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FULL PAPER

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Thieno[2,3-*b*]pyridine amines: Synthesis and evaluation of tacrine analogs against biological activities related to Alzheimer's disease

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Abstract

In search of safer tacrine analogs, various thieno[2,3-*b*]pyridine amine derivatives were synthesized and evaluated for their inhibitory activity against cholinesterases (ChEs). Among the synthesized compounds, compounds **5e** and **5d** showed the highest activity towards acetylcholinesterase and butyrylcholinesterase, with IC_{50} values of 1.55 and 0.23 μM, respectively. The most active ChE inhibitors (**5e** and **5d**) were also candidates for further complementary assays, such as kinetic and molecular docking studies as well as studies on inhibitory activity towards amyloid-beta (βA) aggregation and β-secretase 1, neuroprotectivity, and cytotoxicity against HepG2 cells. Our results indicated efficient anti-Alzheimer's activity of the synthesized compounds.

KEYWORDS

Alzheimer's disease, amyloid beta (βA), BACE1, cytotoxicity, thieno[2,3-*b*]pyridine

1 | INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that accounts for about 70% of all dementia cases. It occurs as people get older, and the main symptoms of the disease include cognitive dysfunction, psychiatric and behavioral disturbances, and serious difficulties in performing daily activities.[1] The exact underlying cause of AD is obscure, and various factors are believed to play important roles in the onset and progression of the disease. In this respect, reduced synaptic levels of acetylcholine (ACh),[2] abnormal deposits of amyloid

This paper is dedicated to the memory of our unique teacher in Chemistry and Medicinal Chemistry, Professor Abbas Shafiee (1937–2016).

β plaques,[3] the presence of neurofibrillary tangles,[4] and dyshomeostasis and miscompartmentalization of metal ions (Fe^{2+}, Cu^{2+}, and Zn^{2+}) in the brain[5] have been comprehensively discussed in the literature. Currently, there is no definite cure for the treatment of AD due to the complicacy of AD pathology, and available treatments are merely palliative. Hence, the design and synthesis of multifactorial compounds, considering various mechanisms involved in AD, have occupied a special position in drug discovery endeavors.[6]

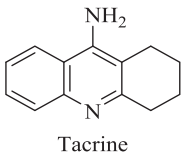
Currently available FDA-approved anti-AD drugs, including donepezil, rivastigmine, and tacrine, profit from the ability of the inhibition of acetylcholinesterase (AChE) and increasing the level of ACh in the brain. Therefore, the cholinergic hypothesis of AD is still important in drug discovery developments.[7] 1,2,3,4-Tetrahydroacridin-9-amine, known as tacrine (Figure 1), was the first biologically important compound for the treatment of AD, which was removed from the market due to serious clinical shortcomings instigating hepatotoxicity probably by mediating the stimulation of reactive oxygen species production and glutathione depletion.[8]

Focusing on the striking effect of tacrine on cognitive defects, a wide range of research has been devoted to modify the structure of tacrine to decrease toxicity and involvement of other approaches in the treatment of AD.[9] In this respect, various tacrine analogs such as tacrine–tryptophan (Figure 2a),[10] tacrine–8-hydroxyquinoline (Figure 2b),[11] tacrine–chromene (Figure 2c,d),[12] and tacrine–1,2,3-triazole–coumarin (Figure 2e–f)[13–15] hybrids were found to be versatile compounds against AD through various mechanisms, along with inhibitory activity against ChEs. Herein, in continuation of our research program on the development of tacrine,[13–17] we planned to synthesize some tacrine analogs possessing different activities against AD.

2 | RESULTS AND DISCUSSION

2.1 | Compound design

Design of target compounds (Figure 3) is related to the potent ChE inhibitory activity of the tacrine scaffold. Herein, we tried to keep the main moiety intact, whereas the aromatic moiety was replaced by the cycloalkylthiophene moiety, as thiophene core had shown a significant BACE1 inhibitory activity (compound **h**, Figure 3)[18] and amyloid-beta aggregation inhibition (compound **i**, Figure 3)[19] to afford multitarget anti-AD agents. The cyclohexane moiety of tacrine was also replaced by cyclopentane and cycloheptane moieties; however, all alterations were considered to investigate how a change in the lipophilicity and polarity affects multiple interactions with complementary sites of ChEs and suppresses toxicity profile, compared with tacrine.



Tacrine

FIGURE 1 The structure of tacrine

2.2 | Chemistry

Synthesis of the target compound **5** is demonstrated in Scheme 1. Initially, an appropriate substrate **4** possessing β-amino nitrile moiety was prepared by the condensation of various cycloalkanones (**1**), malononitrile (**2**), and sulfur (S8, **3**) in the presence of L-proline in dimethylformamide (DMF) at 60°C for 24 hr through the Gewald reaction.[20] It should be mentioned that the reaction was examined in the presence of other reagents, such as morpholine and triethylamine, using different solvents, including dimethyl sulfoxide and acetonitrile, under various conditions. However, using L-proline in DMF at 60°C afforded a higher yield of the reaction. Lewis acid-catalyzed Friedländer condensation reaction of compound **4** and reactive methylene derivative **1** led to the formation of pyridine ring fused with the thiophene moiety. For this purpose, according to our previous experience reported in the literature, [16] reaction of compound **4** and cycloalkanone **1** in the presence of $AlCl_3$ in 1,2-dichloroethane under reflux conditions for 24 hr gave the desired product **5**.

2.3 | Biology

2.3.1 | Anticholinesterase (AChE) activity

Anti-AChE and anti-butyrylcholinesterase (BChE) activities of synthesized compound **5** were evaluated according to the modified Ellman's method,[21] comparing with tacrine as the reference drug. As discussed, different modifications were achieved to afford multitarget anti-AD compounds with lower hepatotoxicity. In this respect, four different classes of tacrine hybrids were prepared (**5a–c**, **5d–f**, **5g–i**, and **5j**/**5k**) according to the ring size of cycloalkyl and cycloalkylthiophene moieties inserted into the target compounds.

As reported in Table 1, compound **5e** was the most active AChE inhibitor (IC_{50} = 1.55 μM). It possessed cyclohexyl moiety connected to both thiophene and pyridine moieties *m* = 1, *n* = 1). Keeping cyclohexylthiophene moiety intact (*n* = 1) and changing the ring size of cycloalkyl moiety connected to the pyridine moiety (*m* = 0 and 2) led to a lower inhibitory activity against AChE as compounds **5d** and **5f** showed IC_{50} values = 3.47 and 22.52 μM, respectively (entries 4–6). Changing the ring size of cycloalkylthiophene moiety (*n* = 0, 2) resulted in a different activity (entries 1–3 and 7–9). In both series, compounds with *m* = 1 showed higher activity. Compound **5b** compared with **5a** and **5c** as well as compound **5h** compared with **5g** and **5i** showed the following order: **5b** > **5c** > **5a** and **5 h** > **5 g** > **5i**. It should be noted that in the series 1–3, the lowest activity was related to compound **5a** (*m* = 0, *n* = 0); however, the lowest activity in the series 7–9 was observed for

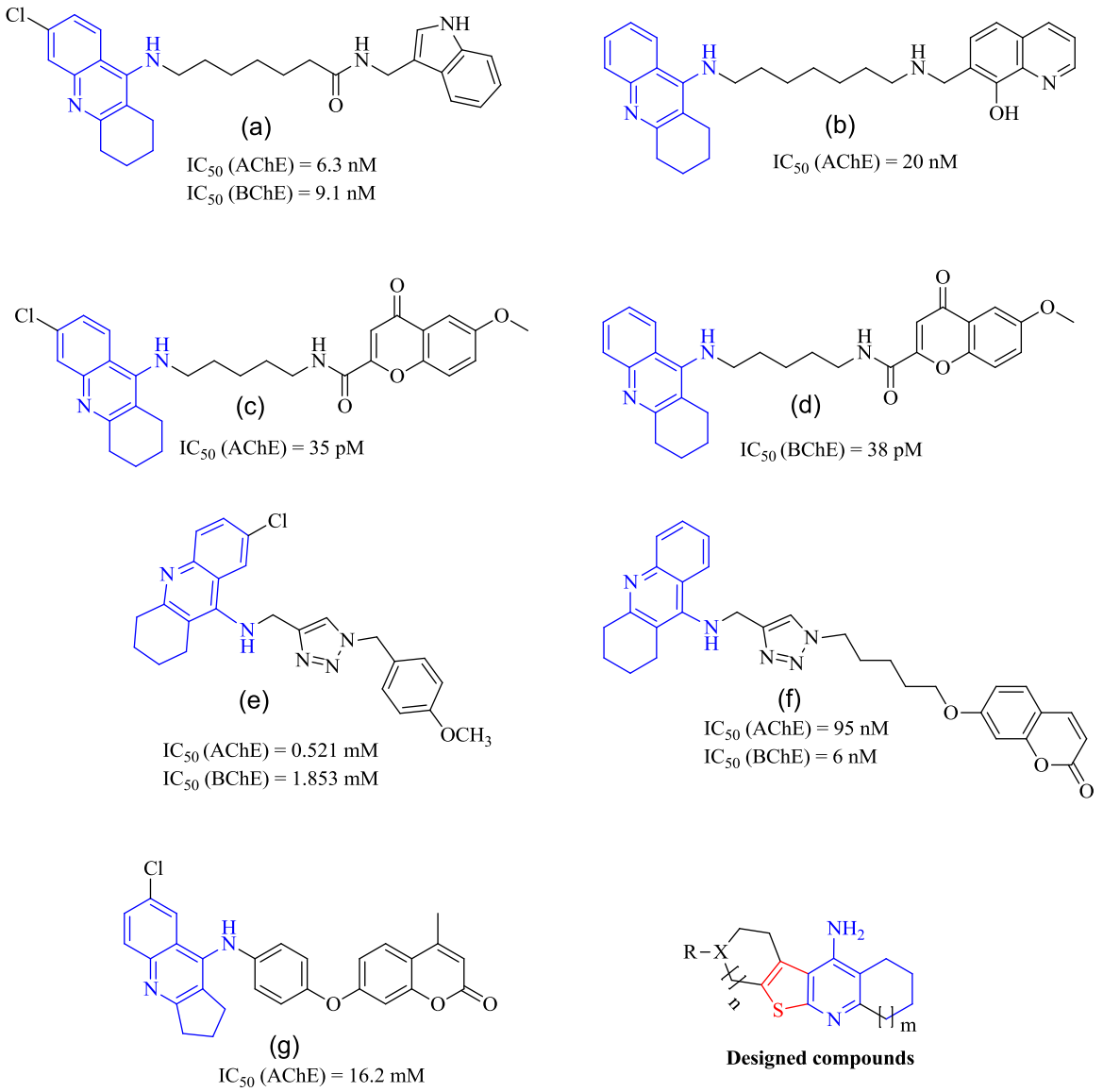


FIGURE 2 The structures of tacrine hybrids and the designed compounds

compound **5i** ($m = 2$, $n = 2$). It seems that the replacement of both aromatic ring and cyclohexyl moieties of tacrine by smaller or larger size rings together led to a weak inhibitory activity. Apparently, both steric hindrance and reduction of lipophilicity could deteriorate the AChEI activity.

Another bioisosteric replacement was performed using benzylpiperidine moiety ($n = 1$, $X = N$) connected to the thiophene moiety (entries 10 and 11). Compound **5j** possessing cyclohexyl moiety connected to the pyridine ring ($m = 1$) was found to be five-fold more potent than compound **5k** having cycloheptyl moiety ($m = 2$). Our result revealed that a good AChEI activity of tacrine analogs **5** was usually achieved by the compounds with $m = 1$ (compounds **5b**, **5e**, **5h**, and **5j**), which mostly mimic the tacrine scaffold from both binding interactions in the active site and lipophilicity points of view.

In the case of BChEI activity of compound **5** derivatives, most compounds showed much better activity than AChEI activity. This can be confirmed by the selectivity index calculated in Table 1. There was an exception related to the most active AChE inhibitor **5e**, which possessed a lower anti-BChE activity.

The best BChEI activity was obtained by compound **5d** having cyclopentyl moiety connected to the pyridine ring ($m = 0$) and cyclohexylthiophene ($n = 1$) with $IC_{50} = 0.23\ \mu M$. The increase in the size of cycloalkyl moiety ($m = 1$ and 2) led to the reduction of activity, and compounds **5e** and **5f** showed an anti-BChE activity with IC_{50} values = 2.33 and 3.08 μM, respectively. In the series of **5a–c** ($n = 0$ and $m = 0$, 1, and 2), a weaker activity was obtained as compared with other compounds, IC_{50} values = 8.94, 3.09, 7.81 μM, respectively; however, the order of BChEI activity, **5b** > **5c** > **5a**, indicated that the cyclohexyl moiety connected to the pyridine ring is effective. In the third category, **5g–i**, the same results were observed and the order of BChEI activity was **5h** > **5i** > **5g** (IC_{50}s = 1.63, 1.81, and 2.07 μM, respectively), which highlighted the efficacy of cyclohexyl moiety ($m = 1$). Our results in the

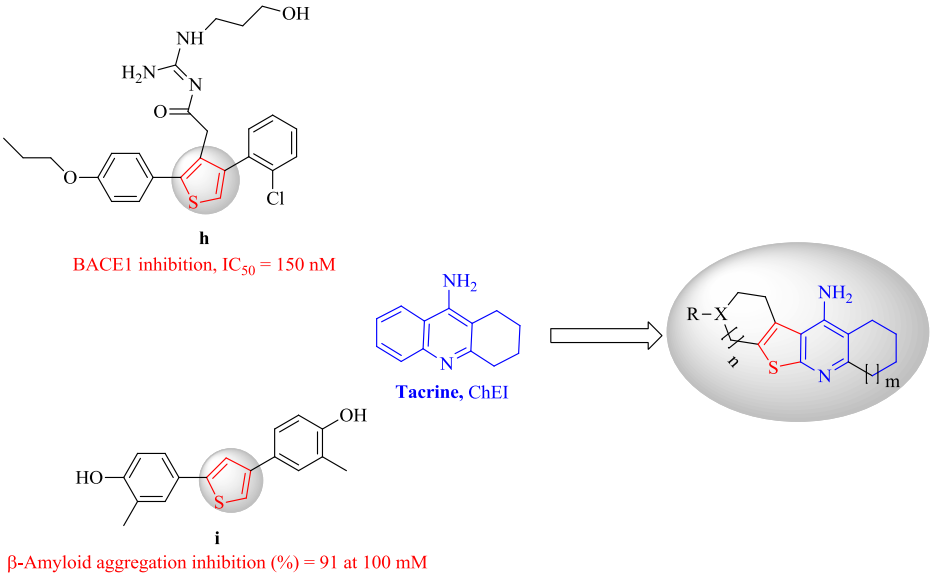


FIGURE 3 The design of the target compounds

case of compounds **5j** and **5k**, which were provided by the connection of benzylpiperidine moiety to the thiophene ring, showed that compound **5k** possessing cycloheptyl moiety showed a more potent activity (IC_{50} = 2.25 μM) than compound **5j** having cyclopentyl moiety (IC_{50} = 3.74 μM). However, there was no definite rule for the effect of cycloalkyl moieties on the BChEI activity.

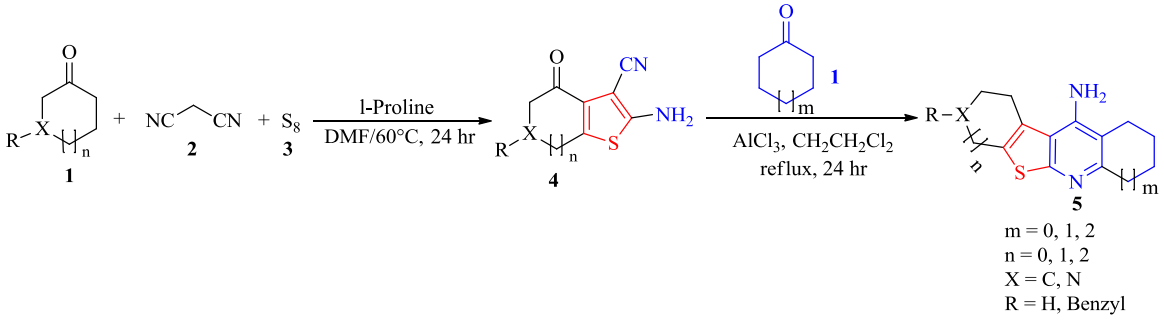
Comparing AChEI and BChEI activity of synthesized compound **5** derivatives showed that cycloalkyl substituents apparently played a more important role in AChE inhibition as mostly compounds possessing cyclohexyl moiety (*m* = 1) depicted a better inhibitory activity. However, benzothienopyridine moiety was an important scaffold for the desired interactions with BChE amino acid residues, and definite structure–activity relationships (SARs) could not be established for the anti-BChE activity.

2.3.2 | Kinetic study

The kinetic studies were performed to determine the mechanism of inhibition by compounds **5e** and **5d** against AChE and BChE, respectively. Graphical analysis of the reciprocal Lineweaver–Burk plot of compound **5d** described a mixed-type inhibition pattern (Figure 4a), indicating that **5e** may bind to AChE, even if it is already bound to the substrate. In addition, the K_i value was calculated using the secondary plot as 2.13 μM (Figure 4b). The kinetic study of compound **5d** toward BChE depicted the same results (Figure 5a). Also, the K_i value was calculated as 0.55 μM (Figure 5b).

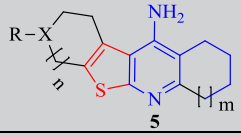
2.3.3 | BACE1 enzymatic assay

β-Secretase (BACE1) inhibitors have emerged as important agents in the treatment of AD due to the role of BACE1 in the proteolytic cleavage of the amyloid protein precursor (APP).[22] Accordingly, the BACE1 inhibitory activities of the most potent ChE inhibitors, compounds **5d** and **5e**, were examined via the fluorescence resonance emission transfer (FRET) method. The used kit consisted of BACE1 enzyme and APP peptide-based substrate (Rh-EVNLDAEFK-quencher), compared with OM99-2 (IC_{50} = 0.014 μM) as the



SCHEME 1 The synthesis of the thieno[2,3-*b*]pyridine amine derivative **5**

TABLE 1 The anticholinesterase activity (IC_{50}, μM)[a] of compound **5**



5

Entry	Compound 5	*m*	*n*	*X*	R	AChEI (IC_{50} [μM])	BChEI (IC_{50} [μM])	SI[b]
1	**5a**	0	0	C	H	28.57 ± 0.04	8.94 ± 0.17	3.2
2	**5b**	1	0	C	H	4.85 ± 0.20	3.09 ± 0.03	1.6
3	**5c**	2	0	C	H	18.92 ± 0.77	7.81 ± 0.19	2.4
4	**5d**	0	1	C	H	3.47 ± 0.20	0.23 ± 0.01	15.1
5	**5e**	1	1	C	H	1.55 ± 0.04	2.33 ± 0.25	0.7
6	**5f**	2	1	C	H	22.52 ± 0.22	3.08 ± 0.33	7.3
7	**5g**	0	2	C	H	17.73 ± 0.31	2.07 ± 0.62	8.6
8	**5h**	1	2	C	H	2.01 ± 0.07	1.63 ± 0.05	1.2
9	**5i**	2	2	C	H	30.71 ± 0.73	1.81 ± 0.01	17.0
10	**5j**	1	1	N	Benzyl	5.50 ± 0.03	3.74 ± 0.23	1.5
11	**5k**	2	1	N	Benzyl	30.44 ± 0.49	2.25 ± 0.55	13.5
12	Tacrine					0.151 ± 0.0035	0.005 ± 0.0003	

Abbreviation: AChE, acetylcholinesterase; BChE, butyrylcholinesterase.
aData are represented in terms of mean ± standard deviation.
bThe selectivity index (SI) is calculated as the ratio of IC_{50} AChE/IC_{50} BChE.

reference drug. It was found that these compounds induced a weak inhibitory activity (8–10% inhibition) at the concentrations of 50 μM.

2.3.4 | Neuroprotective effect against Aβ-induced damage measured in PC12 cells

Compounds **5d** and **5e** were selected to study the neuroprotective ability using PC12 cell injury induced by $A\beta_{25-35}$ by MTT assay, compared with rutin as the reference drug. They demonstrated negligible neuroprotectivity on Aβ-induced PC12 cells up to 50 μM. It can be understood that bioisosterical replacement of cycloalkyl and cycloalkylthiophene induced no desired neuroprotectivity.

2.3.5 | Inhibition of AChE-induced and self-induced Aβ aggregation

Aβ peptide is the major constituent of senile plaques in the brains of patients with AD.[3] In this respect, the effect of the most potent anti-BChE compound **5d** was assessed for the inhibition

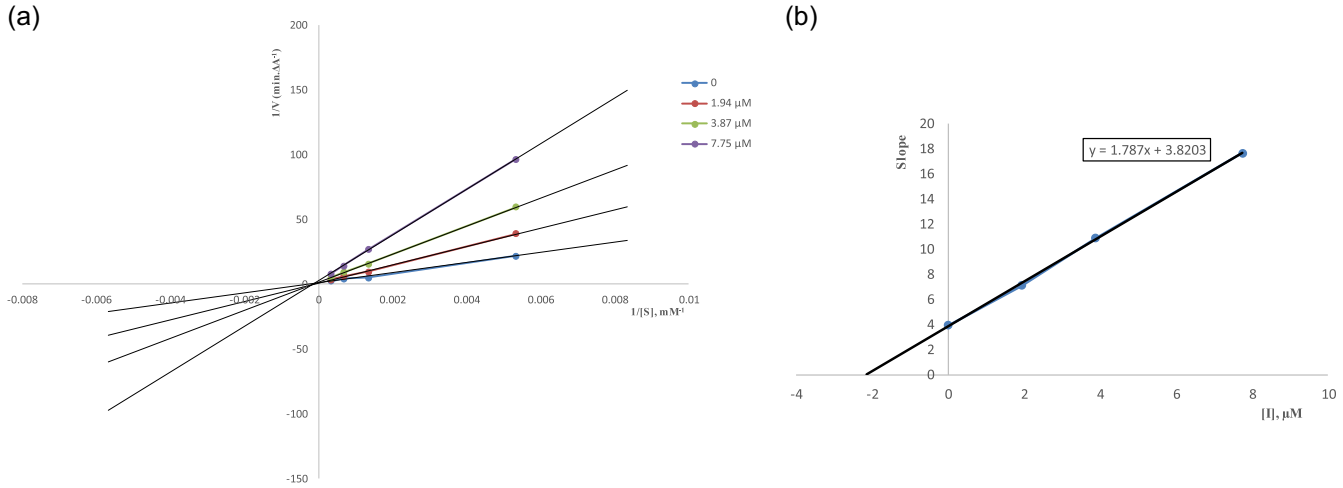


FIGURE 4 (a) Kinetic study of anticholinesterase inhibition by compound **5e**. (b) Inhibition constant (K_i) of compound **5e**

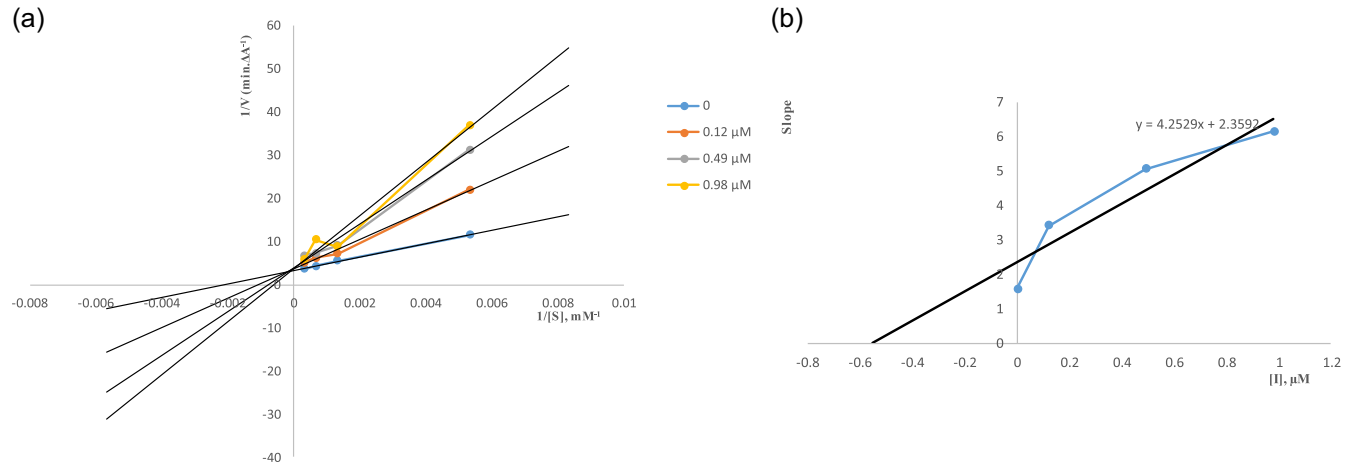


FIGURE 5 (a) Kinetic study of butyrylcholinesterase inhibition by compound **5d**. (b) Inhibition constant (K_i) of compound **5d**

TABLE 2 Inhibitory activities of compound **5d** against $A\beta_{1-42}$ aggregation[a]

Compound	% Inhibition	
	Self-induced $A\beta_{1-42}$ aggregation	AChE-induced Aβ aggregation
5d	29.5 ± 1.2[b]	36.6 ± 1.4[c]
Tacrine	7.9 ± 0.5	7.0 ± 1.4
Donepezil	15.9 ± 1.2 (10 μM)	28.4 ± 3.6 (100 μM)

aValues are expressed as mean ± standard error of the means of three experiments.
bThe inhibition of self-induced $A\beta_{1-42}$ aggregation (25 mM) produced by the tested compound at 10 μM concentration.
cThe co-aggregation inhibition of $A\beta_{1-40}$ and AChE (2 μM; ratio, 100:1) by the tested compound at 100 μM concentration.

against $A\beta_{1-42}$ aggregations and AChE-induced $A\beta_{1-40}$ peptide aggregation using the thioflavin T assay, compared with donepezil and tacrine as the reference compounds. Compound **5d** was more potent than both controls against $A\beta_{1-42}$ self-aggregation, as it depicted 29.5% inhibition at 10 μM (Table 2). Furthermore, compound **5d** inhibited AChE-induced Aβ aggregation by 36.6% at 100 μM.

2.3.6 | Cytotoxic activity

The clinical use of tacrine was restricted due to its poor oral bioavailability and significant side effects mainly related to its hepatotoxicity.[23] In this regard, the design of new nonhepatotoxic analogs is receiving great attention. Compounds **5d** and **5e** were selected to be evaluated in vitro toward HepG2 (Figure 6). It was found that the cytotoxicity of those compounds was comparable with that of tacrine. Compound **5d** demonstrated lower toxicity, as cell viability values were calculated as 100, 100, 100, and 90.2% at concentrations of 2.5, 5, 10, and 20 μg/ml, respectively. Compound **5e** showed cell viability (%) of 100, 100, 97.6, and 65.5% at the same concentrations. It is worth mentioning that tacrine showed higher cytotoxicity against HepG2 than compounds **5d** and **5e**, as cell viability values (%) were obtained as 100, 100, 89.4, and 65.8% at concentrations of 2.5, 5, 10, and 20 μg/ml, respectively.

2.4 | Molecular docking

The best binding modes for both AChE and BChE were ranked according to the minimized affinity values (Table 3).

The proposed binding mode of compound **5e** is illustrated in Figure 7. The interactions were dominated in the region of Phe331, Phe330, and Trp84 amino acid residues due to pronounced existence of the π–alkyl interaction at the anionic site and the π–alkyl interaction with Tyr334 at the edge of the peripheral site region.

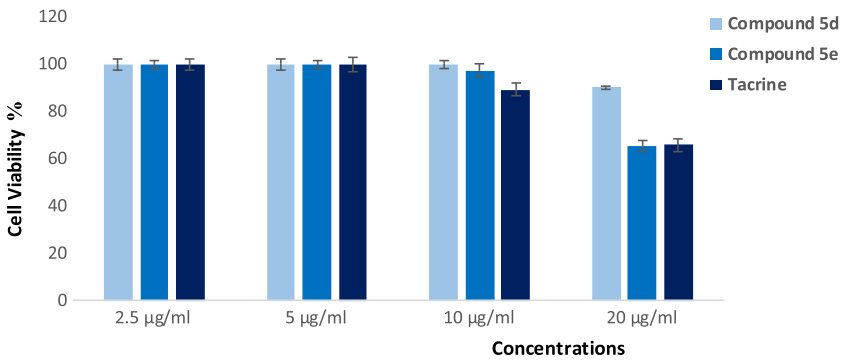


FIGURE 6 Cytotoxicity of compounds **5d** and **5e** against HepG2 cells

TABLE 3 Docking results based on hydrogen bonding, hydrophobic interaction, binding free energy (ΔG_b) of **5d** and **5e**

		Bonds between atoms of compounds and amino acids			
Compound	**ΔG_b[a] (kcal/mol)**	**Atom of the ligand**	**Binding mode**	**Amino acid**	**Distance (Å)**
5d	−9.37 to −7.92	NH_2	H-bond	His438	2.05
		Thienopyridine ring	Four π–π stacking	Trp82	3.81 and 4.92
				Trp82	3.86 and 4.57
			Two π–alkyl interactions	Trp82	4.44 and 4.94
			π–alkyl	Trp82	5.15
		Cyclopentane moiety	Two π–alkyl interactions	Trp430	5.17 and 4.90
			Alkyl (hydrophobic interaction)	Ala328	3.71
5e	−9.78 to −9.11	Cyclohexane moiety	Two π–alkyl interactions	Trp84	4.88 and 4.68
		Cyclopentane moiety	π–alkyl interactions	Phe331	4.74
				Tyr334	4.41
				Phe330	4.66

aBinding free energy.

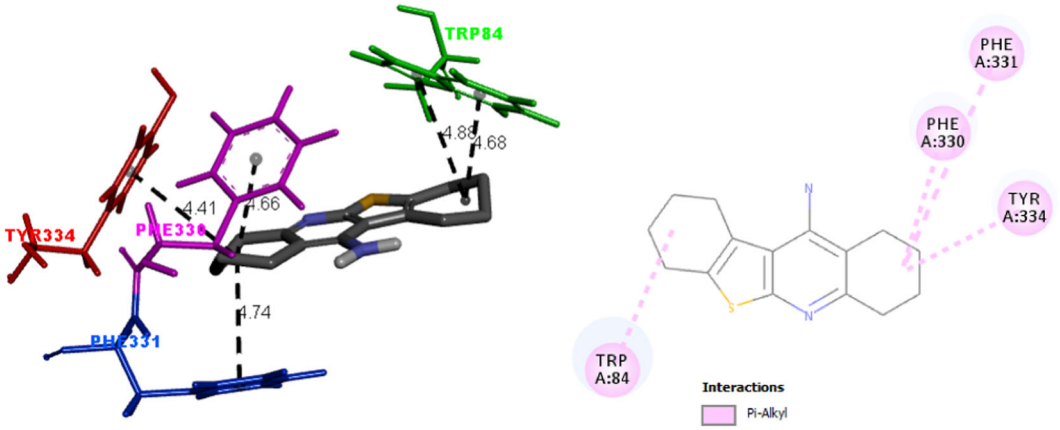


FIGURE 7 Two- and three-dimensional representation of the docked pose of compound **5e** at the 1EVE active site

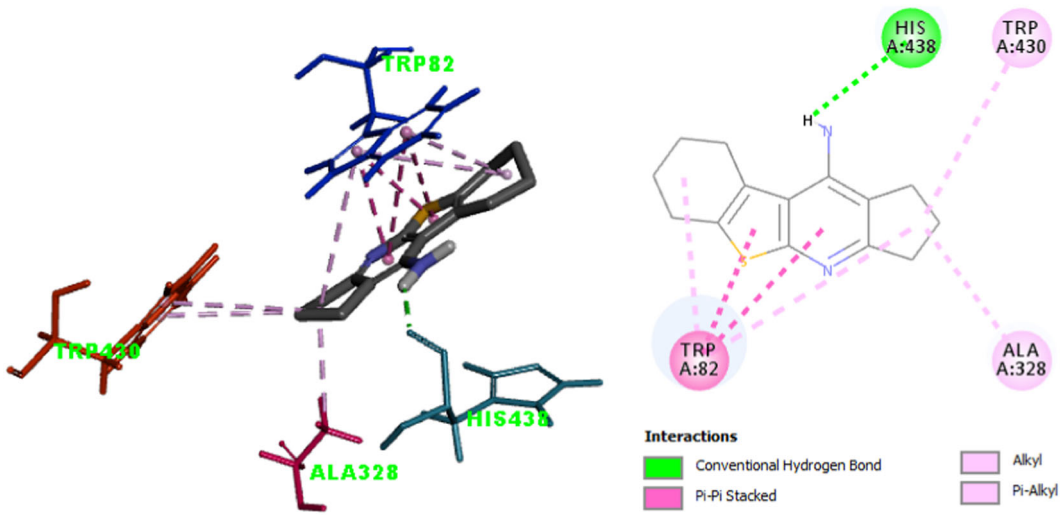


FIGURE 8 Two- and three-dimensional representation of the docked pose of compound **5d** at the 1P0P active site

As observed in Figure 8, compound **5d** was able to form a hydrogen bond with His438 in the catalytic site through the NH_2 group. Benzothienopyridine moiety was trapped in the anionic site through π–π stacking and π–alkyl interactions with Trp82.[24]

These results were in good agreement with those obtained experimentally, indicating the efficacy of compounds **5e** and **5d**. It seems that benzothienopyridine moiety is more significant for desired interactions with BChE amino acid residues, whereas it is not

significant for those of AChE. In this respect, the AChEI activity was more dependent on cycloalkyl substituents than the BChEI activity.

3 | CONCLUSION

In conclusion, various thieno[2,3-*b*]pyridine amines were synthesized as tacrine analogs and evaluated for their anti-ChE activity. They showed a good inhibitory activity; however, the inhibitory activity toward BChE was mostly more significant. It should be mentioned that only compound **5e** showed a better AChEI activity (IC_{50} = 1.55 μM) than the BChEI activity (IC_{50} = 2.33 μM). Also, comprehensive investigations indicated that the most potent AChE and BChE inhibitors (compounds **5e** and **5d**) showed a good activity against AChE-induced and self-induced Aβ aggregation, whereas they exhibited a negligible BACE1 inhibitory activity and neuroprotectivity against Aβ-induced damage in PC12 cells. It is worth mentioning that these compounds showed a lower toxicity than tacrine against the HepG2 cell line.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Melting points were taken on a Kofler hot-stage apparatus and were uncorrected. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded on Bruker FT-500, using tetramethylsilane as an internal standard. The infrared (IR) spectra were obtained on a Nicolet Magna FTIR 550 spectrophotometer (in KBr). Mass spectra were determined on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. The elemental analysis was performed with an Elementar Analysensystem GmbH Vario EL CHNS mode. All reagents and solvents were obtained from Merck and Aldrich and used without purification.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | General procedure for the synthesis of compounds 5a–k

First, a mixture of cycloalkanone (**1**) (1 mmol), malononitrile (**2**) (1.1 mmol), sulfur (S8, **3**) (1.5 mmol), and L-proline (0.1 mmol) in DMF (10 ml) was stirred at 60°C for 24 hr. After completion of the reaction (checked by thin layer chromatography [TLC]), the mixture was poured into water and the precipitated product was filtered off and recrystallized from ethyl acetate to afford pure compound **4**. Then, a mixture of compound **4** (1 mmol) and cycloalkanone (**1**) (1.5 mmol) was added to the suspension of $AlCl_3$ in dry 1,2-dichloroethane (2 mmol in 30 ml) and heated at reflux for 24 hr. After completion of the reaction (checked by TLC), a mixture of H_2O/THF (1:1, 100 ml) was added to the mixture and it was basicified with NaOH (10%). The mixture was stirred at room temperature for 30 min, and the crude product was extracted using CH_2Cl_2 (2 × 50 ml) and washed with brine (2 × 50 ml). The organic phase was dried over Na_2SO_4 and the solvent was evaporated under vacuum. All compounds were recrystallized from EtOH to obtain pure product **5**.

*1,2,3,6,7,8-Hexahydrocyclopenta[e]cyclopenta[4,5]thieno[2,3-b]pyridin-9-amine (**5a**)*

Cream solid, yield: 85%, m.p.: >280°C. IR (KBr): 3,474, 3,293, 2,953, 2,897, 2,843, 1,641, and 1,575 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.29 (s, 2H, NH_2), 3.07 (t, J = 6.5 Hz, 2H, CH_2), 3.03–2.95 (m, 4H, 2 × CH_2), 2.76 (t, J = 7.0 Hz, 2H, CH_2), 2.49 (t, J = 7.0 Hz, 2H, CH_2), and 2.19–2.16 (m, 2H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 163.1, 159.2, 143.6, 137.7, 133.4, 115.5, 110.9, 34.3, 29.7, 29.6, 27.7, 26.7, and 22.9 ppm. MS: *m/z* (%) = 231 $[M+1]^+$ (100), 230 $[M]^+$ (82), 216 (35), 198 (11), 115 (12), 89 (14), 71 (17), and 45 (31). Anal. calcd. for $C_{13}H_{14}N_2S$: C, 67.79; H, 6.13; N, 12.16. Found: C, 67.58; H, 6.31; N, 12.38.

*2,3,6,7,8,9-Hexahydro-1H-cyclopenta[4,5]thieno[2,3-b]quinolin-10-amine (**5b**)*

Cream solid, yield 80%, m.p.: >280°C. IR (KBr): 3,459, 3,326, 2,930, 2,849, 1,619, 1,570, and 1,524 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.35 (s, 2H, NH_2), 3.07 (t, J = 7.0 Hz, 2H, CH_2), 2.96 (t, J = 7.0 Hz, 2H, CH_2), 2.91 (t, J = 6.0 Hz, 2H, CH_2), and 2.5–2.49 (m, 4H, 2 × CH_2), and 1.90–1.86 (m, 4H, 2 × CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 162.5, 154.1, 146.2, 137.3, 134.9, 118.1, 111.0, 33.1, 29.7, 29.6, 27.7, 23.0, 22.9, and 22.8 ppm. MS: *m/z* (%) = 245 $[M+1]^+$ (100), 244 $[M]^+$ (79), 230 (29), 202 (10), 89 (11), and 45 (27). Anal. calcd. for $C_{14}H_{16}N_2S$: C, 68.82; H, 6.60; N, 11.46. Found: C, 68.62; H, 6.42; N, 11.27.

*1,2,3,6,7,8,9,10-Octahydrocyclohepta[e]cyclopenta[4,5]thieno[2,3-b]pyridin-11-amine (**5c**)*

Cream solid, yield 85%, m.p.: >280°C. IR (KBr): 3,481, 3,287, 2,959, 2,841, 1,646, 1,568, and 1,522 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.39 (s, 2H, NH_2), 3.09 (t, J = 6.5 Hz, 2H, CH_2), 3.02 (t, J = 5.0 Hz, 2H, CH_2), 2.97 (t, J = 7.0 Hz, 2H, CH_2), 2.67–2.65 (m, 2H, CH_2), 2.51–2.48 (m, 2H, CH_2), 1.86–1.85 (m, 2H, CH_2), 1.72–1.71 (m, 2H, CH_2), and 1.64 (m, 2H, CH_2) ppm. ^{13}C NMR (dimethyl sulfoxide [DMSO]-d_6, 125 MHz): δ = 159.3, 157.9, 147.8, 136.4, 136.1, 116.4, 116.0, 37.8, 32.0, 29.6, 29.5, 27.8, 27.4, 26.7, and 24.8 ppm. MS: *m/z* (%) = 259 $[M+1]^+$ (100), 258 $[M]^+$ (96), 230 (84), 205 (47), 190 (17), 115 (14), 89 (17), and 45 (38). Anal. calcd. for $C_{15}H_{18}N_2S$: C, 69.73; H, 7.02; N, 10.84. Found: C, 69.57; H, 6.85; N, 10.61.

*1,2,3,6,7,8,9-Heptahydrocyclopenta[e]cyclohexa[4,5]thieno[2,3-b]pyridin-10-amine (**5d**)*

Cream solid, yield 70%, m.p.: 246–248°C. IR (KBr): 3,491, 3,393, 2,925, 2,834, 1,644, 1,615, 1,567, and 1,532 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.43 (s, 2H, NH_2), 3.03–3.00 (m, 4H, 2 × CH_2),

2.80–2.73 (m, 4H, 2 × CH_2), 2.21–2.15 (quint, J = 7.5 Hz, 2H, CH_2), and 1.90–1.88 (m, 4H, 2 × CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 162.7, 160.8, 144.8, 131.7, 125.8, 118.1, 116.1, 34.2, 26.8, 26.6, 25.6, 25.7, 22.8, and 22.6 ppm. MS: *m/z* (%) = 244 $[M]^+$ (25), 216 (14), 66 (15), and 41 (16). Anal. calcd. for $C_{14}H_{16}N_2S$: C, 68.82; H, 6.60; N, 11.46. Found: C, 68.66; H, 6.45; N, 11.28.

*1,2,3,4,7,8,9,10-Octahydrobenzo[4,5]thieno[2,3-b]quinolin-11-amine (**5e**)*

Cream solid, yield 76%, m.p.: 259–261°C. IR (KBr): 3,475, 3,368, 2,930, 2,847, 1,610, 1,566, and 1,531 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.68 (s, 2H, NH_2), 3.00 (m, 2H, CH_2), 2.94–2.92 (m, 2H, CH_2), 2.78 (m, 2H, CH_2), 2.84–2.46 (m, 2H, CH_2), and 1.90–1.87 (m, 8H, 4 × CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 159.5, 153.9, 146.7, 131.9, 125.7, 118.0, 110.9, 33.0, 26.7, 25.6, 23.0, 22.9, 22.8, 22.7, and 22.6 ppm. MS: *m/z* (%) = 259 $[M+1]^+$ (100), 258 $[M]^+$ (65), 231 (82), 216 (23), 202 (15), 179 (11), 71 (14), and 45 (32). Anal. calcd. for $C_{15}H_{18}N_2S$: C, 69.73; H, 7.02; N, 10.84. Found: C, 69.56; H, 6.90; N, 10.71.

*1,2,3,4,7,8,9,10,11-Nonahydrocyclohepta[e]cyclohexa[4,5]thieno-[2,3-b]pyridin-12-amine (**5f**)*

Cream solid, yield 83%, m.p.: 273–275°C. IR (KBr): 3,492, 3,492, 3,301, 2,914, 2,843, 1,644, 1,568, and1,527 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.52 (s, 2H, NH_2), 3.02–3.00 (m, 4H, 2 × CH_2), 2.80–2.78 (m, 2H, CH_2), 2.66–2.64 (m, 2H, CH_2), 1.91–1.84 (m, 4H, 2 × CH_2), 1.72–1.70 (m, 2H, CH_2), and 1.64–1.59 (m, 4H, 2 × CH_2) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 160.3, 157.4, 147.6, 130.2, 127.6, 118.5, 116.5, 38.7, 32.1, 27.7, 26.9, 26.5, 25.7, 24.8, 22.8, and 22.6 ppm. MS: *m/z* (%) = 273 $[M+1]^+$ (100), 272 $[M]^+$ (71), 244 (59), 216 (31), and 41 (23). Anal. calcd. for $C_{16}H_{20}N_2S$: C, 70.55; H, 7.40; N, 10.28. Found: C, 70.39; H, 7.28; N, 10.50.

*1,2,3,6,7,8,9,10-Octahydrocyclopenta[e]cyclohepta[4,5]thieno[2,3-b]pyridin-11-amine (**5g**)*

Cream solid, yield 81%, m.p.: 211–214°C. IR (KBr): 3,493, 3,300, 2,915, 2,843, 1,644, 1,567, and 1,525 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.34 (s, 2H, NH_2), 3.11–3.09 (m, 2H, CH_2), 3.01 (t, J = 7.5 Hz, 2H, CH_2), 2.87–2.85 (m, 2H, CH_2), 2.77–2.75 (m, 2H, CH_2), 2.19–2.16 (m, 2H, CH_2), and 1.89–1.69 (m, 6H, 3 × CH_2) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 161.8, 159.4, 146.4, 133.5, 132.6, 119.0, 116.7, 34.1, 31.0, 29.4, 29.1, 27.7, 27.4, 26.9, and 22.8 ppm. MS: *m/z* (%) = 259 $[M+1]^+$ (100), 258 $[M]^+$ (37), 230 (90), 204 (49), 168 (11), 115 (16), 57 (23), and 41 (40). Anal. calcd. for $C_{15}H_{18}N_2S$: C, 69.73; H, 7.02; N, 10.84. Found: C, 69.57; H, 6.84; N, 10.63.

*2,3,4,7,8,9,10,11-Octahydro-1H-cyclohepta[4,5]thieno[2,3-b]quinolin-12-amine (**5h**)*

Cream solid, yield 73%, m.p.: 230–233°C. IR (KBr): 3,498, 3,301, 2,916, 2,849, 1,624, 1,560, and 1,523 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.45 (s, 2H, NH_2), 3.11–3.09 (m, 2H, CH_2), 2.89 (t, J = 6.0 Hz, 2H, CH_2), 2.85 (t, J = 5.0 Hz, 2H, CH_2), 2.48 (t, J = 6.0 Hz, 2H, CH_2), and 1.90–1.78 (m, 10H, 5 × CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 158.7, 153.8, 146.4, 136.1, 130.6, 119.3, 111.3, 33.1, 30.6, 30.0, 29.0, 27.2, 26.5, 23.1, 22.9, and 22.8 ppm. MS: *m/z* (%) = 273 $[M+1]^+$ (100), 272 $[M]^+$ (49), 244 (94), 218 (47), 202 (17), 115 (13), 77 (14), and 41 (32). Anal. calcd. for $C_{16}H_{20}N_2S$: C, 70.55; H, 7.40; N, 10.28. Found: C, 70.68; H, 7.55; N, 10.41.

*1,2,3,4,5,8,9,10,11,12-Decahydrocyclohepta[e]cyclohepta[4,5]-thieno[2,3-b]pyridin-13-amine (**5i**)*

Cream solid, yield 87%, m.p.: 247–249°C. IR (KBr): 3,481, 3,319, 2,916, 2,846, 1,643, and 1,558 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.45 (s, 2H, NH_2), 3.13 (t, J = 5.0 Hz, 2H, CH_2), 3.01 (t, J = 5.0 Hz, 2H, CH_2), 2.86 (t, J = 5.0 Hz, 2H, CH_2), 2.67 (t, J = 5.0 Hz, 2H, CH_2), 1.90–1.78 (m, 8H, 4 × CH_2), 1.71 (quint, J = 5.5 Hz, 2H, CH_2), and 1.68 (quint, J = 5.5 Hz, 2H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 159.8, 153.7, 147.8, 132.1, 130.0, 119.2, 107.9, 38.9, 32.1, 30.8, 29.9, 29.1, 27.2, 27.1, 26.7, 26.5, and 25.4 ppm. MS: *m/z* (%) = 287 $[M+1]^+$ (100), 286 $[M]^+$ (52), 258 (88), 232 (33), 216 (21), 190 (11), and 41 (29). Anal. calcd. for $C_{17}H_{22}N_2S$: C, 71.29; H, 7.74; N, 9.78. Found: C, 71.45; H, 7.91; N, 9.56.

*2-Benzyl-1,2,3,4,6,7,8,9-octahydropyrido[4′,3′:4,5]thieno[2,3-b]quinolin-5-amine (**5j**)*

Cream solid, yield 78%, m.p.: >280°C. IR (KBr): 3,480, 3,225, 2,920, 2,845, 1,660, 1,643, and 1,558 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.39–7.27 (m, 5H, Ph), 4.56 (s, 2H, NH_2), 3.74–3.69 (m, 4H, 2 × CH_2), 3.10 (s, 2H, CH_2), 2.93–2.88 (m, 4H, 2 × CH_2), 2.48–2.45 (m, 2H, CH_2), and 1.89–1.85 (m, 4H, 2 × CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 158.1, 154.0, 146.9, 137.8, 129.5, 129.1, 128.4, 127.4, 124.2, 117.5, 111.1, 61.9, 52.3, 49.8, 32.8, 29.7, 26.8, 22.9, and 22.7 ppm. MS: *m/z* (%) = 350 $[M+1]^+$ (25), 349 $[M]^+$ (10), 259 (40), 231 (75), 216 (15), 91 (100), 65 (18), and 45 (14). Anal. calcd. for $C_{21}H_{23}N_3S$: C, 72.17; H, 6.63; N, 12.02. Found: C, 72.38; H, 6.45; N, 11.85.

*2-Benzyl-1,2,3,4,6,7,8,9,10-nonahydrocyclohepta[e]pyrido-[4′,3′:4,5]thieno[2,3-b]pyridin-5-amine (**5k**)*

Cream solid, yield 85%, m.p.: >280°C. IR (KBr): 3,482, 3,215, 2,920, 2,845, 1,660,1,645, and 1,555 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.39–7.27 (m, 5H, Ph), 4.59 (s, 2H, NH_2), 3.74–3.70 (m, 4H, 2 × CH_2), 3.11 (s, 2H, CH_2), 3.04–3.02 (m, 2H, CH_2), 2.90–2.88 (m, 2H, CH_2), 2.64–2.63 (m, 2H, CH_2), 1.85 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), and 1.63 (m, 2H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 159.1, 154.3, 146.2, 137.8, 130.1, 129.1, 128.4, 127.4, 124.5, 118.4, 116.7, 61.8, 52.3, 49.8, 38.5, 32.1, 27.1, 26.9, 26.5, and 25.2 ppm. MS: *m/z* (%) = 364 $[M+1]^+$ (42), 363 $[M]^+$ (14), 273 (53), 245 (88), 216 (23), 91 (100), 65 (23), and 45 (17). Anal. calcd. for $C_{22}H_{25}N_3S$: C, 72.69; H, 6.93; N, 11.56. Found: C, 72.44; H, 6.70; N, 11.71.

4.2 | Pharmacological/biological assays

4.2.1 | Anticholinesterase assay

The in vitro anticholinesterase activity of the synthesized compound **5** was assayed using modified Ellman's method, exactly according to our previous study.[21]

4.2.2 | Kinetic study

The kinetic study for the inhibition of AChE and BChE by compounds **5e** and **5d** was carried out according to Ellman's method reported in our previous work,[14] using four different concentrations of inhibitors. For the kinetic study of AChE, compound **5e** was used at the concentrations of 0, 1.94, 3.87, and 7.75 μM. The Lineweaver–Burk reciprocal plot was constructed by plotting 1/*V* against 1/[S] at variable concentrations of the substrate acetylthiocholine (187.5, 750, 1,500, and 3,000 μM). The inhibition constant K_i was achieved by the plot of slopes versus the corresponding concentrations of the compound **5e**. The same method was performed for the kinetic study of BChE using four different concentrations of compound **5d** (0, 0.12, 0.49, and 0.98 μM) and butyrylthiocholine at concentrations of 187.5, 750, 1,500, 3,000 μM.

4.2.3 | BACE1 inhibition

A FRET-based BACE1 enzyme assay kit was used to evaluate the inhibitory activity of the selected compounds toward BACE1. The kit was purchased from Invitrogen (formerly Pan Vera Corporation, Madison, WI) and the evaluation procedure was conducted according to the manufacturer's instructions.[25]

4.2.4 | Neuroprotectivity against damage induced by $A\beta_{25-35}$

The neuroprotective effect of the selected compounds in protecting neuronal PC12 cells against damage induced by $A\beta_{25-35}$ was examined according to our previous report.[25]

4.2.5 | Inhibition of AChE-induced and self-induced Aβ aggregation

The related evaluations were exactly performed according to the previous procedure reported in the literature.[26]

4.2.6 | Cytotoxicity

To investigate the effect of selected compounds **5d** and **5e** on cell viability, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was performed using HepG2 cell line.[26]

4.3 | Molecular docking

To understand the SARs of the most potent AChE and BChE inhibitors (**5e** and **5d**, respectively), Smina in Linux platform was implemented in AutoDock docking program to obtain the predicted binding poses. Crystal structures of human acetylcholinesterase in complex with the anti-Alzheimer drug (1EVE) and butyrylcholinesterase in complex with the substrate analog butyrylthiocholine (1P0P) were fetched and prepared for docking. The accuracy of the docking software was measured using the root mean squared deviations between re-docked proteins and cocrystal ligands.[27]

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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