%) hydrolyzed by AChE, whereas BChE plays a supplementary role. However, with progression of AD, the AChE activity decreases, whereas the activity of BChE gradually increases.[5,6] This phenomenon enhances the significance of BChE as an additional therapeutic target for reducing the cholinergic deficiency inherent in AD.[7,8] Currently, AD therapy is mainly founded on cholinesterase inhibitors, which are able to increase ACh levels in cholinergic synapses.[9] Recent studies have demonstrated that BChE inhibition results in improved cognitive

potential with elevated levels of ACh in brain and hence, it may act as an effective therapeutic strategy for AD.[10-13]

1,5-Benzothiazeoines are considered privileged scaffolds in drug discovery for cardiovascular and neurodegenerative diseases. 1,5-Benzothiazepine derivatives have a broad spectrum of therapeutic applications as coronary vasodilator,[14] Ca^{+2} channel antagonists,[15] antidepressant,[16,17] acetylcholinesterase inhibitors,[18] butyrylcholinesterase inhibitors,[19] and antimicrobial agents.[20-22] Benzofurans, a groups of naturally occurring substances in many plants, exhibit a wide range of biological activities.[23,24] Benzofuran scaffold has emerged as an important pharmacophore for designing antiviral[25,26] and antimicrobial agents[27,28] and inhibitors of cyclin-dependent kinases (CDKs)[29] and cholinesterase.[30,31] Combination of 1,5-benzothiazepine and benzofuran moiety exhibited synergistic effect thereby, enhancing their potency.

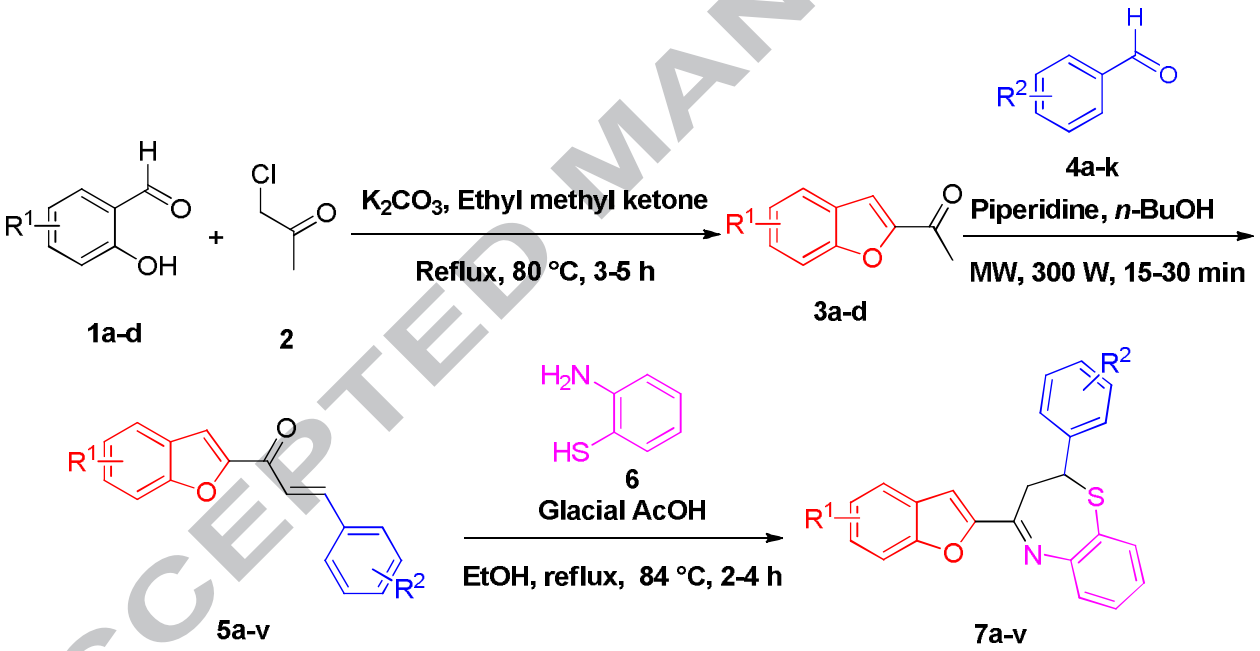
In the light of above-mentioned findings, and as a continuation of our endeavor to identify new candidates that might be advantageous in designing new, potent, selective, and less toxic cholinesterase inhibitors,[32-35] we have reported the synthesis of 1,5-benzothiazepine derivatives containing benzofuran fragments at C-4 position. All synthesized compounds were screened for their ability to inhibit the enzyme activities of BChE and their *in vitro* antimicrobial activity. To better understand the enzyme inhibition mechanisms, in relation to the substituents and their positions in the presented compounds, molecular modeling studies were also performed.

2. Results and Discussion

2.1. Chemistry

The synthesis of 1,5-benzothiazepine derivatives **7a-v** was outlined in Scheme 1. First, 2-acetylbenzofuranes **3a-d** were prepared from the ring closure reaction of salicylaldehyde

derivatives **1a-d** and chloroacetone **2** (Table 1). Then, condensation of 2-acetylbenzofuranes **3a-d** with various benzaldehydes **4a-k** under microwave irradiation in the presence of catalytic amount of piperidine gave α,β-unsaturated carbonyl compounds **5a-v** (Table 2). Finally, the thia Michael addition and further cyclocondensation reaction of compounds **5a-v** with 2-aminothiophenol **6** was accomplished in refluxing ethanol in the presence of catalytic amount of glacial acetic acid to afford designed compounds **7a-v** in good to excellent yields (70-90%). The generality of this sequence was examined by using different salicylaldehyde derivatives in the first step and (het)aryl aldehydes in the second step. It was found that substrates containing various functional groups yielded the corresponding products **7a-v** in good to excellent yields.



Scheme 1. General scheme for the synthesis of 2,3-dihydro-1,5-benzothiazepine derivatives **7a-v**.

Table 1. Synthesis of 2-acetylbenzofuranes **3a-d** from chloroacetone and salicylaldehydes.

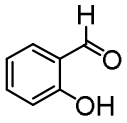
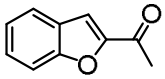
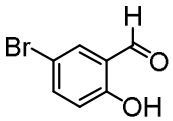
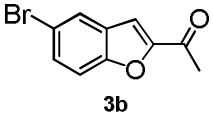
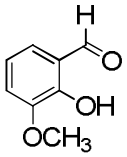
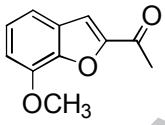
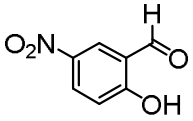
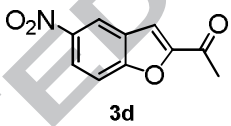
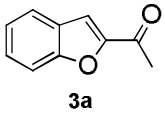
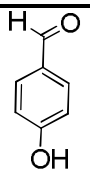
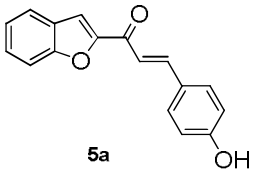
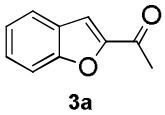
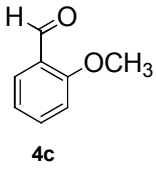
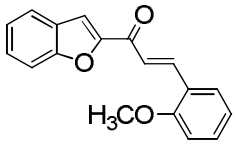
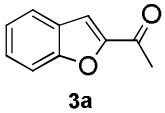
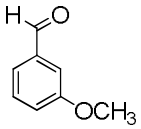
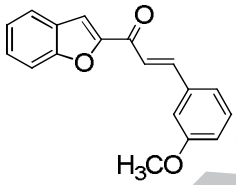
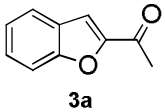
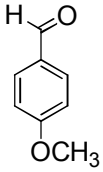
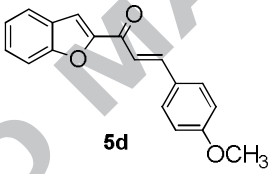
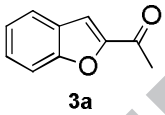
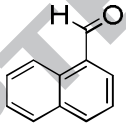
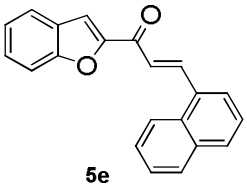
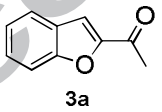
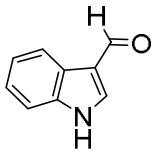
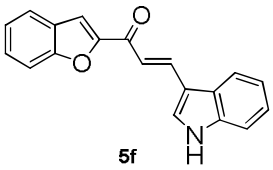
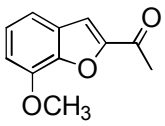
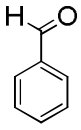
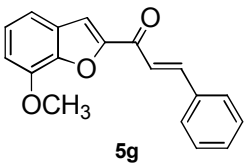
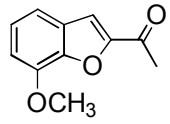
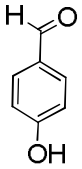
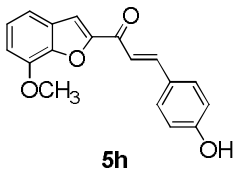
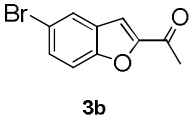
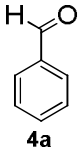
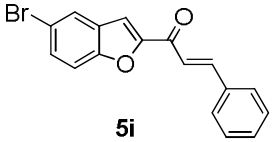
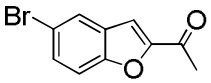
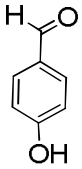
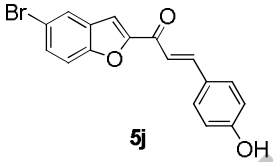
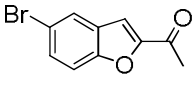
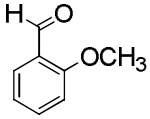
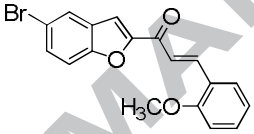
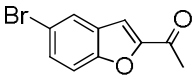
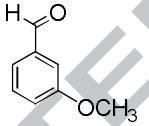
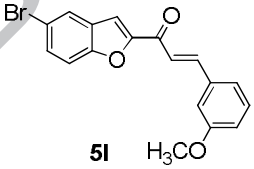
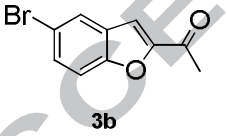
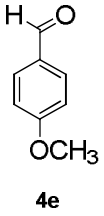
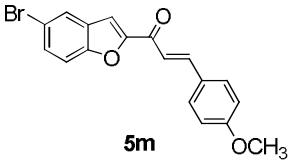
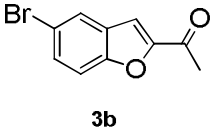
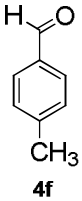
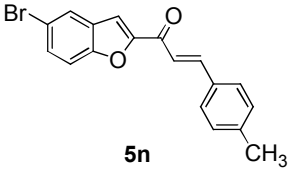
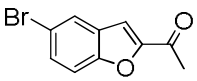
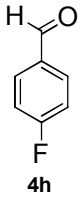
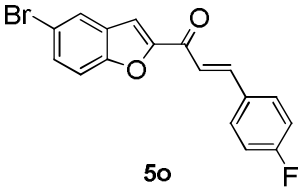
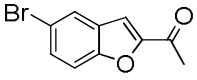
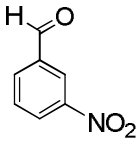
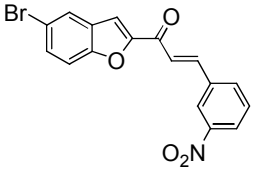
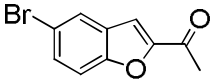
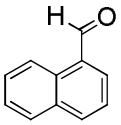
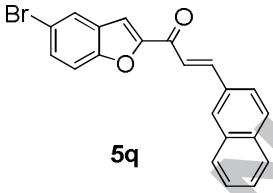
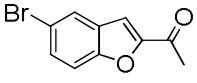
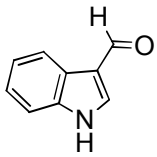
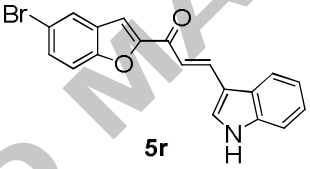
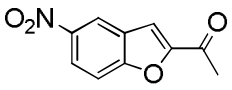
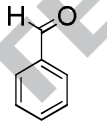
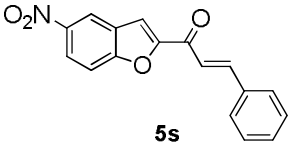
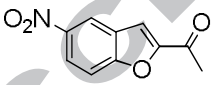
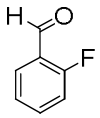
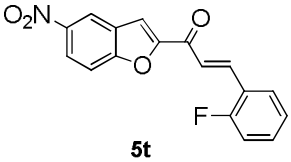
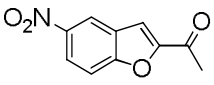
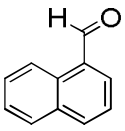
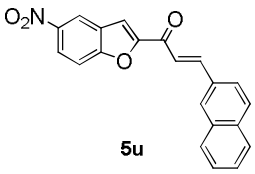
Entry	Salicylaldehyde	Product	Time (h)	Yield (%)	M.P. (ºC)	M.P. [Lit.] (ºC)
1	 **1a**	 **3a**	4	72	74-76	76 [36]
2	 **1b**	 **3b**	3	75	109-111	110 [36]
3	 **1c**	 **3c**	4	78	91-93	91 [36]
4	 **1d**	 **3d**	5	65	174-176	175 [36]

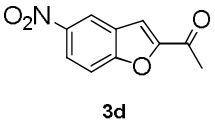
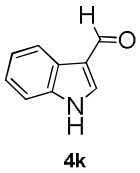
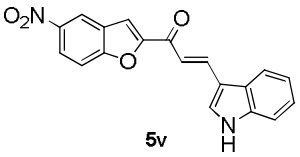
Table 2. Synthesis of chalcones **5a-v** from 2-acetylbenzofuran and benzaldehydes.

Entry	2-Acetyl benzofurane	Benzaldehyde	Chalcone	Time (min)	Yield (%)	M.P (ºC)	M.P. [Lit.] (ºC)

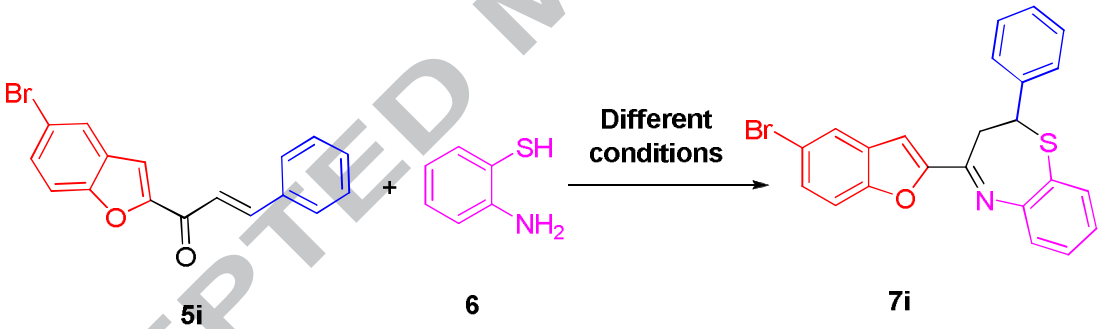
1	 **3a**	 **4b**	 **5a**	20	65	250-252	254 [37]
2	 **3a**	 **4c**	 **5b**	20	80	119-122	118-120 [38]
3	 **3a**	 **4d**	 **5c**	20	88	170-173	New
4	 **3a**	 **4e**	 **5d**	20	73	172-175	178-181 [39]
5	 **3a**	 **4j**	 **5e**	20	72	188-200	New
6	 **3a**	 **4k**	 **5f**	20	87	165-168	159-160 [40]
7	 **3c**	 **4a**	 **5g**	25	77	228-231	New

8	 **3c**	 **4b**	 **5h**	25	83	194-197	New
9	 **3b**	 **4a**	 **5i**	15	70	149-151	145-147 [41]
10	 **3b**	 **4b**	 **5j**	15	65	192-195	New
11	 **3b**	 **4c**	 **5k**	15	80	287-289	New
12	 **3b**	 **4d**	 **5l**	15	78	285-288	New
13	 **3b**	 **4e**	 **5m**	15	69	285-287	286 [42]
14	 **3b**	 **4f**	 **5n**	15	68	279-281	New

15	 **3b**	 **4h**	 **5o**	15	75	287-291	New
16	 **3b**	 **4i**	 **5p**	15	87	203-206	202-204 [41]
17	 **3b**	 **4j**	 **5q**	15	80	223-226	New
18	 **3b**	 **4k**	 **5r**	15	87	230-233	New
19	 **3d**	 **4a**	 **5s**	30	60	221-223	New
20	 **3d**	 **4g**	 **5t**	30	54	230-232	New
21	 **3d**	 **4j**	 **5u**	30	72	184-187	New

22	 3d	 4k	 5v	30	75	236-238	New

As an initial attempt for optimization of the reaction conditions, the effect of various protic and aprotic solvents such as acetonitrile, toluene, dichloromethane, methanol, and ethanol for the cyclocondensation reaction of compound **5i** with 2-aminothiophenol **6** was evaluated (Table 3). The results clearly showed that among the different tested solvents, the best result was obtained using absolute ethanol under reflux condition which led to the formation of the corresponding product **7i** in high yield (80%).



5i **6** **7i**

Table 3. The effect of solvent on the synthesis of 2,3-dihydro-1,5-benzothiazepine **7i**.

Entry	Solvent	Conditions	Time (h)	Yield (%)
1	MeOH	r.t.	24	20
2	MeOH	Reflux	8	50
3	Absolute EtOH	r.t.	24	30
4	Absolute EtOH	Reflux	2	80
5	Dry CH_2Cl_2	60 °C	24	25

6	Dry Toluene	Reflux	6	45
7	Dry CH_3CN	Reflux	6	35
8	DMF	80 °C	6	50

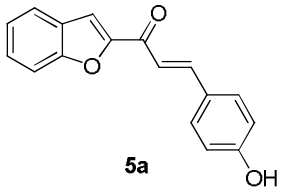
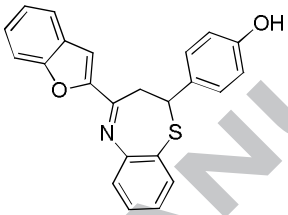
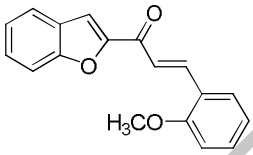
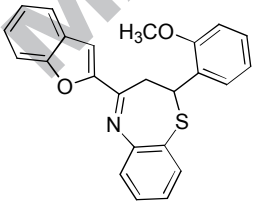
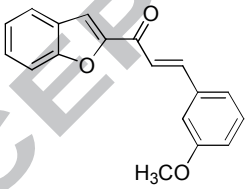
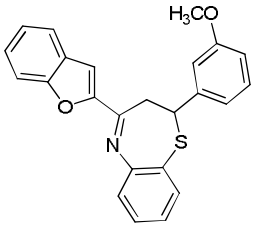
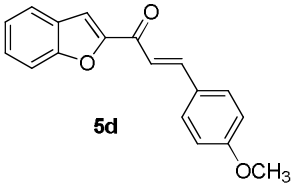
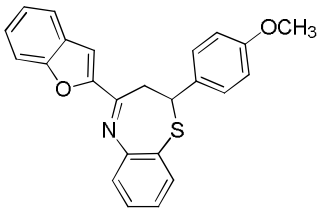
Then, evaluation of various catalytic systems for the formation of target compound **7i** was carried out. It was observed that in the absence of any catalyst, the reaction did not entirely proceed. Several catalysts such as conc. HCl, trifluoroacetic acid (TFA), *p*-toluene sulfonic acid (*p*-TSA), ammonium chloride (NH_3.HCl) and piperidine were applied in this reaction but the reaction time and product yields were not appropriate (Table 4, entries 1-5). The most encouraging result was obtained in the presence of 0.1 equivalent of glacial acetic acid as catalyst for the preparation of the model compound **7i**. Increasing the amount of glacial acetic acid accompanied by the temperature did not lead to an increase in product yields (Table 4, entries 6-8).

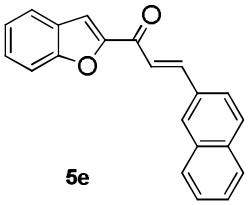
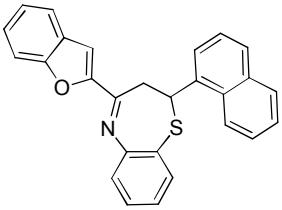
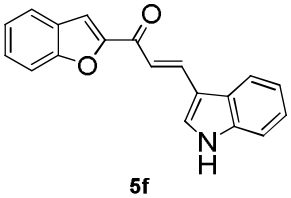
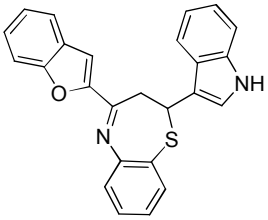
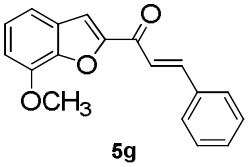
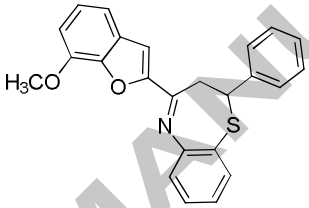
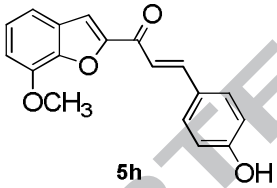
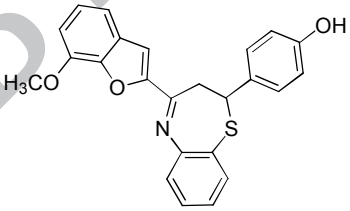
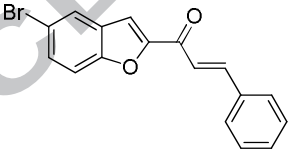
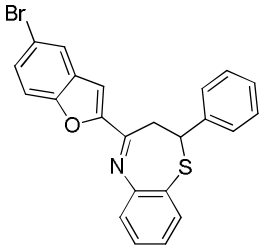
Table 4. The optimization of reaction condition for the synthesis of 2,3-dihydro-1,5-benzothiazepine **7i**.

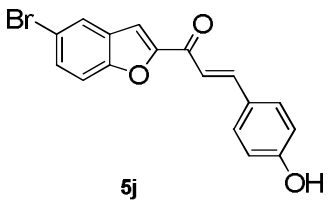
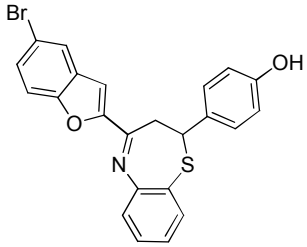
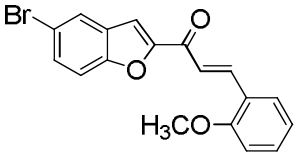
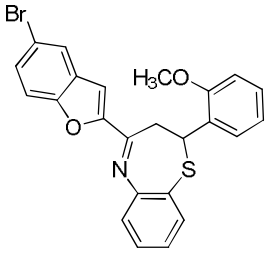
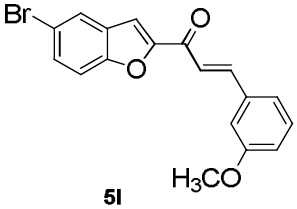
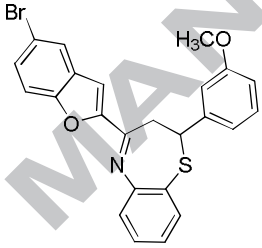
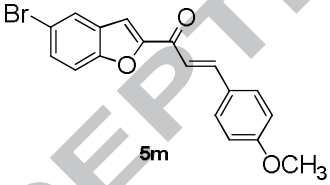
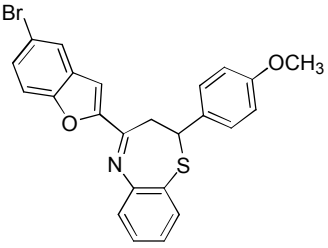
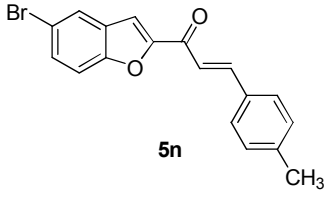
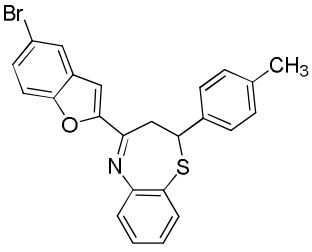
Entry	Catalyst	No. Eq.	Time (h)	Yield (%)
1	Conc. HCl	0.1	5	35
2	*p*-TSA	0.1	6	40
3	TFA	0.1	5	55
4	NH_3.HCl	0.1	5	30
5	Piperidine	0.1	6	35
6	Glacial AcOH	0.1	2	80
7	Glacial AcOH	0.2	2	80
8	Glacial AcOH	0.3	2	80

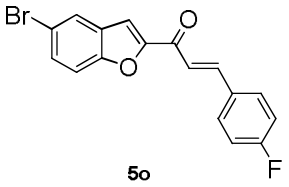
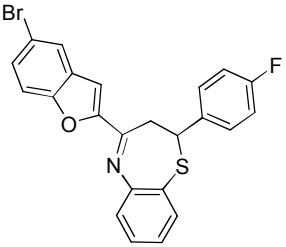
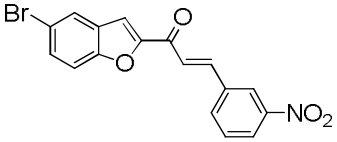
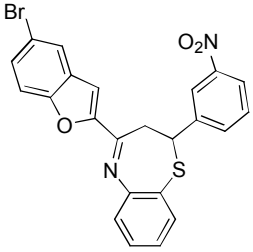
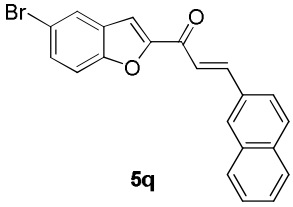
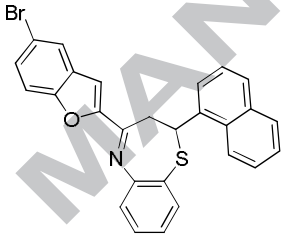
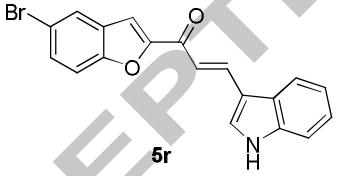
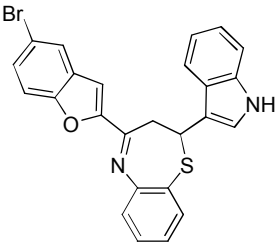
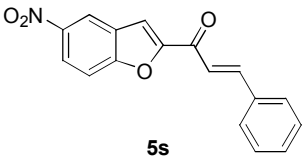
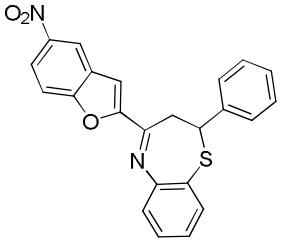
Then, in regard to library construction, we extended our study with 22 different substituted chalcones under optimized reaction condition. All the reactions underwent smoothly and provided corresponding 1,5-benzothiazepines **7a-v** in high yield (Table 5).

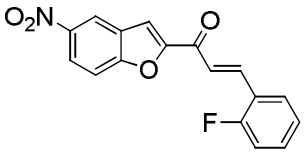
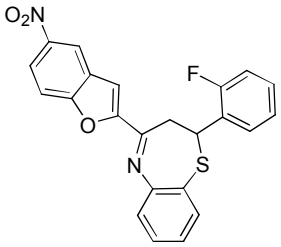
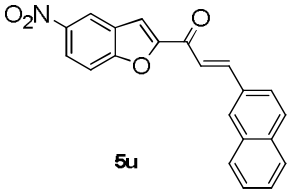
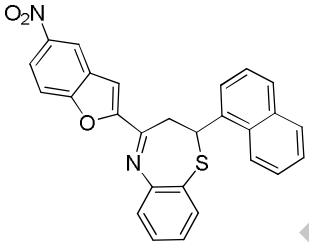
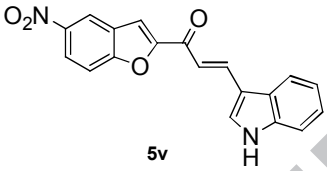
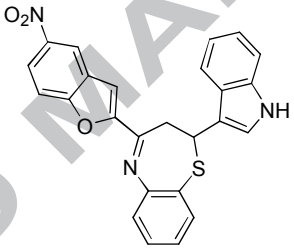
Table 5. Synthesis of 1,5-benzothiazepines **7a-v** from 2-aminothiophenol and chalcone derivatives.

Entry	Chalcone	Product	Time (h)	Yield (%)	M.P. (ºC)
1	 **5a**	 **7a**	3	79	237-239
2	 **5b**	 **7b**	3	72	176-178
3	 **5c**	 **7c**	3	80	138-140
4	 **5d**	 **7d**	3	90	176-178

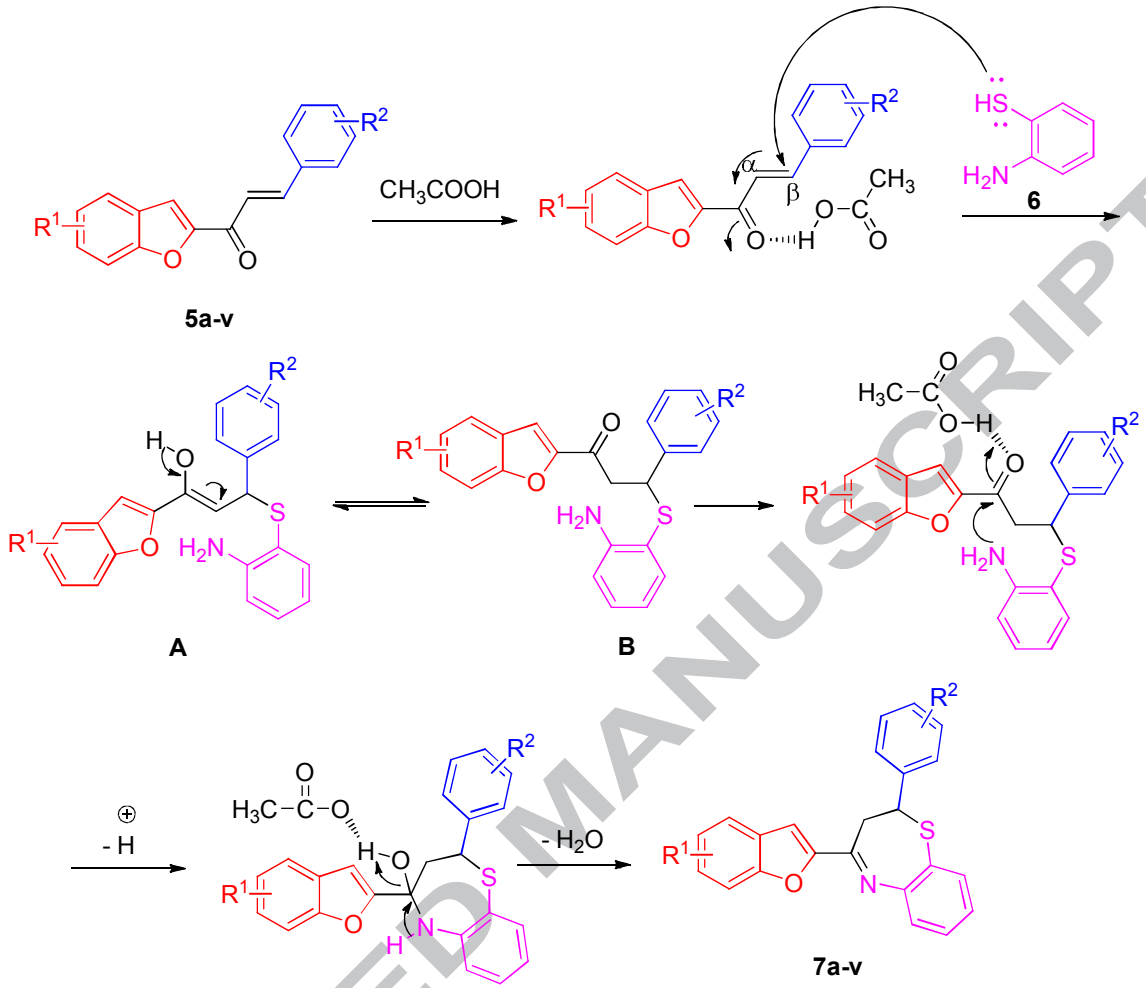
5	 **5e**	 **7e**	3	86	189-191
6	 **5f**	 **7f**	3	80	225-227
7	 **5g**	 **7g**	3	80	227-229
8	 **5h**	 **7h**	3	89	247-249
9	 **5i**	 **7i**	2	80	189-191

10	 **5j**	 **7j**	2	70	253-255
11	 **5k**	 **7k**	2	75	165-167
12	 **5l**	 **7l**	2	73	179-181
13	 **5m**	 **7m**	2	75	181-183
14	 **5n**	 **7n**	2	72	175-177

15	 **5o**	 **7o**	2	83	221-223
16	 **5p**	 **7p**	2	72	175-177
17	 **5q**	 **7q**	2	85	197-199
18	 **5r**	 **7r**	2	80	245-247
19	 **5s**	 **7s**	4	90	222-224

20	 **5t**	 **7t**	4	78	138-140
21	 **5u**	 **7u**	4	73	223-224
22	 **5v**	 **7v**	4	85	228-230

A plausible mechanism for the synthesis of compounds **7a-v** is proposed as illustrated in Scheme 2. The mechanism is most likely to be involved in the intermolecular hydrogen bonding promoted by acetic acid that activates chalcone towards the nucleophilic attack by sulfur in 2-aminothiophenol to afford the enol intermediate **A**. Then, 1,3-hydrogen shift occurs to give an isomeric keto **B** that cyclizes to yield the seven-membered ring product.[43]



Scheme 2. Proposed mechanism for the preparation of benzothiazepine derivatives.

2.2. Butyrylcholinesterase inhibition and preliminary SAR studies

We designed and synthesized 22 benzofuran-appended benzothiazepine analogues and determined their ability to inhibit BChE using Ellman spectrophotometric method.[44] Structurally, the designed compounds can be divided into two series: benzofuran-benzothiazepines with substituents on benzofuran scaffold and benzofuran-benzothiazepines with substituents on aryl ring. According to the enzyme inhibition data, as depicted in Table 6, most of the target compounds showed potent BChE inhibition activity with IC_{50} values within micro molar ranges. As observed, only compounds **7e**, **7f** and **7v** exerted a weak inhibitory activity towards BChE, while all other

compounds, except compounds **7p** and **7s**, inhibited BChE enzymatic activity with a varying efficiency (Table 6). In particular, compounds **7l**, **7m** and **7k** showed the lowest IC_{50} values of 1.0, 1.0, and 1.8 μM, respectively, while IC_{50} of standard donepezil hydrochloride is 2.63 μM. Thus, these compounds were chosen for the kinetic studies.

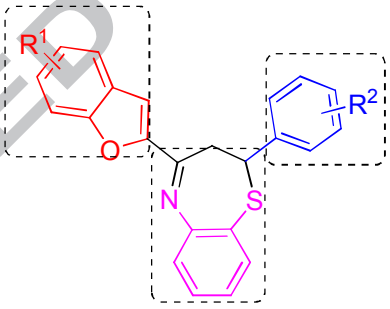
In the compounds with pendant phenyl group at C-2 position of benzothiazepine, the presence of 4-methoxy group on the phenyl ring was favorable for inhibitory activity. It was also revealed that cholinesterase inhibition activity of the target compounds depends largely to the steric and electronic features of the substituents. For example, a twofold reduction in the activity was observed by the movement of methoxy group from position 2 in compound **7k** to position 4 in compounds **7m**. This fact was supported by the docking studies. It can be likely related to the greater extension of the aromatic system of the phenyl, which could better interact with Trp82 through stacking interaction. Based on docking studies, presence of methoxy group at *ortho* position can disrupt the π–π stacking interactions *via* rotation of the phenyl ring as in compounds **7k** and **7b**. Movement of methoxy group to position *meta* as in compound **7l** and **7c** have therefore resulted in better activity due to proper stacking of the pendant phenyl group with Trp82. On the other hand, the activity would slightly improve when the methoxy group or fluoro-substitution shift to the *para* position. It is assumed that the steric hindrance of substituents with amino acids at the bottom of BChE gorge may be responsible for this effect. Furthermore, the activity of target compounds is very sensitive to the size of the substituent at *para* position. The IC_{50} values of 4-substituted phenyl analogs **7m**, **7n** and **7o** revealed that the methoxy group on *para*-position of phenyl ring is more favorable than methyl or fluoro substituents. Thus, compounds such as **7m** and **7d** having bulky groups at *para* position showed stronger activity. It is worth noting here that in case of **7b**, **7c** and **7d**, removing 5-bromo substiuent in benzofuran ring, led to a large decrease

approximately four fold in the activity of the target compounds (IC_{50} = 4.0-4.8 μM). To study the effects of more hydrophilic substituents on benzofuran ring, some compounds bearing methoxy group on 7 position and nitro group on 5 position of benzofuran ring were synthesized. Introduction of 5-nitro substituent on the benzofuran ring resulted in inactive compound **7s**. In contrast, 7-methoxy substitution could improve the activity as observed in compound **7g**. Methoxy-substituted benzofurans showed favorable, but lower activity than bromo-substituted benzofurans whereas nitro-substituted benzofurans showed a significant weak activity. Indeed, the introduction of a methoxyl group on the benzofuran ring (**7g** *vs* **7i**) resulted in a four-fold increase of the activity.

As it could be observed from Table 6, the size of the substituent on the pendant phenyl group, regardless of its electronic properties, is important in order to show considerable activity. Larger substituents have therefore exhibited better activities in comparison to those with the smaller size. The anticholinesterase activity was in the order: OCH_3> OH> F > H according to the Van der Waals radii, respectively. For R groups with similar volumes other factors should be considered, such as hydrogen bond acceptor/donor properties, PSA (polar surface area) and the extension of the aromatic system. This hypothesis was confirmed by docking studies. Astonishing results for BChE inhibition were obtained after synthesis of compounds **7p** and **7s**. The data showed that compounds with more electron-withdrawing substituent on position 3 of pendant phenyl group or on position 5 of benzofuran moiety exhibited no activity (compounds **7p** and **7s**).

Structurally, the most designed compounds possess phenyl ring which enables the molecule to make additional π-π interaction with aromatic or hetero-aromatic amino acid residues of enzyme binding site. On the other hand, the phenyl moiety was selected as it could establish hydrophobic and π-π interactions with the aromatic residues of PAS. Furthermore, in order to obtain

information about the structural requirements for optimally targeting BChE, the phenyl ring was replaced with other aromatic and hetero-aromatic groups with increasing bulkiness. In particular, the phenyl ring was replaced by sterically demanding aromatic substituents: 1-naphthyl and 3-indolyl substitutions (compounds **7e** and **7v**). Interestingly, inhibition of BChE by the 1-naphthyl and 3-indolyl substituted compounds **7e** and **7v** dropped to IC_{50} of 63 and 72 µM, respectively. The replacement of the phenyl ring (**7b**) with the indolyl substitution (**7f**) reduced the inhibitory activity of 12.5-fold. Because of these observations, compounds **7q** and **7u** were prepared carrying a 1-naphthyl moiety in position C-2 of benzothiazepine as well as compounds **7f** and **7r** holding each a 3-indolyl moiety in position C-2 of benzothiazepine. Unfortunately, these compounds gave only minor inhibition of BChE. The bulkiness of the aromatic group seems to play a main role in BChE inhibition. On the other hand, the indole and naphthalene moieties were selected as they could establish hydrophobic and π-π interactions with the aromatic residues of PAS.



7a-v

Table 6. Inhibitory activities of benzofuran linked benzothiazepines **7a-v** against BChE.

Entry	Compound	R^1	R^2	BChE inhibition, $IC_{50} \pm SD^+$(µmol/L)
1	7a	H	4-OH	14±0.71
2	7b	H	2-OCH_3	4.8±0.13
3	7c	H	3-OCH_3	4.1±0.14

4	7d	H	4-OCH_3	4.0±0.12
5	7e	H	1-Naphthyl	63±2.6
6	7f	H	3-Indolyl	60±2.4
7	7g	7-OCH_3	H	2.5±0.12
8	7h	7-OCH_3	4-OH	4.1±0.14
9	7i	5-Br	H	8.5±0.43
10	7j	5-Br	4-OH	6.5±0.35
11	7k	5-Br	2-OCH_3	1.8±0.06
12	7l	5-Br	3-OCH_3	1.0±0.01
13	7m	5-Br	4-OCH_3	1.0±0.01
14	7n	5-Br	4-CH_3	8.5±0.43
15	7o	5-Br	4-F	13.7±0.6
16	7p	5-Br	3-NO_2	Inactive
17	7q	5-Br	1-Naphthyl	17±0.9
18	7r	5-Br	3-Indolyl	28±1.38
19	7s	5-NO_2	H	Inactive
20	7t	5-NO_2	2-F	22±1.13
21	7u	5-NO_2	1-Naphthyl	55±2.1
22	7v	5-NO_2	3-Indolyl	72±2.8
23	Donepezil			2.63±0.28

IC_{50} values are at least from three independent experiments and are expressed as the means ± SD.

2.3. Kinetic study of BChE inhibition

To determine the mechanism of enzyme inhibition, the kinetic study was performed using **7l** as representative compound. The relative velocity of the enzyme was determined on three increasing concentrations (0, 0.5, 1.0 and 2.0 μM) of the substrate BTChI (butyrylthiocholine iodide). The Lineweaver-Burk plot was then schemed using the reciprocal of velocity (1/v) versus reciprocal of substrate concentration (1/[S]) (Figure 1, left). Based on the obtained plot, a mixed-type of inhibition was established for compound **7l**. Using the Lineweaver-Burk secondary plot of slope against concentration of **7l** (Figure 1, right), *K*i value was determined equal to 0.27 μM.

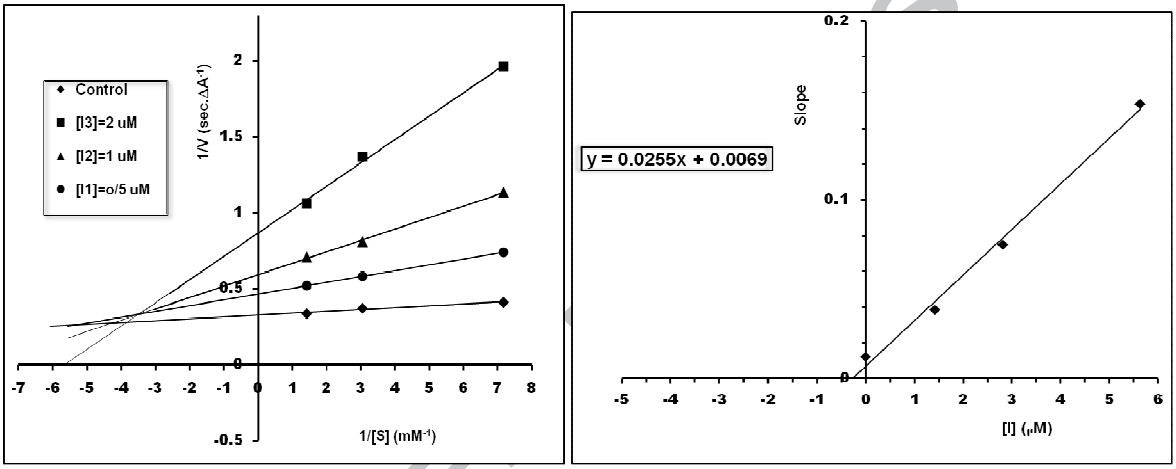


Figure 1. *Left*: Lineweavere-Burk plot for the inhibition of BChE by compound **7l** at different concentrations of substrate (BTChI), *Right:* Secondary plot for calculation of steadystate inhibition constant (*K*i) of compound **7l**.

2.4. Ligand-protein docking study

In order to study the binding mode of the synthesized compounds in active site of BChE, docking studies were performed using Auto Dock Tools (version 1.5.6). The results of SAR show that most potent compounds have a methoxy group on 2, 3, or 4-position of the 2-phenyl ring and a bromine atom on the 5-position of benzofuran. Therefore, the docking studies were performed on the most

active compounds **7k**, **7l**, and **7m** and their hypothetical analogs with two methoxy groups on 2-phenyl ring.

In the compound **7k**, the benzothiazepine moiety created a hydrophobic interaction with important amino acid Trp82 in catalytic anionic site (CAS).[45,46] Also, this moiety interacted with peripheral anionic site (PAS) *via* a π–anion interaction with Asp70. 2-Phenyl ring created a weak hydrophobic interaction with Pro84. Bromine atom of compound **7k** interacted with Trp231, Leu286, and Val288 *via* hydrophobic interactions (Figure 2A).

Movement of methoxy substituent into 3 or 4-position (compounds **7l** and **7m**) caused small increase of potency in comparison with 2-methoxy inhibitor **7k**. In compound **7l**, benzothiazepine and benzofuran moieties interacted with amino acids Trp82 (CAS), Tyr332 (PAS), Ala328, phe329, Leu286, Val288, and Trp231 (Figure 2B). In addition, 2-phenyl ring of this compound formed a π-π interaction with Trp82. As can be seen in Figure 2, compound **7m** established all the interactions mentioned above, but instead of interaction of 2-phenyl ring with Trp82 (Figure 2B), a hydrogen bond formed between 4-methoxy substituent of this ring with NH unit of Gly121(Figure 2C).

Hypothetically, introduction of another methoxy substituent on 2-phenyl ring in most potent compounds, with the exception of two cases (2,4-dimethoxy and 3,5-dimethoxy compounds), does not significantly change the interaction of compounds with the active site (Figure 3). Interaction mode of 2,3-dimethoxy compound exactly is similar to 3-methoxy compound **7l** (Figures 3A and 2B). 2,4-Dimethoxy shows all the interactions of 4-methoxy derivative **7m**, but did not establish π-π interaction with important amino acid Tyr332 (PAS) (Figures 3B and 2C). Interaction mode of 2,5-dimethoxy compound is almost similar to that of 3-methoxy compound **7l** (Figures 3C and

2B). 3,4-Dimethoxy hypothetical derivative shows all interactions that there is between 3 and 4-methoxy compounds **7l** and **7m** with active site of BChE (Figures 3D, 2B, and 2C).

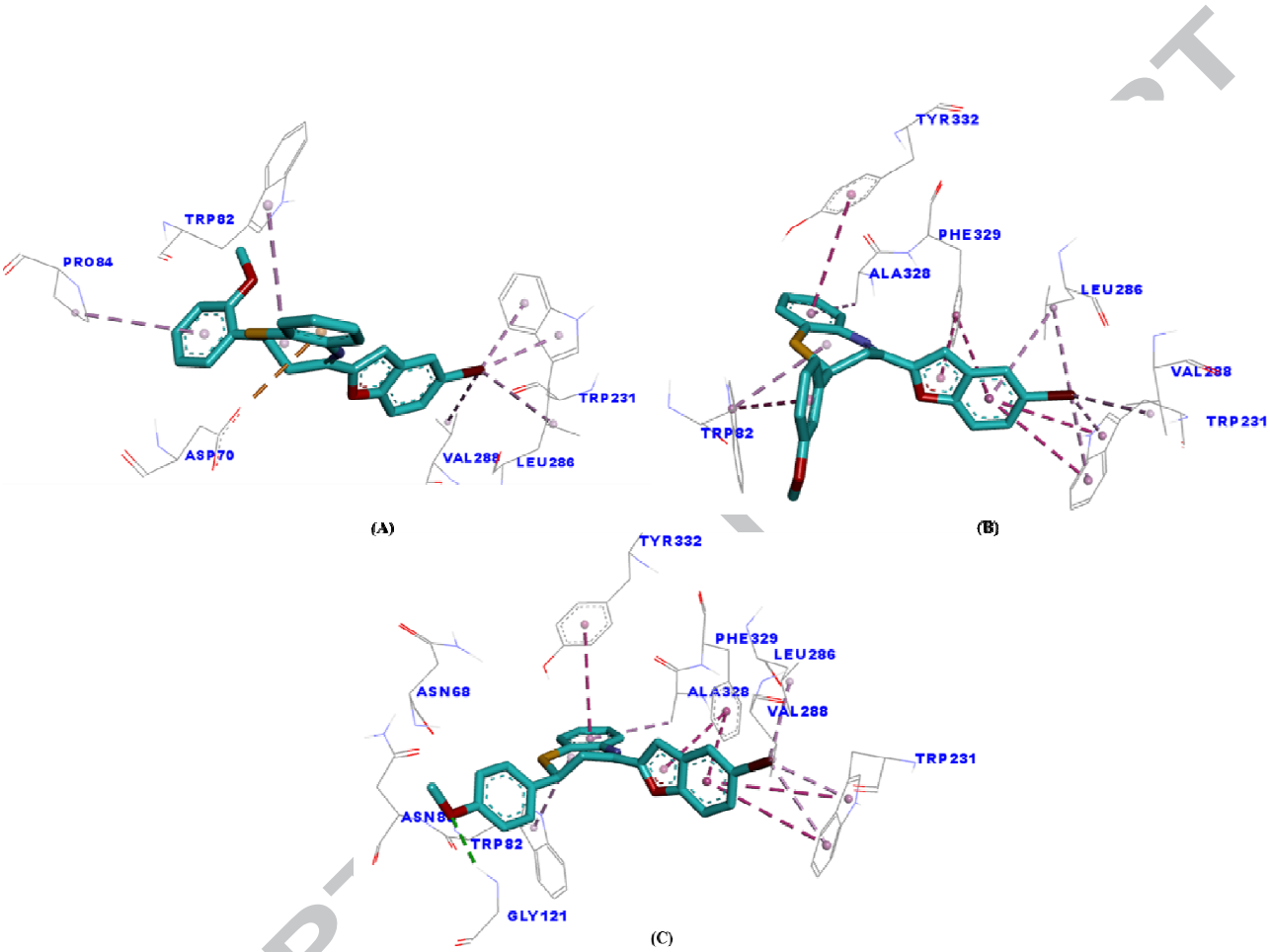


Figure 2. Binding mode of the most potent compounds (A) **7k**, (B) **7l**, and (C) **7m** in the enzyme active site.

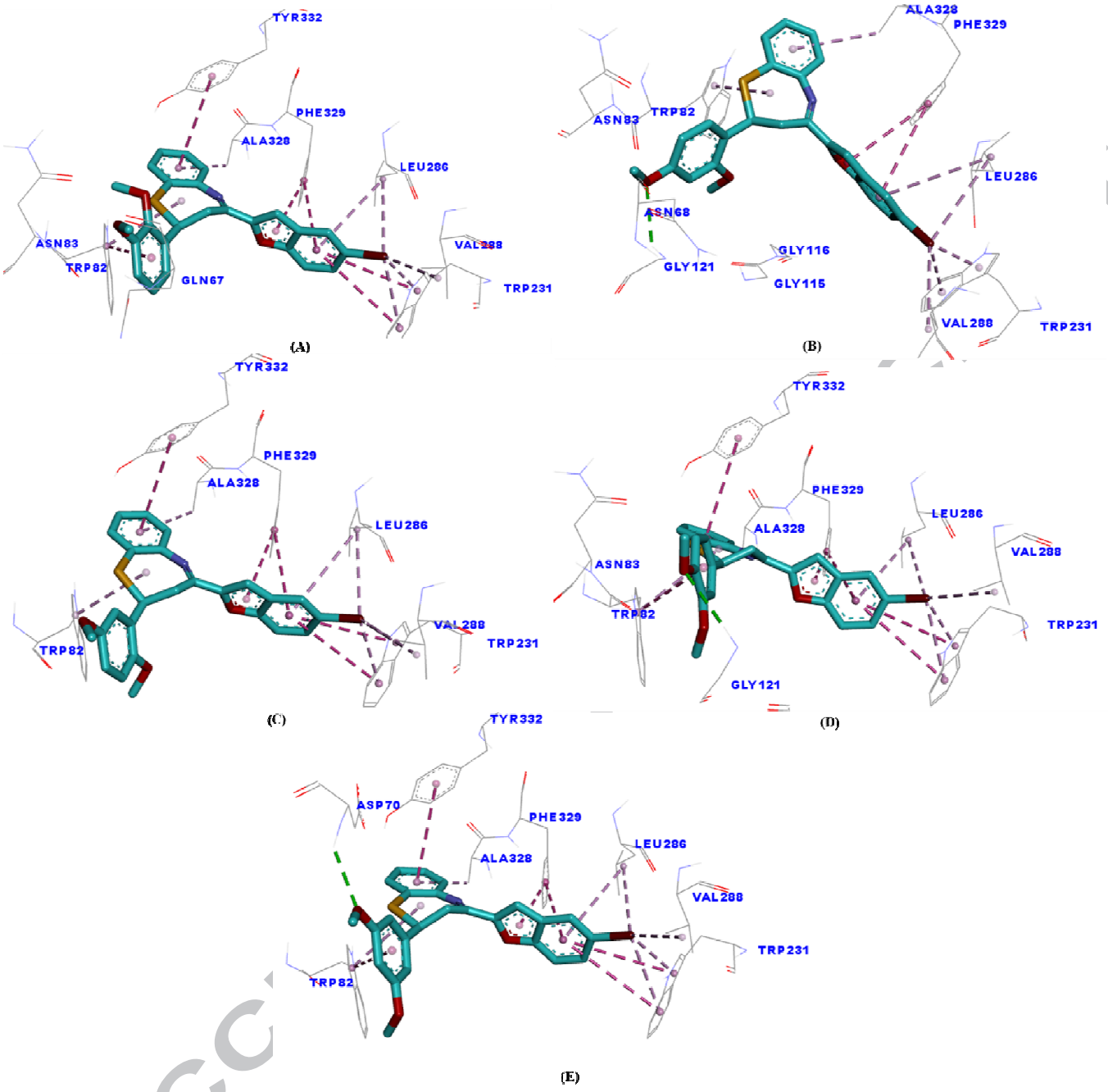


Figure 3. Binding mode of the hypothetical compounds (A) 2,3-dimethoxy , (B) 2,4-dimethoxy, and (C) 2,5-dimethoxy (D) 3,4-dimetoxy and (E) 3,5-dimethoxy analogs of compounds**7k**, **7l**, and **7m** in the enzyme active site.

2.5. Antibacterial activity

All target compounds **7a-v** were screened *in vitro* for their antibacterial activity against three gram-positive bacterial strains [*Bacillus subtilis (ATCC 6633)*, *Staphylococcus epidermidis (ATCC 12228)* and *Staphylococcus aureus* (*ATCC 29737)*] and six gram-negative bacterial strains [*Salmonella paratyphi-A serotype (ATCC 5702)*, *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae (ATCC 10031)*, *Shigella dysenteriae* (PTCC 1188), *Proteus vulgaris* (PTCC 1182) and *Escherichia coli* (ATCC 10536)] using disk diffusion method. Rifampicin and Gentamicin were used as reference drugs.

Tables 7 and 8 represent the zone of inhibition (mm) and minimum inhibitory concentrations (MICs, μg mL^{-1}) obtained for some target compounds. In general, the higher susceptibilities (lower MICs) were observed with gram-positive bacteria and poorer susceptibilities with gram-negative bacteria. The results of survey indicated that compounds **7e**, **7o**, **7p**, **7q**, **7t** and **7u** were active against gram-positive and gram-negative bacterial strains and among them, compound **7u** (IZ 30 mm) was the most active derivative against *S. epidermidis*. This increased potency may be due to the presence of naphthalene moiety on 2 position of benzodiazepine ring and introduction of NO_2 group into the benzofuran ring in this compound. Analysis of the Structure-Activity Relationship (SAR) also revealed that replacement of H atom by halogen or nitro groups on aryl ring at 2 position of benzothiazepine enhanced antimicrobial activity. With respect to gram negative bacteria, compounds **7p** (IZ 23 mm), and **7t** (IZ 23 mm) with nitro or fluoro substituents on phenyl ring at 2 position of benzothiazepine caused a remarkable increase in antimicrobial activity against *S. paratyphi* and *P. vulgaris*, respectively.

Table 7. Antibacterial activity data of tested compounds against Gram positive bacteria.

	Test microorganism

Sample	*S. aureus* (ATCC 29737)		*B. subtilis* (ATCC 6633)		*S. epidermidis* (ATCC 12228)	
	IZ	MIC	IZ	MIC	IZ	MIC
7e	10	5.0	-	-	9	5.0
7o	10	5.0	-	-	9	5.0
7p	-	-	19	12.5	-	-
7q	10	5.0	12	5.0	-	-
7t	17	2.5	-	-	10	5.0
7u	20	0.25	15	0.125	30	0.25
Rifampicin	10	0.25	13	0.125	40	0.25
Gentamicin	21	0.50	21	0.50	35	0.50

Notes: A dash (–) indicates no antimicrobial activity. NA

MIC - minimum inhibitory concentrations (µg mL^{-1})

IZ - Inhibition zone (mm)

Table 8. Antibacterial activity data of tested compounds against Gram negative bacteria.

Sample	Test microorganism											
	S. paratyphi		*E. coli*		*P. aeruginosa*		*K. Pneumoniae*		*S. dysenteriae*		*P. vulgaris*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
7o	11	50	-	-	15	50	-	-	-	-	-	-
7p	23	0.50	-	-	-	-	-	-	-	-	-	-
7q	-	-	-	-	-	-	-	-	14	50	19	12.5
7t	-	-	12	50	-	-	-	-	-	-	23	0.125

7u	11	50	-	-	-	-	11	50	-	-	-	-
Rifampicin	-	-	11	0.50	-	-	7	0.25	8	0.25	10	0.125
Gentamicin	21	0.50	20	0.50	-	-	22	0.25	18	0.50	23	0.50

Notes: A dash (–) indicates no antimicrobial activity. NA

MIC - minimum inhibitory concentrations (μg mL^{-1})

IZ - Inhibition zone (mm)

2.6. Antifungal activity

The antifungal activity of some seven-membered ring products against yeasts [*Candida albicans* (ATCC 10231)] and filamentous fungi [*Aspergillus niger* (ATCC 16404) and *Aspergillus brasiliensis (PTCC 5011*] were evaluated *in vitro*. Antifungal activity was expressed in terms of MIC values and the zone of inhibition (in mm) in comparison with standard drug Nystatin (Table 9). Among the tested compounds, compounds **7e**, **7o**, **7p**, **7q**, **7t** and **7u** showed good biological activities against fungi *C. albicans*, *A. niger* and *A. brasiliensis*. The results showed that compounds **7p** and **7t** were the most potent anti-fungal agents.

By comparing the zone of inhibition values of compounds it could be concluded that the halogen and nitro substitutions had positive effect in some cases depends on its position, significantly improve antifungal activity. Compounds **7o** with *para*-substituent and **7t** with *ortho*-substituent fluoro on phenyl rings were activate against *C. albicans*. Furthermore, in compound **7p** the introduction of *m*-NO_2 group to the aryl ring caused a significant increase in antifungal activity against *A. niger* (IZ = 20 mm) and *A. brasiliensis* (IZ = 23 mm). In compounds **7e**, **7q** and **7u**, the presence of naphthalene moiety on 2 position of benzodiazepine ring resulted in better activity against *C. albicans* (IZ = 13-14 mm).

Table 9. Antifungal activity data of tested compounds.

Sample	Test microorganism					
	C. albicans		*A. niger*		*A. brasiliensis*	
	IZ	MIC	IZ	MIC	IZ	MIC
7e	14	25	-	-	-	-
7o	13	25	-	-	-	-
7p	-	-	20	0.25	23	0.50
7q	14	25	-	-	-	-
7t	19	0.125	-	-	-	-
7u	13	2.5	-	-	-	-
Nystatin	33	0.125	27	0.25	23	0.50

Notes: A dash (–) indicates no antimicrobial activity. NA
MIC - minimum inhibitory concentrations (µg mL^{-1})
IZ - Inhibition zone (mm)

3. Conclusion

In summary, twenty-two substituted 1,5-benzothiazepine derivatives bearing benzofuran moiety have been synthesized and fully characterized. The synthetic pathway was quick and effective. All compounds were evaluated *in vitro* for their ability to inhibit AChE and BChE and the resulting products showed good levels of inhibition against BChE. In particular, compounds **7l**, **7m,** and **7k** expressed the highest BChE-inhibiting activities. Kinetic analysis studies revealed that compound **7l** features a mixed-type inhibition activity on BChE. Furthermore, molecular modeling studies indicate that compounds **7l**, **7m**, and **7k** are able to bind to both CAS and PAS in BChE. In addition, the antibacterial activities of compounds have been evaluated on a panel of pathogenic Gram+ and Gram- bacteria and the results showed that most of compounds exhibited moderate to

good activity against the tested microorganisms. Considering these overall results, our findings could be extended to design and develop new potentially selective BChE inhibitors containing benzofuran moieties as potential agents for the treatment of AD.

4. Experimental section

4.1. General information

All commercially available reagents were purchased from Merck AG, Aldrich or Acros Organics and used without further purification. Column chromatography was carried out on silica gel (70–230 mesh). TLC was conducted on silica gel 250 micron, F254 plates. For the synthesis of compounds 7 the experiments were performed using a microwave oven (ETHOS 1600, Milestone) with a power of 300 W specially designed for an organic synthesis and modified with a condenser and mechanical stirrer. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were taken using Nicolet FT-IR Magna550 spectrographs (KBr disks). Mass spectra of the products were obtained with an HP (Agilent technologies) 5937 Mass Selective Detector. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 and 500 MHz NMR instruments. The chemical shifts (δ) and coupling constants (*J*) are expressed in parts per million and hertz, respectively. Elemental analyses were carried out by a CHN-Rapid Heraeus elemental analyzer. The results of elemental analyses (C, H, N) were within ±0.4% of the calculated values.

4.2. General procedure for the preparation of 2-acetyl benzofuran derivatives 3a-d

A mixture of freshly distilled salicylaldehyde **1a-d** (0.1 mol), chloroacetone **2** (0.1 mol) and anhydrous potassium carbonate (0.1 mol) in ethyl methyl ketone (25 mL) was refluxed for 3-5 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the potassium

salts were filtered off, the filtrate on removal of solvent and on trituration with ethanol gave the pale yellow crystals of 2-acetylbenzofuran derivatives **3a-d**.

*4.2.1. 1-(Benzofuran-2-yl) ethan-1-one (**3a**).* Yield 72%, pale yellow solid; m.p. 75-76 °C (lit.[36] m.p. 76°C); IR ν_{max}/ cm^{-1} (KBr): 1673 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6,), δ ppm: 2.50 (s, 3H, CH_3), 7.36 (td, 1H, H_5-benzofuran, *J* = 8.0 Hz, *J* = 1.2 Hz), 7.53 (td, 1H, H_6-benzofuran, *J* = 8.0 Hz, *J* = 1.2 Hz) 7.71 (dd, 1H, H_7-benzofuran, *J* = 8.0 Hz, *J* = 1.2 Hz), 7.83 (dd, 1H, H_4-benzofuran, *J* = 8.0 Hz, *J*= 1.2 Hz), 7.89 (s, 1H, H_3-benzofuran); ^{13}C NMR (DMSO-d_6, 125 MHz), δ ppm: 26.8, 112.6, 114.7, 124.1, 124.4, 127.3, 128.8, 152.5, 155.4, 188.3.

*4.2.2. 1-(5-Bromobenzofuran-2-yl) ethan-1-one (**3b**).* Yield 75%, yellow solid; m.p. 110-111 °C (lit.[36] m.p. 110 °C); IR ν_{max}/ cm^{-1} (KBr): 1661 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.50 (s, 3H, CH_3), 7.70 (m, 2H, $H_{6,7}$-benzofuran), 7.83 (s, 1H, H_3-benzofuran), 8.06 (s, 1H, H_4-benzofuran); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 26.3, 113.1, 114.2, 116.0, 125.8, 128.9, 130.8, 153.0, 153.6, 187.7.

*4.2.3. 1-(7-Methoxybenzofuran-2-yl) ethan-1-one (**3c**).* Yield 78%, white solid; m.p. 91-93 °C (lit.[36]. m.p. 91 °C); IR ν_{max}/ cm^{-1} (KBr):1660 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.61 (s, 3H, CH_3), 4.01 (s, 3H, OCH_3), 6.93 (d, 1H, H_6-benzofuran, *J* = 8.0 Hz), 7.20 (t, 1H, H_5-benzofuran, *J* = 8.0 Hz), 7.36 (dd, 1H, H_4-benzofuran, *J* = 8.0 Hz, *J*= 2.5 Hz), 7.46 (s, 1H, H_3-benzofuran), ^{13}C NMR (DMSO-d_6, 125 MHz), δ ppm: 26.3, 56.0, 109.4, 112.4, 114.9, 124.4, 128.7, 145.3, 146.0, 153.0, 188.5.

*4.2.4. 1-(5-Nitrobenzofuran-2-yl) ethanone (**3d**):* Yield 65%, mp. 174-176 °C (lit. [36]. m.p. 175–177 °C); IR ν_{max}/ cm^{-1} (KBr): 1665 (C=O), 1615 (C=N), 1570, 1338 (N=O); ^{1}H NMR (300 MHz, DMSO-d_6), δ ppm: 2.59 (3H, CH_3, s), 7.97 (1H, H_7-benzofurane, d, *J*= 9.1 Hz), 8.04 (1H, H_3-benzofuran, s), 8.38 (1H, H_6-benzofuran, dd, *J*= 9.1 Hz , *J*= 2.4 Hz), 8.80 (1H, H_4- benzofuran d, *J*= 2.42).

4.3. General procedure for the preparation of (E)-1-(benzofuran-2-yl)-3-phenylprop-2-en-1-one 5a-v

To a mixture of 2-acetyl benzofuran derivative **3a-d** (0.12 mmol) and benzalaldehyde derivatives **4a-k** (0.12 mmol) in *n*-butanol (3 mL), a catalytic amount of piperidine (3-4 drops) was added. The mixture was heated under microwave irradiation at P= 300 W, for 15-30 min. The reaction was monitored by TLC, and when the starting material disappeared, the mixture was cooled and the precipitated solid was filtered off and washed with ethanol to give the pure products **5a-v**.

*4.3.1. (E)-1-(benzofuran-2-yl)-3-(4-hydroxyphenyl) prop-2-en-1-one (**5a**).* Dark brown semisolid, yield 65%, m.p. 250-252 °C (lit. [37] m.p. 254 °C); IR ν_{max}/ cm^{-1} (KBr): ν = 3116 (O-H), 1630 (C=O), 1206 (C-O).

*4.3.2. (E)-1-(benzofuran-2-yl)-3-(2-methoxyphenyl) prop-2-en-1-one (**5b**).* Light yellow solid, Yield-80%, m.p. 119–122 °C (lit. [38] m.p. 118-120 °C); IR ν_{max}/ cm^{-1} (KBr): 1624 (C=O), 1211, 1254 (C-O).

*4.3.3. (E)-3-(Benzofuran-2-yl)-1-(3-methoxyphenyl)prop-2-en-1-one (**5c**):* Yellow solid, Yield: 88%; m.p. 170-173 ºC; IR ν_{max}/ cm^{-1} (KBr): 2965 (SP^3-CH), 1647(C=O), 1607, 1574, 1519(C=C), 1246 (C-O).

*4.3.4. (E)-1-(benzofuran-2-yl)-3-(4-methoxyphenyl) prop-2-en-1-one (**5d**).* Yellow solid, yield 73%, m.p. 172-177°C (lit.[39]. m.p. 178-181 °C); IR ν_{max}/ cm^{-1} (KBr): 3104 (Ar-CH), 2931-2837 (SP^3-CH), 1668 (C═O), 1595 (C=C), 1256 (C-O).

*4.3.5. (E)- 1-(benzofuran-2-yl)-3-(naphthalen-1-yl)-prop-2en-1-one (**5e**).* White powder; yield: 72%; m.p. 188-200 ºC; IR ν_{max}/ cm^{-1} (KBr): 3142–2963 (Ar–CH), 1634 (C═O).

*4.3.6. (E)-1-(benzofuran-2-yl)-3-(1H-indol-3-yl) prop-2-en-1-one (**5f**).* Orange solid; 87%, yield; m.p. 165-168 °C (lit.[40] m.p. 159–160 ºC); IR ν_{max}/ cm^{-1} (KBr): 3374 (N-H), 3100-3072 (Ar-CH), 1680 (C=O).

*4.3.7. (E)-1-(7-methoxybenzofuran-2-yl)-3-phenylprop-2-en-1-one (**5g**).* Brown solid, Yield 77%; m.p. 228-231 °C; IR ν_{max}/ cm^{-1} (KBr): 3428-3400 (Ar-H), 1676 (C=O).

*4.3.8. (E)-3-(4-hydroxyphenyl)-1-(7-methoxybenzofuran-2-yl) prop-2- en-1-one (**5h**).* Light yellow solid, Yield 83%; m.p. 194-197 °C; IR ν_{max}/ cm^{-1} (KBr): 3305 (O-H), 3059-3003 (Ar-CH), 2936(SP^3-CH), 3115 (O-H); 1651 (C=O), 1571 (C=C), 1272 (C-O).

*4.3.9. (E)-1-(5-Bromo-1-benzofuran-2-yl)-3-phenylprop-2-en-1-one (**5i**).* Yellow solid, Yield: 70%; m.p. 149-151°C (lit. [41] m.p. 145–147 °C); IR ν_{max}/ cm^{-1} (KBr): 3105 (Ar-CH), 1655 (C=O), 1599 (C=C).

*4.3.10. (E)-1-(5-bromobenzofuran-2-yl)-3-(4-hydroxyphenyl) prop-2-en-1-one (**5j**).* Dark yellow solid, yield 65%, m.p. 202-204 ºC; IR ν_{max}/ cm^{-1} (KBr): 3329 (O-H), 1644 (C=O), 1607 (C=C), 1202 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 4.33 (s, 1H, OH), 6.86 (d, 2H, $H_{3,5}$-phenol, *J*= 6.0 Hz), 7.65 (d, 2H, $H_{2,6}$-phenol, *J*= 6.0 Hz); 7.66 (d, 1H alkene, β–CH, *J*=15.4 Hz), 7.75 (d, 1H, H_6-benzofuran, *J*= 8.5 Hz), 7.76 (d, 1H alkene, α–CH, *J*=15.4 Hz), 7.77 (d, 1H, H_7-benzofuran, *J*= 8.5 Hz); 8.08 (s, 1H, H_4-benzofuran); 8.14 (s, 1H, H_3-benzofuran), ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 113.2, 114.3, 115.9, 116.2, 118.0, 125.4, 125.9, 129.2, 130.9, 131.3, 144.5, 153.9, 154.4, 160.6, 178.3.

*4.3.11. (E)-1-(5-bromobenzofuran-2-yl)-3-(2-methoxyphenyl) prop-2-en-1-one (**5k**).* Yield-80%, m.p. 287-289°C (lit. [38] 286 °C); IR ν_{max}/ cm^{-1} (KBr): 3096 (SP^2-CH), 2930-2853 (SP^3-CH), 1657 (C=O), 1594 (C=C), 1246 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 3.94 (s, 3H, OCH_3), 6.95 (d, 1H, H_3-anisole, *J*= 7.5 Hz); 7.01 (t, 1H, H_5-anisole, *J*= 7.5 Hz); 7.41 (t, 1H, H_4-anisole, *J*= 7.5 Hz); 7.50 (d, 1H, H_7-benzofuran, *J*= 8.5 Hz), 7.54 (s, 1H, H_4-benzofuran), 7.56 (d, 1H, H_6-benzofuran, *J*= 8.5 Hz), 7.64 (d, 1H alkene, β–CH, *J*=15.8 Hz), 7.69 (d, 1H, H_6-anisole, *J*= 7.5 Hz); 7.86 (s, 1H, H_3-benzofuran); 8.28 (d, 1H alkene, α–CH, *J*= 15.8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 55.5, 111.2, 111.7, 113.9, 116.8, 120.7, 121.2, 123.3, 125.5, 129.1, 129.3, 130.9, 132.3, 140.7, 154.2, 154.7, 159.0, 179.9.

*4.3.12. (E)-1-(5-bromobenzofuran-2-yl)-3-(3-methoxyphenyl) prop-2-en-1-one (**5l**).* Light yellow solid, yield, 78%, m.p. 285-288 ºC; IR ν_{max}/ cm^{-1} (KBr): 3100–3072 (SP^2–CH), 1658 (C=O), 1608 (C=C), 1261 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 3.88 (s, 3H, CH_3), 7.00 (d, 1H, H_5-anisole, *J*= 7.85 Hz); 7.19 (s, 1H, H_4-benzofuran), 7.26 (s, 1H, H_2-anisole), 7.29 (d, 1H, H_4-anisole, *J*= 7.85 Hz), 7.36 (t, 1H, H_5-anisole, *J*= 7.85Hz); 7.51 (dd, 1H, H_6-benzofuran, *J*= 8.5 and 2.0 Hz), 7.56 (d, 1H, H_7-benzofuran, *J*= 8.5 Hz), 7.57 (d, 1H alkene, β–CH, *J* = 15.7 Hz), 7.86 (s, 1H, H_3-benzofuran), 7.91 (d, 1H alkene, α–CH, *J* = 15.7 Hz), ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 55.3, 112.0, 113.6, 113.9, 116.7, 116.9, 120.9, 121.3, 125.6, 129.0, 129.9, 131.1, 135.7, 145.1, 154.3, 154.4, 159.8, 179.4.

*4.3.13. (E)-1-(5-bromobenzofuran-2-yl)-3-(4-methoxyphenyl) prop-2-en-1-one (**5m**).* Yellow solid, 69%, m.p. 285-287 °C (lit. [42] m.p. 286 ºC). IR ν_{max}/ cm^{-1} (KBr): 3091 (Ar-CH), 2957-2834 (SP^3-CH), 1658 (C=O), 1256 (C-O).

*4.3.14. (E)-1-(5-bromobenzofuran-2-yl)-3-(p-tolyl) prop-2-en-1-one (**5n**).* Yellow solid, 68%, m.p. 279-281 ºC; IR ν_{max}/ cm^{-1} (KBr): 3105 (Ar-CH); 1666 (C=O), 1599 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.59 (s, 3H, CH_3), 7.25 (d, 1H alkene, β–CH, *J* = 15.0 Hz),7.40(s, 1H, H_4-benzofuran), 7.43 (d, 1H, H_7-benzofurane, *J*= 8.5 Hz), 7.47 (d, 2H, $H_{3,5}$-toluene, *J*= 6.0 Hz), 7.53 (d, 1H, H_6-benzofuran, *J*= 8.5 Hz), 7.57 (d, 2H, $H_{2,6}$- toluene, *J*= 6.0 Hz), 7.81 (s, 1H, H_3-benzofuran), 7.91 (d, 1H, α–CH, *J* = 15.0 Hz), ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 26.5, 111.8, 111.9, 113.9, 116.8, 120.1, 125.6, 125.7, 128.8, 131.1, 142.0, 146.0, 153.4, 154.7, 180.0, 188.4.

*4.3.15. (E)-1-(5-bromobenzofuran-2-yl)-3-(4-fluorophenyl) prop-2-en-1-one (**5o**).* Yellow solid, Yield: 75% m.p. 287-291 ºC; IR ν_{max}/ cm^{-1} (KBr): 1652 (C=O), 1623 (C=C), 1080-1095 (C-O-C).

*4.3.16. (E)-1-(5-Bromo-1-benzofuran-2-yl)-3-(3-nitrophenyl) prop-2-en-1-one (**5p**).* Yellow solid Yield: 87%; m.p. 203-206 ºC (lit. [41] m.p. 202–204 ºC); IR ν_{max}/ cm^{-1} (KBr): 1666 (C=O), 1610 (C=C); 1525 (N=O), 1345 (N=O).

*4.3.17. (E)-1-(5-bromobenzofuran-2-yl)-3-(naphthalen-1-yl) prop-2-en-1-one (**5q**).* Yellow solid Yield: 80%; m.p. 223-226 ºC; IR ν_{max}/ cm^{-1} (KBr): 3110-3074 (SP^2-CH), 1664 (C=O), 1592 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 7.25 (s, 1H, H_4-benzofuran), 7.45 (t, 1H, H_3-naphthalene, *J*= 8.5 Hz), 7.52 (dd, 1H, H_6-benzofuran, *J*= 8.5 and 1.5 Hz), 7.57, 2H, $H_{5,8}$-naphthalane, *J*= 8.5 and 4.5 Hz), 7.61 (t, 1H, H_7-naphthalene, *J*= 8.5 Hz), 7.63(d, 1Halkene, β–CH, *J*= 15.4 Hz), 7.84 (s, 1H, H_3-benzofuran), 7.90 (t, 1H, H_6-naphthalene, *J*= 8.5 Hz), 7.97 (dd, 2H, $H_{2,4}$-naphthalene, *J*= 8.5 and 4.5 Hz), 8.30 (d, 1H, H_7-benzofuran, *J*= 8.5 Hz), 8.82 (d, 1Halkene, α–CH, *J*= 15.4 Hz), ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 111.8, 113.9, 117.0, 123.0, 123.3, 125.3, 125.6, 126.3, 127.1, 128.7, 128.8, 129.1, 131.1, 131.3, 131.7, 133.7, 141.9, 154.2, 154.3, 154.5, 179.3.

*4.3.18. (E)-1-(5-bromobenzofuran-2-yl)-3-(1H-indol-3-yl) prop-2-en-1-one (**5r**).* Orange solid Yield: 87%; m.p. 230-233 ºC; IR ν_{max}/ cm^{-1} (KBr): 3193 (NH), 3057 (SP^2-CH), 1639 (C=O), 1573 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 7.27 (dd, 2H, $H_{4,7}$-indole, *J*= 5.5 and *J*=2.5 Hz), 7.51 (t, 1H, H_5-indole, *J*= 5.5 Hz), 7.59 (d, 1Halkene, β–CH, *J*= 15.5 Hz), 7.66 (d, 1H, H_4-benzofuran, *J*= 8.7 Hz), 7.76 (d, 1H, H_7-benzofuran, *J*= 8.7 Hz), 8.06 (s, 1H, H_3-benzofuran), 8.13

(1Halkene, α–CH, *J*= 15.5 Hz), 8.14 (d,1H, H_4-benzofuran, *J*= 3.0 Hz), 8.15 (t, 1H, H_6-indole, *J*= 5.5 Hz), 8.17 (s, 1H, H_2-indole), 12.03 (s, 1H, NH), ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 112.1, 112.6, 112.7, 114.4, 114.7, 116.1, 120.5, 121.4, 122.9, 125.0, 125.6, 129.5, 130.5, 134.4, 137.6, 139.2, 153.8, 154.9, 178.3.

*4.3.19. (E)-1-(5-nitrobenzofuran-2-yl)-3-phenylprop-2-en-1-one (**5s**).* Yellow solid Yield:60% , m.p. 221-223 ºC; IR ν_{max}/ cm^{-1} (KBr): 1648 (C=O), 1618 (C=C), 1524 (N=O), 1342 (N=O), 1080-1095 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6), δ ppm: 6.75 (dd, 1H, H_2-phenyl, *J*=7.8 and *J*= 2.0 Hz), 6.94 (t, 1H, H_4-phenyl, *J*= 7.8 Hz), 7.11 (t, 1H, H_3-phenyl, *J*= 7.8 Hz), 7.22 (t, 1H, H_5-phenyl, *J*= 7.8 Hz); 7.35 (d, 1H alkene, β–CH, *J* = 15.0 Hz), 7.36 (dd, 1H, H_6-phenyl, *J*= 7.8 and 2.0 Hz); 7.38 (d, 1H alkene, α–CH, *J*= 15.0 Hz), 7.47 (dd, 1H, H_6-benzofuran, *J*=9.0 and *J*=1.8 Hz); 7.59 (d, 1H, H_7-benzofuran, *J*= 9.0 Hz); 7.65 (s, 1H, H_3-benzofuran), 7.73 (d, 1H, H_4-benzofuran, *J*= 1.8 Hz).

*4.3.20. (E)- 1-(5-nitrobenzofuran-2-yl) -3-(2-fluorophenyl)- prop-2-en-1-one (**5t**).* Yield: 2.02 g, 54%; 230-232 ºC; IR ν_{max}/ cm^{-1} (KBr): 3120-3067 (Ar-CH), 1659 (C=O), 1611 (C=C), 1576 (N=O), 1350 (N=O), 1292, 1160, 753.

*4.3.21. (E)-1-(5-nitrobenzofuran-2-yl)-3-(naphthalen-1-yl)-prop-2-en-1-one (**5u**).* White powder; yield: 72%; m.p. 184-187 ºC (lit [47] mp: 189–190 °C); IR ν_{max}/ cm^{-1} (KBr): 1666 (C=O), 1610 (C=C); 1526 (N=O), 1342 (N=O).

*4.3.22. (E)-3-(1H-indol-3-yl)-1-(5-nitrobenzofuran-2-yl)prop-2-en-1-one (**5v**).* Yellow powder; yield: 72%; m.p. 236-238 ºC; IR ν_{max}/ cm^{-1} (KBr): 3342 (NH), 1680 (C=O), 1610 (C=C); 1563 (N=O), 1355 (N=O).

4.4. General Procedure for the synthesis of titled compounds 7a-7v

A mixture of chalcone **5a-v** (1 mmol) and 2-aminothiophenol **6** (1 mmol) was dissolved in anhydrous ethanol (5 mL). The reaction mixture was heated to reflux for 2-4 h in the presence of glacial acetic acid (0.1 mL) under stirring condition. Progress of the reaction was monitored by TLC. Also, the reaction was monitored by IR spectroscopy to confirm the formation of the imine moiety by the appearance of the stretching absorption band around 1640 cm^{-1} and the disappearance of the carbonyl band at about 1700 cm^{-1}. Upon completion of the reaction, after allowing the reaction mixture to cool down at room temperature, the resultant solid was separated by filtration and recrystallized from anhydrous ethanol and benzene to give the target compounds **7a-v**.

*4.4.1. 4-(4-(Benzofuran-2-yl)- 2,3-dihydro-1,5-benzothiazepine -2-yl) phenol (**7a**)*: From compound **5a** (R^1= H, R^2= 4-OH, 1 mmol, 0.264 g) and 2- aminothiophenol (1 mmol, 0.125 g), after 3 h, product **7a** was obtained. Yield: 0.29 g (79%) green solid. m.p. 237-239 ºC; IR ν_{max}/ cm^{-1} (KBr):1215, 1256, 1281 (C-O), 1652 (C=N), 3115 (O-H); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.87 (t, 1H, -CH_2, *J*= 12.5 Hz),3.35 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.19 (dd, 1H, -CH, *J*= 12.5Hz, *J*= 4.5 Hz), 6.70 (d, 2H, $H_{3,5}$ phenol, *J*= 8.0 Hz), 7.17 (d, 2H, $H_{2,6}$ phenol, *J*= 8.0 Hz), 7.21 (d, 1H, H_d-phenyl, *J*= 7.5), 7.30 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.35 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.48 (t, 1H, H_5 benzofuran, t, *J*= 8.0 Hz), 7.53 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.57 (t, 1H, H_6 benzofuran, *J*= 8.0 Hz), 7.72 (d, 1H, H_4 benzofuran, *J*= 8.0 Hz), 7.78 (d, 1H, H_7 benzofuran, *J*= 8.0

Hz), 9.45 (s, 1H, O-H phenol); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.4, 59.9, 111.6, 111.7, 115.0, 122.5, 122.6, 123.6, 125.2, 125.6, 127.0,127.2, 127.7, 129.9, 134.4, 134.8, 151.4, 153.1, 155.2, 156.8, 160.0. EI-MS m/z (%) 493). Anal. Calcd for $C_{23}H_{17}NO_2S$: C, 74.37; H, 4.61; N, 3.77. Found: C, 74.10; H, 4.76; N, 3.49.

*4.4.2. 4-(Benzofuran-2-yl)-2-(2-methoxyphenyl)- 2,3-dihydro-1,5-benzothiazepine (**7b**)*: From compound **5b** (R^1= H, R^2= 2-OCH_3, 1 mmol, 0.278 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 3 h, product **7b** was obtained. Yield: 0.28 g (72%) green solid. m. p. 176-178 ºC; IR ν_{max}/ cm^{-1} (KBr): 1217, 1289, 1248 (C-O), 1598 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.94 (t, 1H, -CH_2, *J*=13.0 Hz), 3.31 (dd, 1H, -CH_2, *J*= 13.0 Hz, *J*= 5.0 Hz), 3.85 (s, 3H, -OCH_3), 5.44 (dd, 1H, -CH, *J*= 13.0 Hz , *J*= 5.0 Hz), 6.91(t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.04 (d, 1H, H_d-phenyl, *J*= 7.5 Hz), 7.17 (td, 1H, H_4-anisole, *J*= 8.0 Hz, *J*= 1Hz), 7.28 (td, 1H, H_5 anisole, *J*= 8.0 Hz, *J*= 1.0 Hz), 7.29 (dd, 1H, H_3-anisol, *J*= 8.0 Hz, *J*= 1Hz), 7.35 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.36 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.48 (dd, 1H, H_6 anisole, *J*= 8.0 Hz, *J*= 1.0 Hz), 7.52 (dd, 1H, H_5 benzofuran, *J*= 8.0 Hz, *J*= 1.0 Hz), 7.54 (dd, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 1.0 Hz), 7.72 (d, 1H, H_4 benzofuran, *J*= 8 Hz), 7.77 (s, 1H, H_3 benzofuran), 7.81 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 34.5, 53.9, 55.6, 95.4, 111.0, 111.3, 111.8, 120.3, 122.6, 122.9, 123.6, 125.3, 125.5, 125.9, 127.1, 127.7, 128.8, 129.8, 130.9, 134.9, 151.5, 153.1,155.1, 155.2,160.3. Anal. Calcd for $C_{24}H_{19}NO_2S$: C, 74.78; H, 4.97; N, 3.63. Found: C, 74.99; H, 4.73; N, 3.92.

*4.4.3. 4-(Benzofuran-2-yl)-2-(3-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine (**7c**)*: From compound **5c** (R^1= H, R^2= 3-OCH_3, 1 mmol, 0.278 g) and 2- aminothiophenol (1 mmol, 0.125 g),

for 3 h, product **7c** was obtained Yield: 0.31 g (80%) green solid. m. p. 138-140 ºC; IR ν_{max}/ cm^{-1} (KBr): 1032 ,1175, 1263 (C-O), 1598 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.91 (t, 1H, -CH_2, *J*= 13.0 Hz), 3.39 (dd, 1H, -CH_2 , *J*= 13.0 Hz, J= 5.0 Hz), 3.71 (s, 3H, -OCH_3), 5.23 (dd, 1H, -CH, *J*= 13.0Hz, *J*= 5.0 Hz), 6.85 (dd, 1H, H_5 anisole, *J*= 8.0 Hz, *J*= 2.0 Hz), 6.95 (d, 1H, H_2 anisole, *J*= 7.5 Hz), 7.22 (ddd, 1H, H_4 anisole, *J*= 7.5, *J*=4.5, J=1.5 Hz), 7.24 (t, 1H, H_c-phenyl, *J*= 7.5Hz), 7.31 (dd, 1H, H_5 anisole, *J*= 7.5 Hz, *J*= 1.5 Hz), 7.36 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.49 (td, 1H, H_5 benzofuran, *J*= 8.0 Hz, *J*= 1.0 Hz), 7.55 (td, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 1.0 Hz), 7.60 (d, 2H, $H_{a,d}$-phenyl, *J*= 7.5 Hz), 7.72 (d, 1H, H_4 benzofuran, *J*= 8.0 Hz), 7.93 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 7.88 (s, 1H, H_3 benzofuran); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.1, 54.9, 59.8, 95.3, 111.8, 111.9, 112.9, 118.2, 122.4, 122.5, 123.6, 125.3, 125.6, 127.1, 127.7, 129.6, 130.1, 134.9, 145.4, 144.0, 151.5, 153.0, 155.3, 159.2, 160.0, 162.8. Anal. Calcd for $C_{24}H_{19}NO_2S$: C, 74.78; H, 4.97; N, 3.63. Found: C, 75.04; H, 5.31; N, 3.39.

*4.4.4. 4-(Benzofuran-2-yl)-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine (**7d**)*: From compound **5d** (R^1= H, R^2= 4-OCH_3, 1 mmol, 0.278 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 3 h, product **7d** was obtained. Yield: 0.35 g (90%) green solid. m. p. 176-178 ºC; IR ν_{max}/ cm^{-1} (KBr):1178, 1246 (C-O), 1606 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.88 (t, 1H, -CH_2, *J*= 13.0 Hz),3.36 (dd, 1H, -CH_2 , *J*= 13.0 Hz, *J*= 5.0 Hz), 3.75 (s, 3H, -OCH_3), 5.24 (dd, 1H, -CH, *J*= 13.0 Hz, *J*= 5.0 Hz), 6.88 (d, 2H, $H_{2,6}$ anisole, *J*= 8.0 Hz), 7.21 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.29 (d, 2H, $H_{3,5}$ anisole, *J*= 8.0 Hz), 7.30 (d, 1H, H_d-phenyl, *J*= 7.5 Hz), 7.35 (t, 1H, H_5 benzofuran, *J*= 8.0 Hz), 7.48 (t, 1H, H_6 benzofuran, *J*= 8.0 Hz), 7.53 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.72 (d, 1H, H_4 benzofuran, *J*= 8.0 Hz), 7.78 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 7.84 (s, 1H, H_3 benzofuran); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.3, 55.1, 59.5, 111.7, 111.8, 113.8, 122.5,

123.6, 125.2, 125.6, 127.1, 127.2, 127.7, 129.9, 134.8, 135.4, 136.1, 151.4, 153.1, 155.2, 158.6, 160.0. EI-MS m/z (%) 391 (M^++6, 0.01), 389 (M^++4, 0.1), 387 (M^++2, 2), 386 (M^++1, 6), 385 (M^+, 11), 352 (3), 251 (100), 223 (9), 190 (3), 152 (2), 134 (52), 91 (10), 65 (6), 45 (2). Anal. Calcd for$C_{24}H_{19}NO_2S$: C, 74.78; H, 4.97; N, 3.63. Found: C, 74.87; H, 5.23; N, 3.96.

*4.4.5. 4-(Benzofuran-2-yl)-2-(naphthalen-1-yl)-2,3-dihydro-1,5-benzothiazepine (**7e**)*: From compound **5e** (R^1= H, R^2= naphthalene, 1 mmol, 0.257 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 3 h, product **7e** was obtained. Yield: 0.35 g (86%) green solid. m. p. 189-191 ºC; IR ν_{max}/ cm^{-1} (KBr):1211, 1255 (C-O), 1604 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 3.18 (t, 1H, -CH_2, *J*= 13.0 Hz),3.54 (dd, 1H, -CH_2 , *J*= 13.0 Hz, *J*= 5.0 Hz), 6.12 (dd, 1H, -CH, *J*= 13.0Hz, *J*= 5.0 Hz), 7.18 (td, 1H, H_7 naphthalene, *J*= 8.0 Hz, *J*= 1.5 Hz), 7.36 (t, 2H, $H_{b,c}$-phenyl, *J*= 7.5 Hz), 7.46 (d, 1H, H_d-phenyl, *J*= 7.5 Hz), 7.49 (dd,1H, H_5 naphthalene, *J*= 8.0 Hz, *J*= 1.5 Hz), 7.50 (dd, 1H, H_8 naphthalene, *J*= 8.0 Hz, *J*= 1.5 Hz), 7.54 (td, 1H, H_6 naphthalene, *J*= 8.0 Hz, *J*= 1.5 Hz), 7.60 (t, 1H, H_3 naphthalene, *J*= 8.0 Hz), 7.64 (dd, 2H, $H_{5,6}$ benzofuran, *J*= 8.0 Hz, *J*= 1.0 Hz), 7.74 (d, 1H, H_2 naphthalene, *J*= 8.0 Hz), 7.78 (d, 1H, H_a- phenyl, *J*= 7.5 Hz), 7.84 (s, 1H, H_3 benzofuran); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 35.0, 55.0, 111.5, 111.8, 118.8, 122.6, 123.1, 123.6, 125.4, 125.6, 125.9, 126.5, 127.1, 128.0, 128.8, 129.4, 130.1, 133.5, 135.0, 138.6, 151.6, 155.2, 151.0, 160.5. Anal. Calcd for $C_{27}H_{19}NOS$: C, 79.97; H, 4.72; N, 3.45. Found: C, 79.66; H, 4.81; N, 3.12.

*4.4.6. 4-(Benzofuran-2-yl)-2-(1H-indol-3-yl)-2,3-dihydro-1,5-benzothiazepine (**7f**)*: From compound **5f** (R^1= H, R^2= 3-indolyl, 1 mmol, 0.287 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 3 h, product **5f** was obtained, *green* solid, Yield: 0.31 g (80%). m. p. 225-227 ºC; IR ν_{max}/ cm^{-1}

(KBr): 1100, 1177, 1250 (C-O), 1601 (C=N), 3350 (N-H); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 3.14 (t, 1H, -CH_2, *J*= 13.0 Hz),3.47 (dd, 1H, -CH_2 , *J*= 13.0 Hz, *J*= 5.0 Hz), 5.60 (dd, 1H, -CH, *J*= 13.0Hz, *J*= 5.0 Hz), 6.97 (t, 1H, H_c-phenyl, J= 7.0 Hz), 7.11 (t, 1H, H_b-phenyl, *J*= 7.0 Hz), 7.16 (td, 1H, H_5 indole, *J*= 7.5 Hz, *J*= 1.0 Hz), 7.30 (dd, 2H, $H_{4,7}$ indole, *J*= 7.5 Hz, *J*= 1.0 Hz), 7.33 (s, 1H, H_2 indole), 7.36 (td, 2H, $H_{5,6}$ benzofuran, *J*= 8.0 Hz, *J*= 1.0 Hz), 7.47 (d, 1H, H_d-phenyl, *J*= 7.0 Hz), 7.48 (d, 1H, H_a-phenyl, J= 7.0 Hz), 7.53 (td, 1H, H_6 indole, *J*= 7.5 Hz, *J*= 1.0 Hz), 7.72 (d, 1H, H_6 benzofuran, *J*= 8.0 Hz), 7.75 (s, 1H, H_3 benzofuran), 7.76 (d, 1H, H_4 benzofuran, *J*= 8.0 Hz), 10.10 (s, 1H, N-H); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 35.6, 58.8, 111.3, 111.6, 111.7, 117.5, 118.6, 119.1, 121.4, 122.1, 122.5, 123.3, 123.6, 125.0, 125.2, 125.4, 127.0, 127.7, 128.3, 129.7, 135.0, 136.5, 151.4, 153.4, 155.2, 160.3. EI-MS m/z (%) 400 (M^++6,0.01), 398 (M^++4, 0.02), 396 (M^++2, 0.1) 395 (M^++1, 0.2), 394 (M^+, 1), 393 (M^+-1, 1), 276 (1), 275 (2), 251 (13), 223 (5), 190 (2), 143 (100), 165 (1), 115 (14), 89 (5), 63 (4), 45 (2). Anal. Calcd for $C_{25}H_{18}N_2OS$: C, 76.12; H, 4.60; N, 7.10. Found: C, 76.38; H, 4.94; N, 6.86.

*4.4.7. 4-(7-Methoxybenzofuran-2-yl)-2-phenyl-2,3-dihydro-1,5-benzothiazepine (**7g**)*: From compound **5g** (R^1=7-OCH_3, R^2=H, 1 mmol, 0.278 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 5 h, product **7g** was obtained, green solid, Yield: 0.31 g (80%). m. p. 277-279 ºC; IR ν_{max}/ cm^{-1} (KBr): 1209, 1276 (C-O), 1619 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.70 (t, 1H, -CH_2, *J*= 12.5 Hz),3.25 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 3.95 (s, 3H, -OCH_3), 4.34 (dd, 1H, -CH, *J*= 12.5 Hz, *J*= 4.5 Hz), 6.80 (d, 2H, $H_{a,d}$-phenyl, *J*= 7.5 Hz), 7.03 (t, 1H, H_c phenyl, *J*= 7.5 Hz), 7.05 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.17 (d, 2$H_{a'}$-phenyl, *J*=7.5 Hz), 7.25 (t, 3H, 2$H_{b'}$, 1$H_{c'}$-phenyl, *J*= 7.5 Hz), 7.36 (s, 1H, H_3 benzofuran), 7.48 (t, 1H, H_5 benzofuran, *J*= 8.0 Hz, *J*= 1.5 Hz), 7.69 (dd, 2H, $H_{4,6}$ benzofuran, *J*= 8.0 Hz, *J*= 1.5 Hz), ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.3,

55.1, 59.5, 111.7, 111.8, 113.8, 122.4, 122.5, 123.6, 125.2, 125.6, 127.1, 127.2, 127.7, 129.9, 134.8, 136.1, 151.4, 153.1, 155.2, 158.6, 160.0. Anal. Calcd for $C_{24}H_{19}NO_2S$: C, 74.78; H, 4.97; N, 3.63. Found: C, 75.00; H, 5.08; N, 3.42.

*4.4.8. 4-(4-(7-Methoxybenzofuran-2-yl)-2,3--dihydro-1,5-benzothiazepine-2-yl) phenol (**7h**)*: From compound **5h** (R^1= 7-OCH_3, R^2= 4-OH, 1 mmol, 0.294 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 5 h, product **7h** was obtained, green solid, *Yield*: 0.37 g (89%). m. p. 247-249 ºC; IR ν_{max}/ cm^{-1} (KBr):1095, 1202, 1268 (C-O), 1592 (C=N), 3142 (O-H); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.86 (t, 1H, -CH_2, *J*= 12.5 Hz),3.34 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 3.97 (s, 3H, -OCH_3), 5.17 (dd, 1H, -CH, *J*= 12.5Hz, *J*= 4.5 Hz) 6.70 (d, 2H, $H_{3,5}$ phenol, *J*= 8.0 Hz), 7.08 (d, 1H, H_d-phenyl, *J*=7.5 Hz), 7.16 (d, 2H, $H_{2,6}$ phenol, *J*= 8.0 Hz), 7.20 (d, 1H, H_a-phenyl, *J*= 8.0 Hz), 7.27 (d, 1H, H_6 benzofuran, *J*= 8.0 Hz), 7.31 (t, 2H, $H_{b,c}$-phenyl, *J*= 7.5Hz), 7.53 (t, 1H, H_5 benzofuran, *J*= 8.0 Hz), 7.57 (d, 1H, H_4 benzofuran, *J*= 8.0 Hz), 7.80 (s, 1H, H_3 benzofuran), 9.44 (s, 1H, -OH phenol; ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.4, 55.8, 59.8, 108.9, 111.9, 114.3, 114.9, 115.0, 122.6, 124.4, 125.2, 125.5, 127.2, 129.2, 129.9, 134.4, 134.8, 144.6, 145.2, 151.3, 153.1, 156.8, 159.9. Anal. Calcd for $C_{24}H_{19}NO_3S$: C, 71.80; H, 4.77; N, 3.49. Found: C, 71.63; H, 4.53; N, 3.81.

*4.4.9. 4-(5-Bromobenzofuran-2-yl)-2-phenyl-2,3-dihydro-1,5-benzothiazepine (**7i**)*: From compound **5i** (R^1= 5-Br, R^2= H, 1 mmol, 0.327 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7i** was obtained, 0.35 g (80%), green solid. m. p. 189-191 ºC; IR ν_{max}/ cm^{-1} (KBr): 1135, 1176, 1255 (C-O), 1605 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.92 (t, 1H, -CH_2, *J*= 12.5 Hz),3.39 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.28 (dd, 1H, -CH, *J*= 12.5Hz, *J*= 4.5 Hz),

7.23 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.28 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.30 (d, 1H, H_d –phenyl, *J*= 7.5 Hz), 7.33 (t, 2H, $2H_{b'}$-phenyl, *J*= 7.5 Hz), 7.37 (d, 2H, $2H_{b'}$-phenyl, *J*= 7.5 Hz), 7.37 (d, 2H, $2H_{a'}$ –phenyl, *J*= 7.5 Hz), 7.55 (t, 1H, $H_{c'}$-phenyl, *J*= 7.5 Hz), 7.59 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.62 (dd, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 2.0 Hz), 7.73 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 7.84 (s, 1H, H_3 benzofuran), 8.00 (d, 1H, H_4 benzofuran, *J*= 2.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.0, 59.8, 110.9, 113.9, 115.9, 122.4, 124.8, 125.3, 125.8, 126.0, 127.6, 128.5, 129.6, 129.9, 130.1, 134.9, 143.7, 147.0, 151.3, 154.0, 154.3, 159.9. EI-MS m/z (%) 438 (M^++6, 1), 436 (M^++4, 2), 434 (M^++2, 4), 433 (M^++1, 8), 432(M^+, 3), 400 (3), 300 (44), 368 (12), 354 (4), 353 (5), 351 (1), 329 (34), 331 (38), 330 (12), 313 (16), 285 (9), 264 (12), 236 (30), 211 (8), 189 (17), 171 (8),152 (12), 123 (17), 97 (42), 69 (70),44 (29), 43 (17). Anal. Calcd for $C_{23}H_{16}BrNOS$: C, 63.60; H, 3.71; N, 3.22. Found: C, 63.74; H, 4.00; N, 3.39.

*4.4.10. 4-(-4-(5-Bromobenzofuran-2-yl)-2,3-dihydro-1,5-benzothiazepine -2-yl) phenol (**7j**)*: From compound **5j** (R^1= 5-Br, R^2= 4-OH, 1 mmol, 0.343 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7j** was obtained, Yield: 0.31 g (70%) green solid. m. p. 253-255 ºC; IR ν_{max}/ cm^{-1} (KBr): 1206, 1259 (C-O), 1612 (C=N), 3116 (O-H); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.86 (t, 1H, -CH_2, *J*= 12.5 Hz),3.35 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.95 (dd, 1H, -CH, *J*= 12.5Hz, *J*= 4.5 Hz), 7.16 (d, 2H, $H_{2,6}$ phenol, *J*= 8.0 Hz), 7.21 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.30 (d, 2H, $H_{3,5}$ Phenol, *J*= 8.0 Hz), 7.53 (t, 1H, H_b-Phenyl, *J*= 7.5 Hz), 7.57 (d, 1H, H_d-phenyl, *J*= 7.5 Hz), 7.62 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.72 (d, 2H, $H_{6,7}$ benzofuran, *J*= 8.0 Hz), 7.79 (s, 1H, H_3 benzofuran), 7.99 (s, 1H, H_4 benzofuran), 9.45 (s, 1H, O-H phenol); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.5, 59.9, 110.7, 113.9, 115.0, 115.8, 122.7, 124.8, 125.3, 125.8, 127.2, 124.8, 125.3,

125.8, 127.2, 129.6, 129.9, 134.3, 134.8, 151.2, 154.0, 154.4, 156.8, 159.9. Anal. Calcd for $C_{23}H_{16}BrNO_2S$: C, 61.34; H, 3.58; N, 3.11. Found: C, 61.17; H, 3.86; N, 3.24.

*4.4.11. 4-(5-Bromobenzofuran-2-yl)-2-(2-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine (**7k**)*: From compound **5k** (R^1= 5-Br, R^2= 2-OCH_3, 1 mmol, 0.357 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7k** was obtained. Yield: 0.35 g (75%) green solid. m. p. 165-167 ºC; IR ν_{max}/ cm^{-1} (KBr):1167, 1210, 1239 (C-O), 1602 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.93 (t, 1H, -CH_2, *J*= 12.5 Hz),3.34 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.45 (dd, 1H, -CH, *J*= 12.5Hz, *J*= 4.5 Hz), 6.92 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.05 (d, 1H, H_3 anisole, *J*= 8.0 Hz), 7.19 (t, 1H, H_5 anisole, *J*= 8.0 Hz), 7.28 (d, 1H, H_6 anisole, *J*= 8.0 Hz), 7.30 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.37 (d, 1H, H_d -phenyl, *J*= 7.5 Hz), 7.52 (t, 1H, H_4 anisole, *J*= 8.0 Hz), 7.56 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.63 (dd, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 2.0 Hz), 7.74 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 7.75 (s, 1H, H_3 benzofuran), 8.05 (d, 1H, H_4 benzofuran, *J*= 2.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 34.6, 53.9, 55.6, 110.6, 126.4, 111.0, 113.9, 115.9, 120.3, 122.9,125.0, 125.4, 125.7, 125.9, 128.8, 129.6, 129.9, 130.8, 134.9, 151.3, 154.0, 154.3, 155.1, 160.1. Anal. Calcd for$C_{24}H_{18}BrNO_2S$: C, 62.08; H, 3.91; N, 3.02. Found: C, 62.30; H; 4.13; N, 2.78.

*4.4.12. 4-(5-Bromobenzofuran-2-yl)-2-(3-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine (**7l**)*: From compound **5l** (R^1= 5-Br, R^2= 3-OCH_3 1 mmol, 0.357 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7l** was obtained Yield: 0.34 g (73%) green solid. m. p. 179-181 ºC; IR ν_{max}/ cm^{-1} (KBr): 1165, 1212, 1260, 1294 (C-O), 1594 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.90 (t, 1H, -CH_2, *J*= 13.0 Hz), 3.37 (dd, 1H, -CH_2, *J*= 13.0 Hz, *J*= 5.0 Hz), 3.71 (s, 3H, -OCH_3), 5.24 (dd, 1H, -CH, *J*= 13.0 Hz, *J*= 5.0 Hz) 6.85 (dd, 2H, $H_{4,6}$ anisole, *J*= 7.0 Hz, *J*= 1.5

Hz), 6.94 (d, 1H, H_2 anisole, *J*= 1.5 Hz), 7.22 (d,1H, H_d-phenyl, *J*= 7.5 Hz), 7.25 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.31 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 7.55 (t, 2H, $H_{b,c}$-phenyl, *J*= 7.5 Hz), 7.61 (td, 1H, H_5 anisole, *J*= 7.0 Hz, *J*= 1.5 Hz), 7.72 (d, 1H, H_6 benzofuran, *J*= 8.0 Hz), 7.83 (s, 1H, H_3 benzofuran), 7.99 (d, 1H, H_4 benzofuran, *J*= 1.0 Hz), ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.0, 54.9, 59.8, 110.9, 111.8, 112.9, 113.9, 115.8, 118.1, 122.4, 124.8, 125.3, 125.8, 129.6, 129.9, 130.1, 134.9, 145.3, 151.3, 154.0, 154.2, 159.2, 159.9. Anal. Calcd for $C_{24}H_{18}BrNO_2S$: C, 62.08; H, 3.91; N, 3.02. Found: C: 62.33; H, 4.06; N, 3.24.

*4.4.13. 4-(5-Bromobenzofuran-2-yl)-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine (**7m**):* From compound **5m** (R^1= 5-Br, R^2= 4-OCH_3, 1 mmol, 0.357 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7m** was obtained. Yield: 0.35 g (75%) green solid. m. p. 181-183 ºC; IR ν_{max}/ cm^{-1} (KBr): 1172, 1248 (C-O), 1607 (C=N), 3300 (N-H); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.88 (t, 1H, -CH_2, *J*= 13.0 Hz),3.31 (dd, 1H, -CH_2 , *J*= 13.0 Hz, *J*= 5.0 Hz), 3.73 (s, 3H, -OCH_3), 5.25 (dd, 1H, -CH, *J*= 13.0Hz, *J*= 5.0 Hz), 6.88 (d, 2H, $H_{3,5}$ anisole, *J*= 8.0 Hz) , 7.22 (t, 1H, H_c phenyl, *J*= 7.5 Hz), 7.29 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.53 (d, 2H, $H_{a,d}$-phenyl, *J*= 7.5 Hz), 7.57 (d, 2H, $H_{2,6}$ anisole, *J*= 8.0 Hz), 7.62 (dd, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 2.0 Hz), 7.72 (d, 1H, H_7 benzofuran, d, *J*= 8.0 Hz), 7.81 (s, 1H, H_3 benzofuran), 7.99 (d, 1H, H_4 benzofuran, *J*= 1.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 55.1, 59.5, 95.4, 110.8, 113.8, 113.9, 122.5, 124.8, 125.3, 125.8, 127.2, 129.6, 129.9, 130.0, 134.9, 136.0, 151.2, 154.0, 154.3, 158.7, 159.8. Anal. Calcd for $C_{24}H_{18}BrNO_2S$: C, 62.08; H, 3.91; N, 3.02. Found: C, 61.82; H, 3.99; N, 3.28.

*4.4.14. 4-(5-bromobenzofuran-2-yl)-2-(p-tolyl)- 2,3-dihydro-1,5-benzothiazepine (**7n**).* From compound **5n** (R^1= 5-Br, R^2= 4-CH_3, 1 mmol, 0.341 g) and 2- aminothiophenol (1 mmol, 0.125 g),

for 2 h, product **7n** was obtained. Yield: 0.36 g (72%), green solid. m. p. 175-177 ºC; IR ν_{max}/ cm^{-1} (KBr): 1160,1184, 1266 (C-O), 1680 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.28(s, 3H, -CH_3)2.90 (t, 1H, -CH_2, *J*= 12.5 Hz),3.34 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.24 (dd, 1H, -CH, *J*= 12.5 Hz, *J*= 4.5 Hz), 7.13 (d, 2H, 2$H_{a'}$-toluene, *J*= 7.5 Hz), 7.21 (dd, 1H, H_c-phenyl, *J*= 7.5 Hz, *J*= 1.5 Hz), 7.24 (d, 2H, 2$H_{b'}$-toluene, *J*=7.5 Hz), 7.30 (dd, 1H, H_b-phenyl, *J*= 7.5 Hz, *J*= 1.5 Hz), 7.55 (ddd, 2H, $H_{a,d}$-phenyl, *J*= 7.5 Hz, *J*= 4.5 Hz, *J*= 1.5 Hz), 7.62 (dd, 1H, H_5 benzofuran, *J*= 8.0 Hz, *J*= 2.0 Hz), 7.72 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 7.82 (s, 1H, H_3 benzofuran), 8.51 (s, 1H, H_3 benzofuran), 7.99 (d, 1H, H_4 benzofuran, *J*= 2.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 20.6, 37.0, 59.7, 95.4, 112.7, 110.8, 113.9, 115.9, 122.5, 124.8, 125.3, 125.8, 125.9, 129.0, 129.6, 129.9, 130.1, 134.9, 136.9, 140.9, 151.3, 154.0, 154.3, 159.9. Anal. Calcd for $C_{24}H_{18}BrNOS$: C, 64.29; H, 4.05; N, 3.12. Found: C, 64.59; H, 4.39; N, 3.44.

*4.4.15. 4-(5-bromobenzofuran-2-yl)-2-(2-fluorophenyl)- 2,3-dihydro-1,5-benzothiazepine (**7o**).* From compound **5o** (R^1= 5-Br, R^2= 2-F, 1 mmol, 0.345 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7o** was obtained. Yield 0.41 g (83%), light cream solid. m. p. 221-223 ºC; IR ν_{max}/ cm^{-1} (KBr): 1176, 1215, 1254 (C-O), 1601 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.90 (t, 1H, -CH_2, *J*= 12.5 Hz),3.39 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.33 (dd, 1H, -CH, *J*= 12.5Hz, *J*= 4.5 Hz), 7.17 (t, 2H, $H_{4,5}$ fluorobenzene, *J*= 8.0 Hz), 7.23 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.31 (d, 1H, H_d-phenyl, *J*= 7.5 Hz), 7.42 (dd, 2H, $H_{3,6}$ fluorobenzene, *J*= 8.0 Hz, *J*= 5.0 Hz), 7.56 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.59 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.63 (dd, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 2.0 Hz), 7.73 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 7.85 (s, 1H, H_3 benzofuran), 8.00 (d, 1H, H_4 benzofuran, J= 2.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.5, 58.9, 111.0, 113.9, 115.1, 115.3, 115.9, 122.2, 124.8, 125.4, 125.9, 128.1, 128.2, 129.7, 129.9, 130.2, 134.9, 140.1,

151.3, 154.0,154.2, 159.8. Anal. Calcd for $C_{23}H_{15}BrFNOS$: C, 61.07; H, 3.34; N, 3.10. Found: C, 61.34; H, 3.23; N, 3.31.

*4.4.16. 4-(5-bromobenzofuran-2-yl)-2-(p-tolyl)- 2,3-dihydro-1,5-benzothiazepine (**7p**).* From compound **5p** (R^1= 5-Br, R^2= 4-CH_3, 1 mmol, 0.341 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7p** was obtained. Yield 0.37 g (72%), green solid. m. p. 175-177 ºC; IR ν_{max}/ cm^{-1} (KBr): 1142, 1252 (C-O),1345,1526 (N=O), 1606 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.95 (t, 1H, -CH_2, *J*= 13.0 Hz), 3.47 (dd, 1H, -CH_2 , *J*= 13.0 Hz, J= 5.0 Hz), 5.52 (dd, 1H, -CH, *J*= 13.0Hz, J= 5.0), 7.26 (t, 1H, H_c-phenyl, *J*= 7.0 Hz), 7.33 (d, 1H, H_d, *J*= 7.0 Hz), 7.58 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.62 (t, 1H, H_5 nitrobenzene, *J*= 8 .0Hz), 7.63 (d, 1H, H_6 nitrobenzene, *J*= 8.0 Hz), 7.73 (d, 1H, H_4 nitrobenzene, *J*= 8.0 Hz), 7.86 (d, 1H, H_a-phenyl, *J*= 7.0 Hz), 7.92 (d, 1H, H_7 benzofurane, *J*= 8.0 Hz), 8.01 (d, 1H, H_4 benzofuran, *J*= 2.0 Hz), 8.05 (s, 1H, H_3 benzofuran), 8.15 (d, 1H, H_2 nitrobenzene, *J*= 5.0 Hz), 8.29 (d, 1H, H_6 benzofuran, *J*= 8.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.0, 59.8, 111.7, 112.7, 118.8, 122.2, 122.4, 125.3, 126.0, 127.5, 128.1, 128.4, 130.0, 134.8, 143.5, 144.0, 151.0, 155.8, 157.8, 159.7. Anal. Calcd for$C_{23}H_{15}BrN_2O_3S$: C, 57.63; H, 3.15; N, 5.84. Found: C, 57.52; H, 2.97; N, 6.02.

*4.4.17. 4-(5-bromobenzofuran-2-yl)-2-(naphthalen-1-yl)- 2,3-dihydro-1,5-benzothiazepine (**7q**).* From compound **5q** (R^1= 5-Br, R^2= 1-naphthalene, 1 mmol, 0.376 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7q** was obtained. Yield 0.44 g (85%), green solid. m. p. 197-199 ºC; IR ν_{max}/ cm^{-1} (KBr): 1211, 1254 (C-O), 1605 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 3.17 (t, 1H, -CH_2, *J*= 12.5 Hz),3.52 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 6.13 (dd, 1H, -CH, *J*= 12.5Hz, *J*= 4.5 Hz) 7.19 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.34 (dd, 1H, H_d-phenyl, *J*= 7.5 Hz), 7.45

(t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.48 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.55 (d, 1H, H_7 naphthalene, *J*= 8.0 Hz), 7.59 (dd, 2H, $H_{2,4}$ naphthalene, *J*= 8.0 Hz, *J*= 4.0 Hz), 7.63 (t, 2H, $H_{3,6}$ naphthalene, *J*= 8.0 Hz), 7.74 (d, 1H, H_5 naphthalene, *J*= 8.0 Hz), 7.80 (s, 1H, H_3 benzofuran), 7.88 (d, 1H, H_8 naphthalene, *J*= 8.0 Hz), 7.99 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 8.00 (s, 1H, H_4 benzofuran), 8.17 (d, 1H, H_6 benzofuran, *J*= 8.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 35.0, 55.0, 95.3, 110.6, 113.9, 115.9, 122.6, 122.9, 123.1, 124.9, 125.4, 125.5, 125.8, 125.9, 126.6, 128.0, 128.8, 129.4, 129.6, 130.0, 130.1, 133.5, 135.0, 138.5, 151.4, 154.0, 154.4, 160.4. EI-MS m/z (%) 489 (M^++6, 0.5), 487 (M^++4, 1), 485 (M^++2, 6), 483 (M^+, 7), 452 (1), 451 (1), 450 (1), 356 (1), 332 (10), 329 (41), 250 (4), 222 (11), 178 (4), 155 (16), 154 (100), 153 (61), 152 (27), 127 (3), 102 (1), 69 (4), 45 (3). Anal. Calcd for $C_{27}H_{18}BrNOS$: C, 66.95; H, 3.75; N, 2.89. Found: C, 67.18; H, 3.69; N, 3.14.

*4.4.18. 4-(5-bromobenzofuran-2-yl)-2-(1H-indol-3-yl)- 2,3-dihydro-1,5-benzothiazepine (**7r**).* From compound **5r** (R^1= 5-Br, R^2= 3-indolyl, 1 mmol, 0.366 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 2 h, product **7r** was obtained. Yield 0.38 g (75%), green solid. m. p. 245-247 ºC; IR ν_{max}/ cm^{-1} (KBr):1177, 1247 (C-O), 1602 (C=N), 3374 (N-H); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 3.13 (t, 1H, -CH_2, *J*= 12.5 Hz),3.45 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.62 (dd, 1H, -CH, *J*= 12.5 Hz, *J*= 4.5 Hz), 6.97 (t, 1H, H_5 indole, *J*= 7.5 Hz), 7.10 (t, 1H, H_c-phenyl, *J*= 7.5 Hz),7.17 (t, 1H, H_6 indole, *J*= 7.5 Hz), 7.29 (s, 1H, H_2 indole), 7.32 (d, 1H, H_d-phenyl, *J*= 7.5 Hz), 7.37 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.48 (dd, 2H, $H_{4,7}$ indole, *J*= 7.5 Hz, *J*= 1.0 Hz), 7.53 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.61 (d, 1H, H_7 benzofuran, *J*= 7.5 Hz), 7.70 (s, 1H, H_4 benzofuran), 7.71(d, 1H, H_6 benzofuran, *J*= 7.5 Hz), 7.97 (s, 1H, H_3 benzofuran), 10.99 (s, 1H, N-H); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 35.6, 53.8, 110.4, 111.6, 113.9, 115.8, 117.3, 118.6, 119.1, 121.4,

122.0, 122.1, 123.4, 124.8, 125.0, 125.2, 125.6, 129.5, 129.8, 129.9, 135.0, 136.3, 136.5, 151.3, 154.0, 154.6, 160.2. EI-MS m/z (%) 478 (M^++6, 0.02), 476 (M^++4, 0.1), 474 (M^++2, 1), 472 (M^+, 1), 438 (1), 345 (3), 329 (12), 301 (1), 275 (1), 250 (2), 222 (7), 196 (2), 178 (3), 160 (1), 144 (15), 143 (100), 140 (4), 115 (6), 89 (5), 63 (6), 45 (3). Anal. Calcd for $C_{25}H_{17}BrN_2OS$: C, 63.43; H, 3.62; N, 5.92. Found: C, 63.30; H, 3.51; N, 6.04.

*4.4.19. 4-(5-nitrobenzofuran-2-yl)-2-phenyl-2,3-dihydro-1,5-benzothiazepine (**7s**).* From compound **5s** (R^1= 5-NO_2, R^2= H, 1 mmol, 0.293 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 4 h, product **7s** was obtained. Yield 0.39 g (90%), dark yellow solid. m. p. 222-224 ºC; IR ν_{max}/ cm^{-1} (KBr): 1178, 1266 (C-O), 1342, 1524 (N=O), 1604 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.95 (t, 1H, -CH_2, *J*= 12.5 Hz),3.42 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.31 (dd, 1H, -CH, *J*= 12.5 Hz, *J*= 4.5 Hz), 7.26 (t, 2H, $H_{b,c}$-phenyl, *J*= 7.5 Hz), 7.29 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.34 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.38 (d, 2H, $2H_{a'}$ -phenyl, *J*= 7.5 Hz), 7.57 (t, 2H, $2H_{b'}$-phenyl, *J*= 7.5 Hz), 7.60 (d, 1H, H_a-phenyl, *J*= 7.5 Hz), 7.98 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 8.05 (s, 1H, H_3 benzofuran), 8.35 (dd, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 2.0 Hz), 8.70 (s, 1H, H_4 benzofuran); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.1, 59.9, 112.0, 112.9, 119.0, 122.3, 122.5, 125.4, 126.1, 127.1, 128.2, 128.5, 130.1, 134.9, 143.0, 143.7, 144.1, 151.1, 155.9, 157.9, 159.8. Anal. Calcd for $C_{23}H_{16}N_2O_3S$: C, 68.99; H, 4.03; N, 7.00. Found: C, 68.78; H, 4.26; N, 7.19.

*4.4.20. 2-(2-fluorophenyl)-4-(5-nitrobenzofuran-2-yl)- 2,3-dihydro-1,5-benzothiazepine (**7t**).* From compound **5t** (R^1= 5-NO_2, R^2= 2-F, 1 mmol, 0.311 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 4 h, product **7t** was obtained. Yield 0.37 g (78%), dark yellow solid. m. p. 138-140 ºC; IR ν_{max}/

cm^{-1} (KBr): 1216, 1259 (C-O), 1347,1524 (N=O); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 2.94 (t, 1H, -CH_2, *J*= 13.0 Hz), 3.42 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.36 (dd, 1H, -CH, *J*= 12.5 Hz, *J*= 4.5 Hz), 7.17 (t, 2H, $H_{b,c}$-phenyl, *J*=7.5 Hz), 7.24 (td, 1H, H_5 fluorobenzene, *J*= 7.5 Hz, *J*=1.0 Hz), 7.33 (dd, 1H, H_6 fluorobenzene, *J*= 7.5 Hz, *J*= 1.0 Hz), 7.42 (dd, 2H, $2H_{a,d}$-phenyl, *J*= 7.5 Hz, *J*= 5.5 Hz), 7.57(td, 1H, H_4 fluorobenzene, *J*= 7.5 Hz, *J*= 1.0 Hz), 7.60 (dd, 1H, H_3 fluorobenzene, *J*= 7.5 Hz, *J*= 1.0 Hz), 7.99 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 8.07 (s, 1H, H_3 benzofuran), 8.36 (dd, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 2.0 Hz), 8.73 (d, 1H, H_4 benzofuran, *J*= 2.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 37.1, 59.0, 95.4, 112.1, 112.9, 115.2, 115.4, 122.2, 122.4, 125.4, 126.1, 128.1, 128.2, 128.3, 130.2, 134.9, 140.0, 144.1, 151.1, 155.8, 157.9, 159.7, 162.4. Anal. Calcd for $C_{23}H_{15}FN_2O_3S$: C, 66.02; H, 3.61; N, 6.69. Found: C, 65.84; H, 3.81; N, 6.36.

*4.4.21. 2-(naphthalen-1-yl)-4-(5-nitrobenzofuran-2-yl)- 2,3-dihydro-1,5-benzothiazepine (**7u**).* From compound **5u** (R^1= 5-NO_2, R^2= naphthyl, 1 mmol, 0.343 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 4 h, product **7u** was obtained. Yield 0.38 g (73%), dark yellow solid. m. p. 223-224ºC; IR ν_{max}/ cm^{-1} (KBr): 1214, 1291 (C-O),1338, 1570 (N=O), 1615 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 3.16 (t, 1H, -CH_2, *J*= 12.5 Hz), 3.48 (dd, 1H, -CH_2 , *J*= 12.5 Hz, *J*= 4.5 Hz), 5.61 (dd, 1H, -CH, *J*= 12.5 Hz, *J*= 4.5 Hz), 7.18 (d, 1H, H_5 naphthalene, *J*= 7.5 Hz), 7.26 (d, 2H, $H_{a,d}$ -phenyl, *J*= 7.5 Hz), 7.34 (t, 1H, H_7 naphthalene, *J*= 7.5 Hz), 7.38 (t, 2H, $H_{3,6}$ naphthalene, *J*= 7.5 Hz), 7.48 (dd, 2H, $H_{2,4}$ naphthalene, *J*= 7.5 Hz, *J*= 1.0 Hz), 7.58 (t, 2H, $H_{b,c}$ -phenyl, *J*= 7.5 Hz), 7.64 (d, 1H, H_8 naphthalene, *J*= 7.5 Hz), 8.14 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 8.18 (s, 1H, H_3 benzofuran), 8.28 (d, 1H, H_6 benzofuran, *J*= 8.0 Hz), 8.72 (s, 1H, H_4 benzofuran); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 35.0, 55.0, 111.5, 117.5, 122.0, 122.6, 124.3, 125.3, 125.6, 125.9,

126.5, 127.1, 128.8, 129.4, 130.1, 133.5, 135.0, 139.9, 144.1, 151.6, 155.2, 161.3. Anal. Calcd for $C_{27}H_{18}N_2O_3S$: C, 71.98; H, 4.03; N, 6.22. Found: C, 72.23; H, 4.18; N, 6.44.

*4.4.22. 2-(1H-indol-3-yl)-4-(5-nitrobenzofuran-2-yl)- 2,3-dihydro-1,5-benzothiazepine (**7v**).* From compound **5v** (R^1= 5-NO_2, R^2= 3-indolyl, 1 mmol, 0.332 g) and 2- aminothiophenol (1 mmol, 0.125 g), for 4 h, product **7v** was obtained. Yield 0.43 g (85%), light green solid. m. p. 228-230 ºC; IR ν_{max}/ cm^{-1} (KBr):1178, 1246 (C-O), 1600 (C=N), 3374 (N-H); ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 3.16 (t, 1H, -CH_2, *J*= 12.5 Hz), 3.48 (dd, 1H, -CH_2 , *J*= 12.5 Hz, J= 4.5 Hz), 5.61 (dd, 1H, -CH, *J*= 12.5 Hz, *J*= 4.5 Hz), 6.97 (t, 1H, H_5 indole, *J*= 7.5 Hz), 7.10 (t, 1H, H_c-phenyl, *J*= 7.5 Hz), 7.19 (t, 1H, H_6 indole, *J*= 8.0 Hz), 7.30 (s, 1H, H_2 indole), 7.34 (d, 1H, H_7 indole, *J*= 8.0 Hz), 7.37 (d, 1H, H_4 indole, *J*= 8.0 Hz), 7.49 (d, 2H, $H_{a,d}$-phenyl, *J*= 7.5 Hz), 7.54 (t, 1H, H_b-phenyl, *J*= 7.5 Hz), 7.93 (s, 1H, H_3 benzofuran), 7.96 (d, 1H, H_7 benzofuran, *J*= 8.0 Hz), 8.34 (dd, 1H, H_6 benzofuran, *J*= 8.0 Hz, *J*= 2.0 Hz), 8.70 (d, 1H, H_4 benzofuran, J= 2.0 Hz), 10.99 (s, 1H, -NH); ^{13}C NMR (125 MHz, DMSO-d_6), δ ppm: 35.7, 53.8, 111.6, 111.7, 112.8, 117.3, 118.6, 119.0, 121.4, 122.1, 122.2, 123.5, 125.0, 125.3, 125.8, 128.3, 129.8, 135.0, 136.5, 144.1, 151.1, 156.2, 157.8, 160.0. Anal. Calcd for $C_{25}H_{17}N_3O_3S$: C, 68.32; H, 3.90; N, 9.56. Found: C, 68.55; H, 3.84; N, 9.81.

4.5. Cholinesterases inhibition assay

The inhibitory activities of target compounds against AChE and BChE were determined using the spectrophotometric method of Ellman.[48] Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from electric eel, 1000 unit), butylcholinesterase (BChE, E.C. 3.1.1.8, from equine serum), acetylthiocholine iodide, and butylthiocholine iodode (BTC) were purchased from Sigma-Aldrich. Ellman's reagent [5,5´-dithiobis(2-nitrobenzoic acid)] (DTNB), potassium

dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, sodium hydrogen carbonate, and acetylthiocholine iodide were obtained from Fluka. Ethanol/DMSO (9:1, 1 mL) was used as a solvent system for dissolving test compounds. The stock solutions of tested compounds were prepared in a mixture of DMSO (1 mL) and methanol (9 mL) followed by dilution in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to obtain final assay concentrations. The previously reported method was applied to evaluate enzyme inhibition activity of tested compounds. All solution temperatures were adjusted to 25 ºC prior to use. Five different concentrations of each compound were tested in triplicate. The assay medium contained 3 mL of 0.1 M phosphate buffer pH 8.0, 100 µL of 0.01 M DTNB, and 100 µL of 2.5 unit/ mL enzyme solution (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from electric eel) (Sigma Chemical). 100 µL of each tested compounds were added to the assay medium and incubated at 25 ºC for 15 min followed by addition of 20 µL of substrate (acetylthiocholine iodide). After that the rate of absorbance change was measured at 412 nm for 6 minutes. The same protocol was applied for determination of BChE activity by using butyrylthiocholine iodide as substrate. The blank reading solution was used to justify non-enzymatic hydrolysis of substrate during the assay. The blank solution contained 3 mL buffer, 200 µL water, 100 µL DTNB and 20 µL substrate. As a reference, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The rate of the substrate enzymatic hydrolysis was calculated, and inhibition percent of the test compounds was determined. Spectrophotometric measurements were performed on a UV-2100 Rayleigh Double Beam Spectrophotometer. The same method was used for BChE inhibition assay.

4.5.1. Kinetic analysis of BChE inhibition. Determination of steady-state inhibition constants

To elucidate the inhibition mechanisms for the active compounds, the BChE residual activities were determined in the presence of three concentrations of the test compounds and three concentrations of of the substrates. The test compounds were preincubated with the enzymes at 25 °C for 10 min, followed by the addition of the substrates. Parallel controls were made to find the rate of hydrolysis of the same concentrations of substrates in the solutions with no inhibitor. The kinetic parameters of substrate hydrolysis were determined. The measurements were carried out using a Bio Rad Benchmark Plus microplate spectrophotometer (France). Each experiment was performed in triplicate. The results were fitted into Lineweaver-Burk double-reciprocal kinetic plots of 1/V versus 1/[S] and the value of inhibition constant *Ki* was calculated using Origin 6.1 software for Windows.

4.5.2. Molecular modeling

Docking studies were carried out using Auto Dock Tools (version 1.5.6) and the pdb structure of 1P0I was taken from the Brookhaven protein database (http://www.rcsb.org). The 3D structures of the selected inhibitors were created by MarvineSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and by Auto dock Tools converted to pdbqt coordinate. Also, the pdbqt coordinate of enzyme was prepared using the Auto dock Tools. Before preparation of pdbqt form of enzyme, the water molecules and the inhibitors were removed from it. Then, using Auto dock Tools, Polar hydrogen atoms were added, Koullman charges were assigned, and the obtained protein structure was used as an input file for the AUTOGRID program. In AUTOGRID for each atom type in the inhibitor, maps were calculated with 0.375 Å spacing between grid points and the center of the grid box was placed at x = 137.985, y = 122.725, z = 38.78. The dimensions of the active site box were set at 55 × 55 × 55 Å. Flexible ligand dockings were accomplished for the

selected inhibitors. Each docked system was performed by 50 runs of the AUTODOCK search by the Lamarckian genetic algorithm (LGA). The best pose of each inhibitor was selected for analyzing the interactions between BChE and the inhibitor and the results were visualized using Discovery Studio 4.0 Client.[49]

4.6. Antimicrobial assay

The antimicrobial activity of the tested samples was determined by the agar disc diffusion method. Solution of each synthesized compound was prepared at the concentration of 20 mg mL^{-1} using DMSO as a solvent and filtered by 0.45 μm Millipore filters for sterilization. 100 μL of the suspension containing 10^8 CFU mL^{-1} of bacteria were spread on to the nutrient agar. The discs (6 mm in diameter) impregnated with 10 μl of the synthesized compound (200 μg per disc) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 24 hours at 37 ºC for bacterial strains and 48 hours and 72 hours at 30 ºC for the yeast and mold isolated, respectively. As positive controls, gentamicin (10 μg per disc) and rifampin (5 μg per disc) were used for bacteria and nystatin (30 μg per disc) was used for fungi. After incubation, the growth inhibition zones around the discs were observed, which indicated that the examined compound inhibited the growth of microorganisms. The diameters of inhibition zones were used as a measure of antimicrobial activity. Each assay in this experiment was repeated for three times.[50-52] For the purpose of easier visualization, the zone date from these assays indicates the average diameter (from 3 trails) of the growth inhibition zones. The error margin of these measurements is ±1 mm. The antibacterial activity was classified as highly active (>14 mm), moderately active (10–14 mm), slightly active (6–10 mm) and less than 6 mm was regarded as inactive.

Acknowledgements

The authors are thankful for the financial support from the Research Council of Alzahra University and the Research Council of Tehran University of Medical Sciences and Iran National Science Foundation (INSF). N. L. thanks the National Elites Foundation of Iran, Tehran (BMN) for support of this work.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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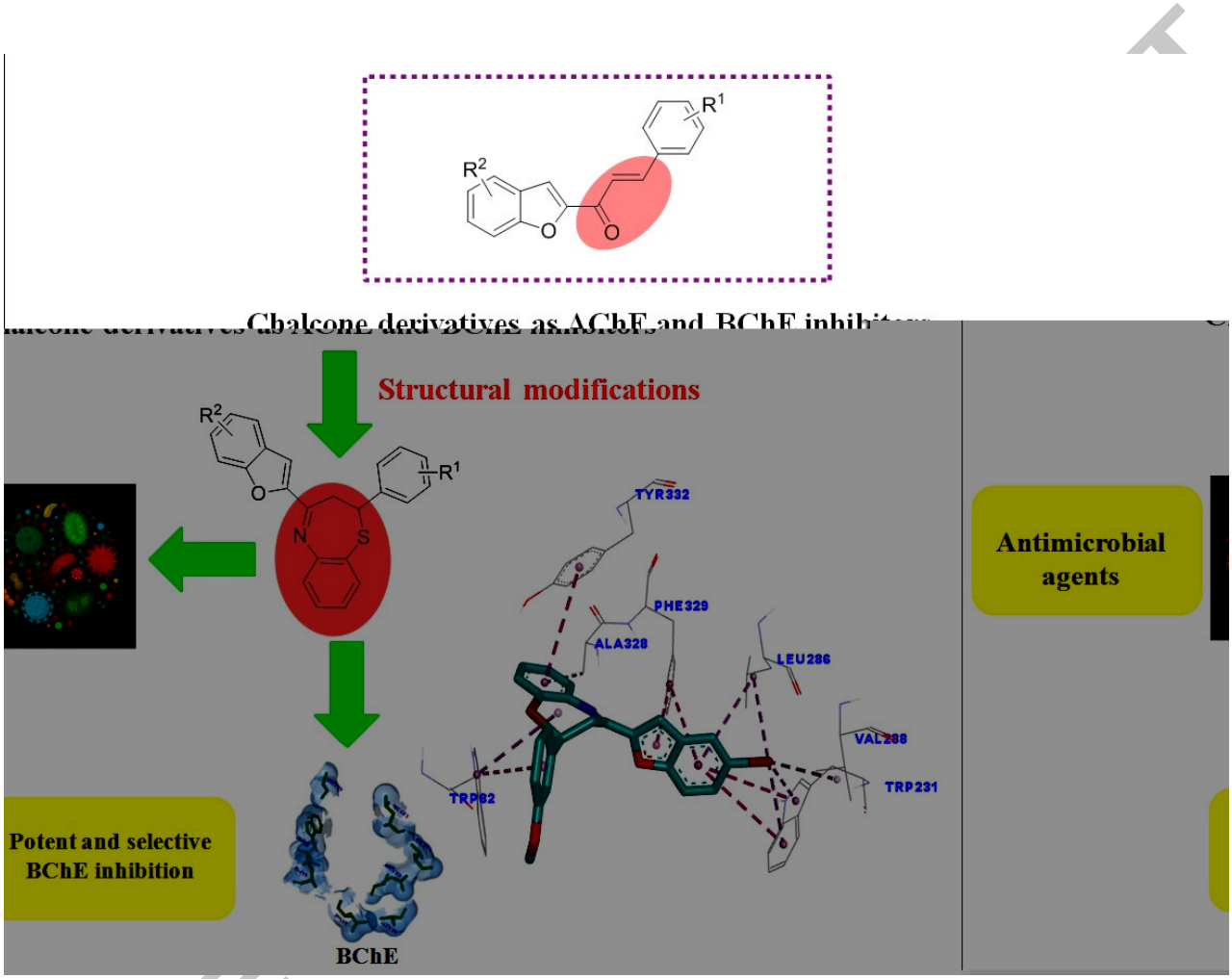
Chalcone derivatives as AChE and BChE inhibitors
Structural modifications
Potent and selective BChE inhibition
Antimicrobial agents
BChE
TYR332
PHE329
ALA328
LEU286
VAL288
TRP231
TRP82