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Ionic liquid mediated synthesis of mono- and bis-spirooxindole-hexahydropyrrolidines as cholinesterase inhibitors and their molecular docking studies



Yalda kia^a, Hasnah Osman^{a,*}, Raju Suresh Kumar^{b,*}, Alireza Basiri^c, Vikneswaran Murugaiyah^c

^a School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

^b Department of Chemistry, College of Science, King Saud University, PO Box 2455, Riyadh, Saudi Arabia

^c School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

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ABSTRACT

One pot, three-component reaction of 1-acryloyl-3,5-bisarylmethylidenepiperidin-4-ones with isatin and sarcosine in molar ratios of 1:1:1 and 1:2:2 furnished to mono- and bis-spiropyrrolidine heterocyclic hybrids comprising functionalized piperidine, pyrrolidine and oxindole structural motifs. Both mono and bis-spiropyrrolidines displayed good inhibitory activity against acetylcholinesterase (AChE) with IC₅₀ values of 2.36–9.43 μ M. For butyrylcholinesterase (BChE), mono-cycloadducts in series **8** with IC₅₀ values of lower than 10 μ M displayed better inhibitory activities than their bis-cycloadduct analogs in series **9** with IC₅₀ values of 7.44–19.12 μ M. The cycloadducts **9** and **8e** were found to be the most potent AChE and BChE inhibitors with IC₅₀ values of 2.35 and 3.21 μ M, respectively. Compound **9** was found to be competitive inhibitor of AChE while compound **8e** was a mixed-mode inhibitor of BChE with calculated *K*_i values of 2.01 and 6.76 μ M, respectively. Molecular docking on *Torpedo californica* AChE and human BChE showed good correlation between IC₅₀ values and free binding energy values of the synthesized compounds docked into the active site of the enzymes.

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1. Introduction

Alzheimer's disease (AD), the most common cause of dementia among the elderly individuals is an irreversible neurodegenerative disorder characterized by diverse cognitive impairments, neuropsychiatric symptoms and inability to perform routine activities.¹ AD is associated with the degeneration of cholinergic neurons in the basal forebrain, which leads to substantial decrease in generation of acetylcholine (ACh) neurotransmitter. ACh deficiency severely affects the cognitive abilities, memory function and emotional responses in AD patients. Pathogenesis of AD is largely characterized by the presence of extracellular amyloid plaques (Aβ plaques) and intracellular neurofibrillary tangles (NFT) composed of phosphorylated tau proteins.²⁻⁴ Based on so-called cholinergic hypothesis, one of the most promising therapeutic approach to enhance cholinergic function is by the use of cholinesterase inhibitors.^{5,6} In spite of tremendous efforts to develop novel disease modifying agents working via β-amyloid or tau pathways, none is clinically available due to their side effects. Thus, the search for new cholinesterase inhibitor is still ongoing worldwide.

Two major cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), involved in hydrolysis and regulation of ACh in vertebrates.⁷ These two cholinesterases share similar structure topology of possessing a 20 Å long, narrow active site channel located in the center of the enzyme, ending to a catalytic triad where the hydrolysis of acetylcholine takes place.⁸ This channel is composed of five major binding regions, namely (i) peripheral anionic site,⁹ (ii) oxyanion hole,¹⁰ (iii) choline binding pocket,¹⁰ (iv) acyl binding site¹¹ and (v) catalytic triad. In AChE, amino acid residues having aromatic side chains such as tryptophan (Trp) and tyrosine (Tyr) facilitate the insertion and transition of substrate or inhibitor inside the active site cavity. In BChE these aromatic residues are mostly replaced with hydrophobic ones such as valine (Val) and leucine (Leu) resulting in more spacious channel to accommodate bulkier substrates.¹²

lonic liquid mediated, multi-component reactions owing to their unrivaled features such as high solvating abilities, interesting catalytic behavior and recyclability have gained great importance among the chemists for the synthesis of biologically active heterocycles.^{13–15} Under this context and as a part of our ongoing



^{*} Corresponding authors. Tel.: +60 4 6533558; fax: +60 4 6574854.

E-mail addresses: osman.hasnah2012@yahoo.com (H. Osman), sraju@ksu.edu.sa (R.S. Kumar).

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research in the construction and biological evaluation of novel heterocyclic hybrids,^{16–19} we report ionic liquid mediated synthesis of a library of novel mono- and bis-spiropyrrolidines and their *in vitro* cholinesterases inhibitory activities. Mode of inhibition, enzyme kinetics and molecular docking studies were also conducted for the most active derivatives to investigate their nature of inhibition and binding interaction template to the active site of the enzymes.

2. Results and discussion

2.1. Chemistry

The highly functionalized dipolarophiles *viz.* 1-acryloyl-3,5-diarylmethylidenepiperidin-4-ones (**5**) required for the synthesis of spiro-heterocycles were prepared by Claisen–Schmidt condensation of 4-piperidone hydrochloride (**1**) with a series of aromatic aldehydes (**2**) to furnish *N*-unsubstituted 3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones (**3**), which were subsequently acylated with acryloyl chloride, following the previously reported procedure.²⁰ The dipolarophiles (**5**) are versatile synthons for the construction of more complex spiro-heterocycles owing to presence of diverse dipolarophile functions such as three C=C and two C=O groups.

As depicted in Scheme 1, three-component [3+2]-cycloaddition reaction of dipolarophiles (**5**) with isatin (**6**) and sarcosine (**7**) in [bmim]Br as ionic solvent afforded a library of spiro-cycloadducts in good yields (Table 1). Initially, the reaction of an equimolar mixture of 1-acryloyl-3,5-diphenylidenepiperidin-4-ones (**5**), isatin (**6**) and sarcosine (**7**) was investigated under refluxing methanol for 5 h, furnishing to mono-spiropyrrolidine (**8a**) as the sole product in 62% yield. The reaction of **5**, **6** and **7** in a molar ratio 1:2:2 for a longer period of time (15 h) afforded more complex bis-spiropyrrolidine (**9a**) in moderate yield of 48%.

The above reactions were also performed in [bmim]Br, as a green and eco-friendly reaction medium. By refluxing an equimolar mixture of **5**, **6**, **7** and 1 molar equiv of [bmim]Br, the monospiropyrrolidine (**8a**) was obtained in 30 min and 83% yield. The bis-spiropyrrolidine (**9a**) was also afforded in a shorter reaction time of 2 h and 71% yield by refluxing 1:2:2 mixture of the reactants and 2 molar equiv of ionic solvent. In both reactions, the spiropyrrolidines [(**8a**) and (**9a**)] were purified through flash column chromatography. Further attempt to obtain the more complex tri-spiropyrrolidine cycloadduct were unsuccessful, plausibly due to the steric hindrance exerted by the cycloadduct **9** for subsequent cycloaddition. Performing aforementioned reactions in ionic solvent had noticeable advantages over methanol in terms of reaction time and yield. The high product yields were probably due to the catalytic abilities of [bmim]Br that also improved the reaction rates.

Structures of the mono and bis-spiropyrrolidines 8 and 9 are in agreement with the combustion data, IR, 1D and 2D NMR spectroscopic data. The elemental analysis results are within ±0.4% of the theoretical values. In the ¹H NMR spectrum of **8a**, a doublet of doublets at 5.00 ppm with J = 10.5 and 8.7 Hz is due to H-4 of the pyrrolidine ring. The HMOC correlation of H-4 assigns the carbon signal at 44.0 ppm to C-4. Further, H-4 shows HMBC correlation with the C=O of the piperidone ring at 196.5 ppm and the spiro carbon C-3" at 63.2 ppm, besides showing correlation with the adjacent carbon C-5 at 57.1 ppm. The HMQC correlation of C-5 assigns the triplets at 3.47 ppm (I = 8.7 Hz) and 4.10 ppm(I = 10.5 Hz) to 5-CH₂. The two doublets at 2.54 and 4.33 ppm with I = 14.3 Hz are assigned to 2"-CH₂ while the doublet of doublets at 3.31 ppm with I = 18.8, 2.0 Hz and a doublet at 4.27 ppm with I = 18.8 Hz are due to 6"-CH₂ of the piperidone ring. From HMQC correlation, the carbon signals at 46.1 and 45.5 ppm are assigned to C-2" and C-6", respectively (Fig. 1).

Further, the piperidone ring protons, 2"-CH₂ and 6"-CH₂ show HMBC correlation with the carbonyl carbon of acryloyl moiety at 176.7 ppm. The doublet of doublets at 6.62 ppm is due to β -hydrogen of the acryloyl moiety whereas the doublets at 5.68 and 6.25 ppm are due to two α -hydrogens. The singlets at 7.82 and 8.36 ppm are due to the arylmethylidene hydrogen and NH hydrogen of the oxindole, respectively. The aromatic hydrogens appear as doublets and multiplets in region between 6.87 and 7.56 ppm. The ¹H and ¹³C NMR chemical shifts of the bis-spiropyrrolidines (9) were also assigned by similar considerations. As the cycloadduct **9** possess two oxindolo-pyrrolidine rings, to distinguish the ¹H and ¹³C chemical shifts of these two rings, the ring attached to C-3" of 4-piperidone moiety is considered as ring A and the other ring attached to 1"-N—C=O is considered as ring B. Further. the structure and stereochemistry of mono and bis-spiropyrrolidines were confirmed by the single crystal X-ray crystallographic analysis of 8a and 9a (Figs. 2 and 3).

The plausible mechanism for the formation of spiropyrrolidines **8** and **9** in ionic solvent is depicted in Scheme 2. The electrondeficient hydrogen atom of [bmim]Br, forms hydrogen bonding interaction with the carbonyl function of isatin, whereby facilitates



Scheme 1. Synthesis of mono- and bis-spiropyrrolidines 8(a-k) and 9(a-k).

Tabl	e 1				
AChE	E and BChE inhibitory	activities of	compounds	8 (a-k) and	9(a-k)

Entry	Compound	Ar	Yield (%)	AChE inhibition IC ₅₀		BChE inhibition IC ₅₀		Selec	Selectivity	
				µg/mL	μΜ	μg/mL	μΜ	AChE ^a	BChE ^b	
1	8a	C ₆ H ₅	83	4.39 ± 0.14	8.74	3.78 ± 0.21	7.52	0.86	1.16	
2	8b	2-CH ₃ C ₆ H ₄	71	1.28 ± 0.11	3.97	2.05 ± 0.14	3.86	0.97	1.03	
3	8c	2-(CH ₃ O)C ₆ H ₄	75	1.41 ± 0.15	4.10	2.37 ± 0.19	4.22	1.03	0.97	
4	8d	2-ClC ₆ H ₄	77	2.93 ± 0.12	5.12	2.41 ± 0.15	4.20	0.82	1.22	
5	8e	2-FC ₆ H ₄	71	3.71 ± 0.14	6.88	1.72 ± 0.12	3.21	0.47	2.14	
6	8f	3-(NO2)C6H4	82	3.16 ± 0.09	5.34	4.58 ± 0.21	7.74	1.45	0.69	
7	8g	2,4-Cl ₂ C ₆ H ₃	80	3.92 ± 0.15	6.14	3.40 ± 0.19	5.32	0.87	1.15	
8	8h	$4-CH_3C_6H_4$	77	3.98 ± 0.14	7.49	4.92 ± 0.22	9.28	1.24	0.81	
9	8i	4-ClC ₆ H ₄	81	4.79 ± 0.19	8.40	3.87 ± 0.24	6.77	0.81	1.24	
10	8j	$4-FC_6H_4$	77	4.23 ± 0.11	7.80	1.82 ± 0.14	3.39	0.43	2.30	
11	8k	1-Naphthyl	85	4.58 ± 0.21	7.59	5.32 ± 0.25	8.85	1.17	0.86	
12	9a	C ₆ H ₅	71	1.78 ± 0.21	2.63	6.31 ± 0.21	9.34	3.55	0.28	
13	9b	2-CH ₃ C ₆ H ₄	60	6.65 ± 0.19	9.43	8.80 ± 0.24	12.51	1.33	0.75	
14	9c	2-(CH ₃ O)C ₆ H ₄	64	3.49 ± 0.22	4.73	7.69 ± 0.18	10.42	2.20	0.45	
15	9d	2-ClC ₆ H ₄	52	3.88 ± 0.17	5.19	6.97 ± 0.17	9.38	1.81	0.55	
16	9e	2-FC ₆ H ₄	54	4.41 ± 0.25	6.18	5.29 ± 0.22	7.44	1.20	0.83	
17	9f	3-(NO ₂)C ₆ H ₄	57	1.81 ± 0.18	2.47	9.45 ± 0.26	12.34	5.00	0.20	
18	9g	2,4-Cl ₂ C ₆ H ₃	69	3.60 ± 0.15	4.42	14.53 ± 0.17	17.90	4.05	0.25	
19	9h	$4-CH_3C_6H_4$	65	5.41 ± 0.25	7.67	7.94 ± 0.27	11.29	1.47	0.68	
20	9i	4-ClC ₆ H ₄	61	4.59 ± 0.28	6.14	6.94 ± 0.18	9.44	1.54	0.65	
21	9j	$4-FC_6H_4$	64	1.76 ± 0.14	2.35	5.56 ± 0.21	7.82	3.33	0.30	
22	9k	1-Naphthyl	72	3.84 ± 0.28	4.93	14.83 ± 0.29	19.12	3.88	0.26	
23	Reference standard	Galanthamine	-	0.60 ± 0.09	2.09	5.55 ± 0.15	19.34	3.47	0.28	

^a Selectivity for AChE is defined as IC₅₀(BChE)/IC₅₀(AChE).

^b Selectivity for BChE is defined as IC₅₀(AChE)/IC₅₀(BChE).



Figure 1. Selected HMBC correlations and ¹H and ¹³C chemical shifts of 8a.

the formation of reactive azomethine ylide *via* the decarboxylative condensation of isatin and sarcosine. The newly formed ylide, attacks chemo-selectively to a C=C bond of piperidone ring of dipolarophile furnishing the mono-spiropyrrolidine (**8**). To afford bis-spiropyrrolidines (**9**), an additional azomethine ylide adds to the C=C bond of acryloyl moiety of **8**. These reactions proceeded regio-selectively due to preferential addition of azomethine ylide nucleophilic carbon to the β -carbon of enone moiety in piperidone ring, and stereo-selectively owing to formation of only one stereoisomer in both mono- and bis-cycloadducts.

2.2. Cholinesterase inhibitory activity

All the newly synthesized spiropyrrolidine derivatives were evaluated *in vitro* for their inhibitory activities against AChE and BChE. Both mono and bis-cycloadducts displayed good inhibitory activity against AChE enzyme with IC₅₀ values of lower than

10 μ M (Table 1). In series **8**, *ortho*-substituted derivatives *viz*. **8b** with *o*-CH₃, **8c** with *o*-methoxy, **8d** with *o*-Cl and **8e** bearing *o*-F moiety on phenyl rings, with IC₅₀ values in the range of 3.97–6.88 μ M, displayed better AChE inhibitory activity than *para*-substituted derivatives (**8h**–**j**) with IC₅₀ values in the range of 7.49–8.40 μ M. Remaining compounds in this series also showed good AChE inhibitory activity with IC₅₀ values ranging from 7.49 to 8.74 μ M. Bis-spiropyrrolidine cycloadducts in series **9** also displayed good AChE inhibitory activities. The un-substituted **9a**, dichloro-substituted **9g** and fluoro-substituted **9j** showed potent inhibitory activity with IC₅₀ values of lower than 3 μ M, comparable to the reference standard, galanthamine with IC₅₀ value of 2.09 μ M. Among the synthesized derivatives, compound **9j** displayed the most potent AChE inhibitory activity with IC₅₀ value of 2.36 μ M.

Unlike AChE, for BChE, compounds in series **8** displayed better inhibitory activities than bis-cycloadducts in series **9**. The



Figure 2. ORTEP diagram of 8a.



Figure 3. ORTEP diagram of 9a.

mono-spiropyrrolidines bearing fluoro atom on either *ortho* or *para* position of phenyl ring *viz.* **8e** and **8j**, showed the highest inhibitions with IC_{50} values of 3.21 and 3.39 μ M. Similar to AChE, *ortho*-substituted derivatives in series **8** displayed higher inhibitory activities compared to *para*-substituted analogs. On the other

In addition, except for compound **8e** and **8j** that showed more selectivity for BChE, the rest of the compounds in series **8**, displayed equal selectivity toward both AChE and BChE with selectivity indexes ranging from 0.81 to 1.24. Recent studies have showed that as the AD progresses, the activity of AChE decreases while the activity of BChE remains unaffected or even increases. Moreover, in the advance staged AD patients, BChE can compensate for the AChE to diminish the already depleted level of acetylcholine.^{21,22} Thus, novel cholinesterase inhibitors with dual AChE and BChE inhibitory activity or more selectivity toward BChE are valuable therapeutic agents. Unlike series **8**, the bis-spiropyrrolidines in series **9** were more selective toward AChE with selectivity indexes ranging from 1.20 to 5.00 whereby compound **8f** displayed highest selectivity for AChE.

2.3. Enzyme inhibition kinetics

The types of inhibition and enzyme kinetics were investigated for the most active AChE and BChE inhibitors, namely **9j** and **8e**. The inhibition data and types of inhibitions of these compounds are summarized as kinetic data (Table 2) and Lineweaver–Burk plots (Figs. 4 and 5), respectively. Compound **9j** was found to be a competitive inhibitor of AChE while compound **8e** was a mixed-mode inhibitor of BChE. The calculated K_i values were 2.01 and 6.76 μ M for **9j** and **8e**, respectively.

2.4. Molecular docking

To further validation of *in vitro* cholinesterase inhibitory results and get better insight into the binding interactions at molecular level, all the newly synthesized compounds were docked into the active site of AChE and BChE derived from crystal structure of *Torpedo californica* AChE and human BChE enzymes. The free energies of binding interactions for the synthesized compounds showed good correlations to their *in vitro* median inhibitory concentrations, with regression values of 0.667 and 0.712 for AChE and BChE enzymes, respectively (Figs. 6 and 7).

Both mono- and bis-cycloadducts in series **8** and **9** displayed good activity against AChE. Active site gorge of AChE is composed of residues possessing aromatic side chains such as phenylalanine (Phe) and tyrosine (Tyr), which due to its high aromatic content establishes extensive hydrophobic interactions with the inhibitors and substrates. Although the overall structure of BChE is similar to that of AChE, but the active site of BChE has many of the aromatic residues replaced by residues with aliphatic side chains, such as leucine (Leu) and valine (Val). Due to this, the bis-cycloadducts in series **9**, comprising more aromatic cores, displayed better AChE inhibitory activities than mono-cycloadducts in series **8**, plausibly owing to more π - π and σ - π interactions with aromatic channellining residues. However, for BChE, both series displayed moderate inhibition probably due to less favorable interactions with aliphatic residues lining the gorge wall in the BChE enzyme.

The molecular docking analysis of the most potent compounds, namely **9j** against AChE and **8e** against BChE are depicted in Figures 6 and 7 and described in detail. Docking of compound **9j** into the active site of AChE disclosed that it preferentially binds to peripheral anionic site (PAS) of the enzyme through hydrogen bonding interactions with Tyr334 as well as hydrophobic interactions with Trp279, Tyr70 and Tyr121. The effective binding to PAS of the AChE efficiently blocked the entry of the channel and



Scheme 2. Mechanism for the formation of mono- and bis-cycloadducts 8 and 9.

Table 2Kinetic parameters for cholinesterase inhibition

Compound	Enzyme	μΜ	$K_{\rm m}({ m mM})$	$V_{\rm max}$ ($\Delta OD412/min$)	Ki
9j	AChE	0	0.331	0.021	2.01
		0.70	0.462	0.021	
		1.40	0.560	0.021	
8e	BChE	0	0.150	0.011	6.76
		0.90	0.221	0.012	
		1.81	0.497	0.015	

prohibits insertion and accommodation of substrate into the active site of the enzyme (Fig. 8). This result completely coincided with the kinetic findings, which indicates **9j** is a competitive inhibitor of AChE.

Docking analysis for compound **8e** on BChE revealed that this inhibitor is effectively anchored to the active site of BChE enzyme through hydrophobic and π , π -stacking interactions with Trp82 and

Phe329 at choline binding site as well as hydrophobic interaction with Leu286 and Val288 at the peripheral anionic site of the enzyme. Compound **8e** also displayed mild polar interaction with Gly116 and Gly117 composing oxyanion hole, in addition to Ser198 and His438 at the catalytic triad of the enzyme (Fig. 9). Based on the above findings, compound **8e** completely filled in the BChE channel and prevents substrate insertion and hydrolysis at the active site of enzyme. The interaction data of the enzymes with amino acid residues involved in ligand–receptor complex and their bonding types are presented Table 3 for the most active inhibitors **9j** and **8e**.

3. Conclusion

Two novel series of piperidone-grafted, mono- and bis-spiropyrrolidine derivatives were prepared *via* one pot, three-component reaction of azomethine ylide generated from isatin and sarcosine with highly functionalized dipolarophiles in good yield and short reaction time using [bmim]Br as ionic solvent. Both mono and biscycloadducts in series **8** and **9** displayed good activity against AChE



Figure 4. Lineweaver-Burk plot of cholinesterase inhibition by compounds 9j on AChE.



Figure 5. Lineweaver–Burk plot of cholinesterase inhibition by compounds 8e on BChE.



Figure 6. Correlation diagram of plC_{50} versus free binding energy of compounds 8(a-k) and 9(a-k) for AChE inhibitory activity.



Figure 7. Correlation diagram of pIC_{50} versus free binding energy of compounds **8**(**a**-**k**) and **9**(**a**-**k**) for BChE inhibitory activity.

with IC_{50} values of lower than 10 μ M. However mono-spiropyrrolidines better BChE inhibitory activity than the bis-spiropyrrolidines. The cycloadducts **9** and **8e** were found to be the most potent AChE



Figure 8. Binding orientations and interactions of compound 9j at the TcAChE active site.

and BChE inhibitors with IC₅₀ values of 2.35 and 3.21 μ M, respectively. The docking analysis of the newly synthesized compounds showed good correlations between IC₅₀ values and free binding energies of the docked compounds.

4. Experimental

4.1. General methods

The melting points were measured in open capillary tubes and are uncorrected. FT-IR spectra were recorded on a Perkin Elmer 2000 instrument. The ¹H, ¹³C and the 2D NMR spectra were recorded on a Bruker (Avance) 500 MHz NMR instrument using



Figure 9. Binding orientations and interactions of compound 8e at the *h*BChE active site.

TMS as internal standard, DMSO- d_6 and CDCl₃ as solvents. Chemical shifts are given in parts per million (δ -scale) and the coupling constants are given in Hertz. Silica gel-G plates (Merck) were used for TLC analysis with a mixture of petroleum ether (60–80 °C) and ethyl acetate as eluent. Elemental analyses were performed using a Perkin Elmer 2400 Series II Elemental CHN analyzer.

4.1.1. General procedure for synthesis of 8(a-k) and 9(a-k)

1 molar equiv of [bmim]Br were added to equimolar mixture of 1-acryloyl-3,5-diarylidenepiperidin-4-ones (**5**, 0.364 mmol), isatin (**6**, 0.364 mmol) and sarcosine (**7**, 0.364 mmol) and refluxed for 30 min. After completion of the reaction, as evident by TLC, the mono-spiropyrrolidines (**8**) were afforded in pure form through column chromatography. The same reaction was performed employing 1:2:2 ratio of (**5**, 0.364 mmol), (**6**, 0.728 mmol) and (**7**, 0.728 mmol) in 2 molar equiv of refluxing [bmim]Br, which furnished to bis-spiropyrrolidines (**9**) after 2 h that subsequently purified through column chromatography. The purity of both (**8**) and (**9**) examined using TLC and ¹H NMR techniques.

4.1.1.1. 1-Methyl-4-(phenyl)pyrrolo-(spiro[2.3']oxindole)spiro[3.3"]-5"-(phenylmethylidene)-1"-*N*-acrolylpiperidin-4"-

one (8a). White solid; (0.137 g, 83%); mp 192–195 °C; IR (KBr) v_{max} : 3421, 1719, 1617 cm⁻¹; Anal. Calcd for $C_{32}H_{28}N_3O_3$:

C, 76.47; H, 5.62; N, 8.36. Found: C, 75.25; H, 5.12; N, 7.96. ¹H NMR (500 MHz, CDCl₃): δ 2.19 (s, 3H, CH₃), 2.54 (d, *J* = 14.35, H-2"), 3.31 (dd, *J* = 18.81, 2.09, 1H, H-6"), 3.47 (t, *J* = 8.79, 1H, H-5), 4.10 (t, *J* = 10.59, 1H, H-5), 4.27 (d, *J* = 18.81, 1H, H-6"), 4.33 (d, *J* = 14.35, 1H, H-2"), 5.00 (dd, *J* = 10.59, 8.79 1H, H-4), 5.68 (d, *J* = 11.89 Hz, 1H, H-α), 6.25 (d, *J* = 16.88 Hz, 1H, H-α), 6.62 (dd, *J* = 16.88, 11.89 Hz, 1H, H-β), 6.87–7.56 (m, 14H, H-aromatic), 7.82 (s, 1H, H-arylmethylidene), 8.36 (br. s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 34.53, 44.04, 45.52, 46.18, 57.19, 63.27, 74.91, 110.43, 122.55, 125.13, 126.74, 127.20, 127.45, 128.62, 128.88, 128.92, 129.60, 129.69, 129.78, 129.84, 130.40, 134.07, 137.47, 140.49, 142.80, 167.23, 176.75, 196.56.

4.1.1.2. 1-Methyl-4-(2-methylphenyl)pyrrolo-(spiro[2.3']oxindole)-spiro[3.3"]-5"-(2-methylphenylmethylidene)-1"-*N*-acro-

lylpiperidin-4"-one (8b). White solid: (0.121 g. 71%); mp 150–153 °C; IR (KBr) v_{max}: 3423, 1720, 1615 cm⁻¹; Anal. Calcd for C₃₄H₃₂N₃O₃: C, 76.96; H, 6.08; N, 7.92. Found: C, 75.34; H, 6.17; N, 6.99. ¹H NMR (500 MHz, CDCl₃): δ 2.11 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 2.46 (d, *J* = 14.30, H-2"), 3.22 (dd, *J* = 18.28, 2.39 Hz, 1H, H-6"), 3.37 (t, *J* = 8.49 Hz, 1H, H-5), 3.98 (t, I = 9.51 Hz, 1H, H-5, 4.18 (d, I = 18.28, 1H, H-6''), 4.24 (d, *J* = 14.30, 1H, H-2"), 4.88 (dd, *J* = 9.51, 8.49 Hz, 1H, H-4), 5.63 (d, I = 10.54 Hz, 1H, H- α), 6.22 (d, I = 16.72 Hz, 1H, H- α), 6.51–6.57 $(dd, J = 16.71, 10.54 \text{ Hz}, 1\text{H}, \text{H}-\beta), 6.73-7.35 (m, 12\text{H}, \text{H}-aromatic)$ 7.70 (s, 1H, H-arylmethylidene), 9.01 (br. s, 1H, NH) ¹³C NMR (125 MHz, CDCl₃): δ 20.02, 20.37, 33.49, 43.15, 44.15, 45.17, 56.27, 62.24, 73.91, 109.16, 121.54, 124.27, 125.73, 126.29, 127.77, 128.06, 128.27, 128.35, 128.55, 128.68, 129.59, 130.35, 133.35, 136.01, 139.06, 139.42, 141.47, 165.98, 175.64, 195.56.

4.1.1.3. 1-Methyl-4-(2-methoxyphenyl)pyrrolo-(spiro[2.3']oxindole)-spiro[3.3"]-5"-(2-methoxyphenylmethylidene)-1"-N-acrolylpiperidin-4"-one (8c). White solid; (0.134 g, 75%); mp 125–127 °C; IR (KBr) v_{max} : 3419, 1711, 1601 cm⁻¹; Anal. Calcd for C₃₄H₃₂N₃O₅: 72.58; H, 5.73; N, 7.47. Found: 72.07; H, 5.23; N, 7.07. ¹H NMR (500 MHz, CDCl₃): δ 2.21 (s, 3H, CH₃), 2.45 (d, *I* = 14.05, H-2"), 3.20 (dd, *J* = 17.95, 2.37 Hz, 1H, H-6"), 3.39 (t, I = 9.22 Hz, 1H, H-5), 3.70 (s, 3H, O-CH₃), 3.72 (s, 3H, O-CH₃), 3.95 (t, J = 8.27 Hz, 1H, H-5), 4.17 (d, J = 17.95, 1H, H-6"), 4.22 (d, *J* = 14.05, 1H, H-2"), 4.85 (dd, *J* = 9.22, 8.27 Hz, 1H, H-4), 5.62 (d, I = 10.51 Hz, 1H, H- α), 6.22 (d, I = 16.12 Hz, 1H, H- α), 6.57 (dd, I = 16.12, 10.51 Hz, 1H, H- β), 6.72–7.43 (m, 12H, H-aromatic) 7.69 (s, 1H, H-arylmethylidene), 9.20 (br. s, 1H, NH) ¹³C NMR $(125 \text{ MHz}, \text{ CDCl}_3)$: δ 33.46, 43.20, 43.67, 45.16, 54.17, 54.27, 56.43, 62.00, 73.98, 109.13, 112.87, 113.12, 121.44, 124.18, 125.77, 126.16, 126.65, 127.74, 128.41, 128.46, 129.78, 131.61, 139.07, 141.40, 157.74, 159.68, 166.02, 175.71, 195.52.

Table	3
Tuble	•

Rinding int	araction data	for compounds	Oi and	Re docked into	active site c	of TcAChE and	hRChE
Dinung int	craction uata	i ioi compounds	Janu	be ubered mito	active site e	n nenenie anu	noch

Entry	Ligand	Enzyme	Interacting site	Amino acid residue	Bond type
1	9j	<i>Tc</i> AChE	PAS ^a	Trp279	Hydrophobic
				Tyr70	Hydrophobic
				Tyr121	Hydrophobic
				Tyr334	H-bonding (2.38 Å)
2	8e	hBChE	PAS	Leu286	Hydrophobic
				Val288	Hydrophobic
			OH ^b	Gly116 & 117	Mild polar
			Choline binding site	Phe329	$\pi - \pi$ Stacking
				Trp82	Hydrophobic
			CT ^c	His438	Mild polar
				Ser198	Mild polar

^a Peripheral anionic site.

^b Oxyanion hole.

^c Catalytic triad.

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4.1.1.4. 1-Methyl-4-(2-chlorophenyl)pyrrolo-(spiro[2.3']oxindole)-spiro[3.3"]-5"-(2-chlorophenylmethylidene)-1"-N-acrolylpiperidin-4"-one (8d). White solid; (0.144 g, 77%); mp 155–157 °C; IR (KBr) v_{max}: 3420, 1717, 1621 cm⁻¹; Anal. Calcd for C₃₂H₂₆Cl₂N₃O₃: C, 67.25; H, 4.59; N, 7.35. Found: C, 66.51; H, 4.14; N, 7.08. ¹H NMR (500 MHz, CDCl₃): δ 2.12 (s, 3H, CH₃), 2.46 (d, J = 14.22, H-2"), 3.20 (dd, J = 18.25, 2.52 Hz, 1H, H-6"), 3.41 (t, J = 8.32 Hz, 1H, H-5), 4.05 (t, J = 9.58 Hz, 1H, H-5), 4.13 (d, *J* = 18.25, 1H, H-6"), 4.42 (d, *J* = 14.22, 1H, H-2"), 4.92 (dd, *J* = 9.58, 8.32 Hz, 1H, H-4), 5.92 (d, J = 10.19 Hz, 1H, H- α), 6.31 (d, J = 16.51 Hz, 1H, H- α), 6.68 (dd, J = 16.51, 10.19 Hz, 1H, H- β), 6.70-7.57 (m, 12H, H-aromatic) 7.75 (s, 1H, H-arylmethylidene), 9.25 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 34.28, 43.68, 44.48, 48.02, 56.40, 62.08, 75.46, 110.48, 122.65, 126.01, 126.70, 127.78, 129.28, 129.40, 129.55, 130.37, 130.84, 131.47, 132.21, 132.47, 134.74, 136.29, 136.32, 140.23, 141.14, 177.03, 179.89, 198.60.

4.1.1.5. 1-Methyl-4-(2-fluorophenyl)pyrrolo-(spiro[2.3']oxindole)-spiro[3.3"]-5"-(2-fluorophenylmethylidene)-1"-*N*-acro-

lylpiperidin-4"-one (8e). White solid; (0.107 g, 71%); mp 145–148 °C; IR (KBr) v_{max}: 3423, 1717, 1618 cm⁻¹; Anal. Calcd for C₃₂H₂₆F₂N₃O₃: C, 71.36; H, 4.87; N, 7.80. Found: C, 70.52; H, 4.38; N, 7.92. ¹H NMR (500 MHz, CDCl₃): δ 2.20 (s, 3H, CH₃), 2.74 (d, J = 14.22, H-2''), 3.13 (d, J = 18.09, 1H, H-6''), 3.47 (t, J = 18.09, 1H, H-6'')J = 8.15 Hz, 1H, H-5), 4.08 (t, J = 9.71 Hz, 1H, H-5), 4.12–4.18 (m, 2H, H-2", H-6"), 5.20 (dd, J=9.71, 8.15 Hz, 1H, H-4), 5.65 (d, J = 16.64 Hz, 1H, H- α), 6.23 (d, J = 10.43 Hz, 1H, H- α), 6.55 (dd, J = 16.64, 10.43 Hz, 1H, H- β), 6.81–7.65 (m, 12H, H-aromatic), 7.88 (s, 1H, H-arylmethylidene), 8.78 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 34.90, 37.69, 44.49, 46.13, 56.36, 62.85, 75.53, 110.83, 115.52, 115.82, 116.05, 116.34, 116.47, 123.22, 124.41, 124.45, 124.79, 125.01, 126.96, 127.69, 129.19, 130.24, 131.07, 131.69, 131.81, 132.27, 133.53, 142.53, 167.01, 176.81, 196.31.

4.1.1.6. 1-Methyl-4-(3-nitrophenyl)pyrrolo-(spiro[2.3']oxindole)-spiro[3.3"]-5"-(3-nitrophenylmethylidene)-1"-N-acro-

lylpiperidin-4"-**one (8f).** White solid; (0.135 g, 82%); mp 157–159 °C; IR (KBr) ν_{max} : 3294, 1712, 1615 cm⁻¹; Anal. Calcd for C₃₂H₂₆N₅O₇: C, 64.86; H, 4.42; N, 11.82. Found: C, 64.17; H, 4.79; N, 12.25. ¹H NMR (500 MHz, CDCl₃): δ 2.20 (s, 3H, CH₃), 2.54 (d, *J* = 14.07, H-2"), 3.25 (dd, *J* = 18.25, 2.07 Hz, 1H, H-6"), 3.57 (t, *J* = 8.38 Hz, 1H, H-5), 4.07 (t, *J* = 9.19 Hz, 1H, H-5), 4.25 (d, *J* = 18.25, 1H, H-6"), 4.29 (d, *J* = 14.07, 1H, H-2"), 5.05 (dd, *J* = 9.19, 8.38 Hz, 1H, H-4), 5.74 (d, *J* = 10.52 Hz, 1H, H-α), 6.27 (d, *J* = 16.24 Hz, 1H, H-α), 6.55 (dd, *J* = 16.24, 10.52 Hz, 1H, H-β), 6.83–8.41 (m, 13H, H-aromatic, H-arylmethylidene), 8.79 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 34.88, 44.42, 45.30, 46.53, 57.76, 63.62, 75.34, 110.74, 123.08, 125.32, 126.96, 127.77, 129.14, 129.42, 129.57, 130.25, 130.54, 131.71, 132.07, 132.80, 133.73, 136.22, 136.35, 139.52, 142.80, 167.44, 176.92, 196.53.

4.1.1.7. 1-Methyl-4-(2,4-dichlorophenyl)pyrrolo-(spiro[2.3'] oxindole)-spiro[3.3'']-5''-(2,4-dichlorophenylmethylidene)-1''-

N-acrolylpiperidin-4^{*ν*}**-one (8g).** White solid; (0.144 g, 80%); mp 171–174 °C; IR (KBr) ν_{max} : 3419, 1701, 1640 cm⁻¹; Anal. Calcd for C₃₂H₂₄Cl₄N₃O₃: C, 60.02; H, 3.78; N, 6.56. Found: C, 60.45; H, 4.28; N, 7.81. ¹H NMR (500 MHz, CDCl₃): δ 2.17 (s, 3H, CH₃), 2.70 (d, *J* = 14.40, H-2^{*ν*}), 2.81 (d, *J* = 17.40, 1H, H-6^{*ν*}), 3.59 (t, *J* = 8.27 Hz, 1H, H-5), 4.05 (t, *J* = 9.49 Hz, 1H, H-5), 4.06 (d, *J* = 17.40, 1H, H-6^{*ν*}), 4.09 (d, *J* = 14.40, 1H, H-2^{*ν*}), 5.07 (dd, *J* = 9.49, 8.27 Hz, 1H, H-4), 5.67 (d, *J* = 11.07 Hz, 1H, H-α), 6.12 (d, *J* = 17.15 Hz, 1H, H-α), 6.79 (dd, *J* = 17.15, 11.07 Hz, 1H, H-β), 6.89–7.41 (m, 10H, H-aromatic) 7.76 (s, 1H, H-arylmethylidene), 8.02 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 34.65, 42.06, 43.87, 46.04, 56.95, 62.32, 76.03, 110.89, 123.01, 124.36, 126.71, 127.20, 127.35, 127.73, 128.96, 129.65, 129.98, 130.50, 131.52, 131.95, 132.61, 134.03, 134.41, 135.94, 136.19, 136.56, 136.88, 142.70, 167.05, 176.95, 197.63.

4.1.1.8. 1-Methyl-4-(4-methylphenyl)pyrrolo-(spiro[2.3']oxindole)-spiro[3.3"]-5"-(4-methylphenylmethylidene)-1"-*N*-acro-

White solid; (0.117 g, 77%); mp lylpiperidin-4"-one (8h). 139–141 °C; IR (KBr) v_{max} : 3421, 1707, 1618 cm⁻¹; Anal. Calcd for C₃₄H₃₂N₃O₃: C, 76.96; H, 6.08; N, 7.92. Found: C, 75.12; H, 5.99; N, 6.81. ¹H NMR (500 MHz, CDCl₃): δ 2.12 (s, 3H, CH₃), 2.28 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 2.45 (d, J = 14.39, H-2"), 3.25 (dd, J = 18.32, 2.77 Hz, 1H, H-6"), 3.40 (t, J = 7.98 Hz, 1H, H-5), 4.01 (t, J = 8.87 Hz, 1H, H-5), 4.23 (d, J = 18.32, 1H, H-6"), 4.27 (d, *J* = 14.39, 1H, H-2"), 4.90 (dd, *J* = 8.87, 7.98 Hz, 1H, H-4), 5.69 (d, I = 11.15 Hz, 1H, H- α), 6.22 (d, I = 17.19 Hz, 1H, H- α), 6.57 (dd, I = 17.19, 11.15 Hz, 1H, H-B), 6.84–7.51 (m, 12H, H-aromatic) 7.72 (s, 1H, H-arylmethylidene), 8.97 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 20.05, 20.40, 33.52, 43.17, 44.19, 45.17, 56.26, 62.28, 73.92, 110.11, 121.52, 124.31, 125.82, 126.32, 127.71, 128.11, 128.34, 128.42, 128.62, 128.74, 129.68, 130.44, 133.41, 136.12, 139.15, 139.47, 141.51, 165.74, 175.64, 195.72.

1-Methyl-4-(4-chlorophenyl)pyrrolo-(spiro[2.3']oxin-4.1.1.9. dole)-spiro[3.3"]-5"-(4-chlorophenylmethylidene)-1"-N-acrolylpiperidin-4"-one (8i). White solid; (0.127 g, 81%); mp 154–157 °C; IR (KBr) v_{max}: 3422, 1729, 1615 cm⁻¹; Anal. Calcd for C₃₂H₂₆Cl₂N₃O₃: C, 67.25; H, 4.59; N, 7.35. Found: C, 66.71; H, 4.21; N, 7.19. ¹H NMR (500 MHz, CDCl₃): δ 2.19 (s, 3H, CH₃), 2.54 (d, J = 14.17, H-2"), 3.24 (dd, J = 18.12, 2.41 Hz, 1H, H-6"), 3.49 (t, J = 8.11 Hz, 1H, H-5), 4.02 (t, J = 9.24 Hz, 1H, H-5), 4.20 (d, *J* = 18.12, 1H, H-6"), 4.27 (d, *J* = 14.17, 1H, H-2"), 4.95 (dd, *J* = 9.24, 8.11 Hz, 1H, H-4), 5.72 (d, J = 10.25 Hz, 1H, H- α), 6.26 (d, J = 17.32 Hz, 1H, H- α), 6.59 (dd, J = 17.32, 10.25 Hz, 1H, H- β), 6.82-7.52 (m, 12H, H-aromatic) 7.75 (s, 1H, H-arylmethylidene), 9.17 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 34.83, 44.37, 45.26, 46.49, 57.71, 63.56, 75.29, 110.73, 123.04, 125.32, 126.96, 127.58, 129.19, 129.35, 129.52, 130.18, 130.49, 131.58, 132.03, 132.80, 133.73, 136.22, 136.35, 139.52, 142.80, 167.45, 176.97, 196.55.

4.1.1.10. 1-Methyl-4-(4-fluorophenyl)pyrrolo-(spiro[2.3']oxindole)-spiro[3.3"]-5"-(4-fluorophenylmethylidene)-1"-*N*-acro-

lylpiperidin-4["]-one (8j). White solid; (0.142 g, 77%); mp 181–184 °C; IR (KBr) v_{max} : 3423, 1725, 1615 cm⁻¹; Anal. Calcd for C₃₂H₂₆F₂N₃O₃: C, 71.36; H, 4.87; N, 7.80. Found: C, 70.87; H, 4.21; N, 7.89. ¹H NMR (500 MHz, CDCl₃): δ 2.09 (s, 3H, CH₃), 2.45 (d, J = 14.50, H-2"), 3.20 (dd, J = 18.05, 2.45 Hz, 1H, H-6"), 3.40 (t, J = 8.35 Hz, 1H, H-5), 3.94 (t, J = 9.45 Hz, 1H, H-5), 4.16 (d, *J* = 18.05, 1H, H-6"), 4.20 (d, *J* = 14.35, 1H, H-2"), 4.86 (dd, *J* = 9.45, 8.35 Hz, 1H, H-4), 5.63 (d, J = 10.71 Hz, 1H, H- α), 6.19 (d, J = 16.86 Hz, 1H, H- α), 6.52 (dd, J = 16.86, 10.71 Hz, 1H, H- β), 6.74-7.44 (m, 12H, H-aromatic) 7.69 (s, 1H, H-arylmethylidene), 9.52 (s, 1H, NH) ¹³C NMR (125 MHz, CDCl₃):δ 33.43, 44.00, 44.70, 46.18, 56.45, 62.07, 73.90, 109.42, 114.36, 114.53, 114.77, 115.04, 121.50, 124.10, 125.70, 126.17, 128.07, 128.46, 128.60, 129.23, 130.31, 131.44, 131.51, 132.17, 138.25, 141.81, 160.11, 161.12, 162.07, 163.13, 166.31, 175.76, 195.19.

4.1.1.11. 1-Methyl-4-(1-naphthyl)pyrrolo-(spiro[2.3']oxindole)spiro[3.3"]-5"-(1-naphthylmethylidene)-1"-N-acrolylpiperidin-

4"-one (8k). White solid; (0.177 g, 85%); mp 171–173 °C; IR (KBr) v_{max} : 3429, 1716, 1617 cm⁻¹; Anal. Calcd for C₄₀H₃₂N₃O₃: 79.71; H, 5.35; N, 6.97. Found: 80.15; H, 6.12; N, 7.49. ¹H NMR (500 MHz, CDCl₃): δ 2.15 (s, 3H, CH₃), 2.57 (d, *J* = 14.21, H-2"), 3.39 (d, *J* = 18.07, 1H, H-6"), 3.55 (t, *J* = 7.87 Hz, 1H, H-5), 4.27 (t,

J = 9.12 Hz, 1H, H-5), 4.41 (d, *J* = 18.07, 1H, H-6″), 4.48 (d, *J* = 14.21, 1H, H-2″), 5.15 (dd, *J* = 9.12, 7.87 Hz, 1H, H-4), 5.71 (d, *J* = 11.02 Hz, 1H, H-α), 6.13 (d, *J* = 17.21 Hz, 1H, H-α), 6.32 (dd, *J* = 17.21, 11.02 Hz, 1H, H-β), 6.41–7.69 (m, 16H, H-aromatic) 7.79 (s, 1H, H-arylmethylidene), 8.24 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 29.07, 47.86, 48.02, 51.81, 56.83, 61.17, 76.34, 112.14, 125.53, 126.57, 127.44, 128.33, 128.80, 129.86, 130.26, 130.42, 131.62, 131.74, 132.68, 133.44, 134.41, 136.67, 137.41, 137.81, 138.06, 139.15, 139.47, 143.65, 175.08, 180.96, 198.42.

4.1.1.12. Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro [2.3']oxindole-N-methyl pyrrolo)-5"-(phenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(phenyl) pyrrolidine (9a). White solid; (0.125 g, 71%); mp 160-162 °C; IR (KBr) v_{max} : 3321, 1712, 1615 cm⁻¹; Anal. Calcd for C₄₂H₃₈N₅O₄: C, 74.54; H, 5.66; N, 10.35. Found: C, 75.12; H, 6.11; N, 11.21. ¹H NMR (500 MHz, CDCl₃): δ 0.48 (d, 1H, J = 14.45 Hz, H-6"), 1.63 (s, 3H, CH₃), 2.02 (s, 3H, CH₃), 2.08-2.14 (m, 1H, H-4 of ring A), 2.38-2.44 (m, 2H, H-2", H-4 of ring A), 2.91-3.01 (m, 2H, CH₂-5 of ring A), 3.21 (d, 1H, J = 14.45 Hz, H-6"), 3.25 (d, 1H, I = 14.50 Hz, 2"), 3.36-3.40 (m, 1H, H-3 of ring A), 4.12 (t, 1H, *I* = 9.00 Hz, H-5 of ring B), 5.21 (t, 1H, *I* = 9.00 Hz, H-4 of ring A), 5.50-8.08 (m, 19H, H-aromatic, H-arylmethylidene), 8.09 (s, 1H, NH), 10.61 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 24.63, 34.01, 34.12, 43.22, 43.40, 45.58, 51.83, 56.61, 62.68, 72.18, 74.77, 108.55, 110.21, 119.19, 121.83, 122.52, 124.54, 124.65, 124.99, 125.27, 125.43, 125.77, 126.03, 126.24, 126.51, 126.79, 127.27, 128.09, 128.49, 129.15, 129.49, 129.76, 130.75, 130.78, 132.59, 132.74, 132.76, 133.44, 133.59, 137.43, 141.12, 142.97, 170.21, 176.38, 178.01, 199.51.

Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro 4.1.1.13. [2.3']oxindole-N-methyl pyrrolo)-5"-(2-methylphenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(2-methylphenyl) pyrrolidine (9b). White solid; (0.118 g, 60%); mp 142–144 °C; IR (KBr) v_{max}: 3432, 1714, 1619 cm⁻¹; Anal. Calcd for C₄₄H₄₂N₅O₄: C, 74.98; H, 6.01; N, 9.94. Found: C, 75.51; H, 6.44; N. 10.32. ¹H NMR (500 MHz, CDCl₃): δ 2.07 (s, 3H, CH₃), 2.15 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 2.27 (s, 3H, CH₃), 2.34-2.38 (m, 1H, H-4 of ring A), 2.71-2.79 (m, 2H, H-6", H-4 of ring A), 3.17-3.29 (m, 2H, CH₂-5 of ring A), 3.48-3.53 (m, 1H, H-5 of ring B), 3.45 (d, J = 14.07 Hz, 1H, H-2"), 3.59 (d, J = 17.32 Hz, 1H, H-6"), 3.90 (d, *J* = 14.07, 1H, H-2"), 3.95-4.02 (m, 1H, H-3 of ring A), 4.15-4.21 (m, 1H, H-5 of ring B), 5.21-5.27 (m, 1H, H-4 of ring B), 6.17-7.52 (m, 16H, H-aromatic), 7.72 (s, 1H, H-arylmethylidene), 7.91 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 21.45, 21.74, 26.15, 34.61, 35.42, 44.32, 45.25, 45.52, 49.02, 54.32, 57.21, 63.29, 73.52, 75.19, 109.16, 110.12, 122.21, 122.75, 124.45, 125.32, 126.58, 127.27, 129.36, 129.60, 130.09, 130.28, 130.43, 130.89, 131.78, 135.47, 136.68, 140.18, 140.37, 141.32, 142.69, 171.88, 177.65, 179.50, 198.19.

Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro 4.1.1.14. [2.3']oxindole-N-methyl pyrrolo)-5"-(2-methoxyphenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(2-methoxyphenyl) pyrrolidine (9c). White solid; (0.138 g, 64%); mp 164–166 °C; IR (KBr) v_{max}: 3430, 1718, 1617 cm⁻¹; Anal. Calcd for C₄₄H₄₂N₅O₆: C, 71.72; H, 5.75; N, 9.50. Found: C, 72.41; H, 6.22; N, 10.17. ¹H NMR (500 MHz, CDCl₃): δ 1.98 (s, 3H, CH₃), 2.15 (s, 3H, CH₃), 2.19–2.25 (m, 1H, H-4 of ring A), 2.62–2.74 (m, 2H, H-6", H-4 of ring A), 3.13-3.22 (m, 2H, CH₂-5 of ring A), 3.34-3.38 (m, 1H, H-5 of ring B), 3.39 (d, *J* = 13.43 Hz, 1H, H-2"), 3.49 (d, *I* = 17.05 Hz, 1H, H-6"), 3.69 (d, *I* = 13.43, 1H, H-2"), 3.72 (s, 3H, O-CH₃), 3.76 (s, 3H, O-CH₃), 3.80-3.85 (m, 1H, H-3 of ring A), 4.12-4.17 (m, 1H, H-5 of ring B), 5.09-5.16 (m, 1H, H-4 of ring B), 6.25–7.71 (m, 16H, H-aromatic), 7.65 (s, 1H, H-arylmethylidene), 8.02 (s, 1H, NH). 13 C NMR (125 MHz, CDCl₃): δ 24.51, 29.32, 38.29, 38.44, 41.17, 47.96, 48.67, 51.71, 56.92, 59.43, 64.31, 66.52, 76.17, 78.90, 113.22, 114.51, 118.45, 119.17, 119.36, 119.42, 125.59, 126.54, 127.91, 127.97, 128.19, 128.37, 129.11, 129.89, 130.31, 132.44, 132.59, 133.14, 134.12, 134.68, 135.54, 135.76, 135.97, 136.55, 144.71, 146.39, 175.11, 180.59, 182.15, 199.27.

41115 Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro [2.3']oxindole-N-methyl pyrrolo)-5"-(2-chlorophenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(2-chlorophenyl) pyrrolidine (9d). White solid; (0.105 g, 52%); mp 147–149 °C; IR (KBr) v_{max}: 3412, 1719, 1617 cm⁻¹, Anal. Calcd for C42H36Cl2N5O4: C, 67.65; H, 4.87; N, 9.39. Found: C, 68.19; H, 5.29; N, 9.71. ¹H NMR (500 MHz, CDCl₃): δ 1.95 (s, 3H, CH₃), 2.15-2.18 (s, 4H, CH₃, H-4 of ring A), 2.52 (d, J = 16.80, 1H, H-6"), 2.71-2.81 (m, 1H, H-4 of ring A), 3.05–3.25 (m, 3H, CH₂-5 of ring A, H-5 of ring B), 3.35 (d, J = 13.10 Hz, 1H, H-2"), 3.40 (d, J = 16.80 Hz, 1H, H-6"), 3.47 (d, J = 13.10, 1H, H-2"), 3.67-3.73 (m, 1H, H-3 of ring A), 4.04–4.11 (m, 1H, H-5 of ring B), 4.86–4.92 (m, 1H, H-4 of ring B), 6.06–7.54 (m, 16H, H-aromatic) 7.61 (s, 1H, H-arylmethylidene), 8.13 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 26.24, 34.82, 35.15, 42.52, 44.11, 44.93, 48.89, 53.53, 56.99, 62.96, 72.51, 75.77, 76.97, 77.39, 77.59, 77.81, 109.10, 110.67, 122.02, 123.40, 125.10, 126.83, 127.53, 127.94, 128.77, 129.64, 129.81, 129.87, 130.41, 130.70, 131.31, 132.20, 132.71, 132.95, 135.21, 136.89, 137.21, 140.46, 170.89, 179.17, 181.19, 198.73.

4.1.1.16. Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro [2.3']oxindole-N-methyl pyrrolo)-5"-(2-fluorophenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(2-fluorophenyl) pyrrolidine (9e). White solid; (0.115 g, 54%); mp 171–174 °C; IR (KBr) v_{max}: 3425, 1702, 1618 cm⁻¹; Anal. Calcd for C42H36F2N5O4: C, 70.77; H, 5.09; N, 9.83. Found: C, 71.29; H, 5.44; N, 10.29. ¹H NMR (500 MHz, CDCl₃): δ 2.05 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.22-2.29 (m, 1H, H-4 of ring A), 2.64-2.70 (m, 2H, H-6", H-4 of ring A), 3.12-3.20 (m, 2H, CH₂-5 of ring A), 3.30–3.32 (m, 1H, H-5 of ring B), 3.35 (d, J = 13.70 Hz, 1H, H-2"), 3.44 (d, J = 17.15 Hz, 1H, H-6"), 3.55 (d, J = 13.70, 1H, H-2"), 3.72-3.75 (m, 1H, H-3 of ring A), 4.00-4.04 (m, 1H, H-5 of ring B), 4.88-4.90 (m, 1H, H-4 of ring B), 6.02-7.48 (m, 16H, H-aromatic) 7.56 (s, 1H, H-arylmethylidene), 7.86 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 24.77, 29.14, 38.07, 38.56, 41.05, 47.93, 48.60, 51.54, 56.84, 59.64, 64.54, 66.03, 76.67, 78.89, 113.19, 114.25, 118.90, 119.08, 119.36, 119.53, 125.54, 126.51, 127.81, 127.84, 128.29, 128.37, 128.97, 129.89, 130.62, 132.37, 132.44, 133.08, 133.94, 134.68, 135.33, 135.39, 135.96, 136.77, 144.71, 146.29, 175.46, 180.93, 183.05, 199.17.

Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro 4.1.1.17. [2.3']oxindole-N-methyl pyrrolo)-5"-(3-nitrophenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(3-nitrophenyl) pyrrolidine (9f). White solid; (0.105 g, 57%); mp 160–162 °C; IR (KBr) v_{max} : 3427, 1707, 1619 cm⁻¹; Anal. Calcd for C₄₂H₃₆N₇O₈: C, 65.79; H, 4.73; N, 12.79. Found: C, 65.95; H, 4.92; N, 12.58. ¹H NMR (500 MHz, CDCl₃): δ 1.78 (s, 3H, CH₃), 2.19 (s, 3H, CH₃), 2.20-2.24 (m, 1H, H-4 of ring A), 2.49-2.62 (m, 2H, H-6", H-4 of ring A), 2.98-3.02 (m, 1H, CH₂-5 of ring A), 3.13-3.18 (m, 1H, H-5 of ring B), 3.28 (d, J = 13.95 Hz, 1H, H-2"), 3.47-3.53 (m, 2H, H-2", H-6"), 3.64–3.68 (m, 1H, H-3 of ring A), 4.30–4.34 (m, 1H, H-5 of ring B), 5.01-5.05 (m, 1H, H-4 of ring B), 6.12-8.03 (m, 17H, H-aromatic, H-arylmethylidene), 8.14 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 21.54, 22.15, 26.39, 31.25, 37.19, 37.61, 45.19, 46.51, 46.97, 53.29, 64.79, 68.03, 76.19, 78.42, 111.19, 122.47, 125.79, 127.21, 127.72, 128.14, 128.56, 129.79, 130.44, 130.96, 131.12, 131.67, 132.17, 132.23, 132.44, 136.21, 138.71, 143.20, 145.32, 177.15, 179.21, 181.29, 199.05.

4.1.1.18. Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro [2.3']oxindole-N-methyl pyrrolo)-5"-(2,4-dichlorophenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(2,4dichlorophenyl) pyrrolidine (9g). White solid; (0.138 g, 69%); mp 151–153 °C; IR (KBr) v_{max}: 3429, 1711, 1621 cm⁻¹, Anal. Calcd for C₄₂H₃₄Cl₄N₅O₄: C, 61.93; H, 4.21; N, 8.60. Found: C, 62.44; H, 4.79; N, 9.07. ¹H NMR (500 MHz, CDCl₃): δ 1.99 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 2.30–2.34 (m, 1H, H-4 of ring A), 2.72–2.79 (m, 2H, H-6", H-4 of ring A), 2.94-3.03 (m, 2H, CH₂-5 of ring A), 3.06-3.14 (m, 1H, H-5 of ring B), 3.29-3.35 (m, 1H, H-2"), 3.45-3.53 (m, 1H, H-6"), 3.73 (d, J = 14.10, 1H, H-2"), 3.79 (d, J = 17.42, 1H, H-6"), 3.89-3.94 (m, 1H, H-3 of ring A), 3.96-4.02 (m, 1H, H-5 of ring B), 4.74-4.80 (m, 1H, H-4 of ring B), 6.08-7.32 (m, 14H, H-aromatic), 7.53 (s, 1H, H-arylmethylidene), 8.03 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 22.44, 22.91, 26.43, 35.34, 36.02, 45.05, 45.72, 46.19, 54.18, 57.69, 64.49, 64.71, 72.64, 74.91, 109.79, 110.44, 123.78, 124.91, 128.18, 128.85, 129.51, 131.29, 131.60, 141.45, 141.61, 173.77, 178.80, 181.05, 199.45.

Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro 4.1.1.19. [2.3']oxindole-N-methyl pyrrolo)-5"-(4-methylphenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(4-methylphenyl) pyrrolidine (9h). White solid; (0.119 g, 65%); mp 121–124 °C; IR (KBr) v_{max}: 3429, 1712, 1613 cm⁻¹; Anal. Calcd for C₄₄H₄₂N₅O₄: C, 74.98; H, 6.01; N, 9.94. Found: C, 75.71; H, 6.52; N, 10.44. ¹H NMR (500 MHz, CDCl₃): δ 2.03 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 2.19 (s, 3H, CH₃), 2.21 (s, 3H, CH₃), 2.27-2.34 (m, 1H, H-4 of ring A), 2.69-2.75 (m, 2H, H-6", H-4 of ring A), 3.14-3.22 (m, 2H, CH₂-5 of ring A), 3.36-3.43 (m, 1H, H-5 of ring B), 3.48 (d, J = 13.82 Hz, 1H, H-2"), 3.69 (d, J = 17.24 Hz, 1H, H-6"), 3.83 (d, J = 13.82, 1H, H-2"), 4.02-4.07 (m, 1H, H-3 of ring A), 4.10-4.14 (m, 1H, H-5 of ring B), 4.67-4.72 (m, 1H, H-4 of ring B), 6.15-7.53 (m, 14H, H-aromatic) 7.52 (s, 1H, H-arylmethylidene), 7.80 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 21.52, 21.83, 26.28, 34.75, 35.33, 44.54, 45.19, 45.43, 48.87, 53.48, 57.03, 63.82, 73.22, 74.78, 109.46, 110.58, 122.18, 122.96, 124.98, 125.74, 126.96, 127.51, 129.36, 129.63, 130.01, 130.20, 130.33, 130.86, 131.68, 135.45, 136.48, 140.28, 140.48, 141.19, 142.64, 172.75, 177.71, 179.85, 197.18.

Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro 4.1.1.20. [2.3']oxindole-N-methyl pyrrolo)-5"-(4-chlorophenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(4-chlorophenyl) pyrrolidine (9i). White solid; (0.132 g, 61%); mp 170–172 °C; IR (KBr) v_{max}: 3431, 1718, 1611 cm⁻¹; Anal. Calcd for C₄₂H₃₆Cl₂N₅O₄: C, 67.65; H, 4.87; N, 9.39. Found: C, 68.22; H, 5.37; N, 9.78. ¹H NMR (500 MHz, CDCl₃): δ 1.99 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 2.30-2.35 (m, 1H, H-4 of ring A), 3.15-3.35 (m, 4H, H-6", H-4 of ring A, 5-CH₂ of ring A), 3.71–3.80 (m, 2H, H-5 of ring B, H-2"), 3.38-4.05 (m, 2H, H-2", H-6"), 4.26-4.32 (m, 1H, H-3 of ring A), 4.54-4.60 (m, 1H, H-5 of ring B), 4.74-4.82 (m, 1H, H-4 of ring B), 6.09-7.32 (m, 16H, H-aromatic) 7.54 (s, 1H, H-arylmethylidene), 9.22 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 21.11, 21.46, 25.93, 34.34, 34.90, 44.05, 44.72, 44.93, 53.18, 56.56, 63.49, 72.54, 74.35, 108.79, 109.79, 122.71, 124.74, 127.18, 128.85, 129.22, 130.08, 130.54, 140.45, 140.61, 171.77, 179.10, 182.07, 197.42.

4.1.1.21. Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro [2.3']oxindole-N-methyl pyrrolo)-5"-(4-fluorophenylmethylidene)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(4-fluorophenyl) pyrrolidine (9j). White solid; (0.129 g, 64%); mp 169–171 °C; IR (KBr) v_{max} : 3422, 1707, 1618 cm⁻¹; Anal. Calcd for C₄₂H₃₆F₂N₅O₄: C, 70.77; H, 5.09; N, 9.83. Found: C, 71.86; H, 5.52; N, 10.38. ¹H NMR (500 MHz, CDCl₃): δ 2.04 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 2.19–2.25 (m, 1H, H-4 of ring A), 2.59–2.65 (m, 2H, H-6", H-4 of ring A), 3.04–3.15 (m, 2H, 5-CH₂ of ring A), 3.43–3.48 (m, 1H, H-5 of ring B), 3.62 (d, J = 13.55 Hz, 1H, H-2"), 3.75 (d, J = 17.49 Hz, 1H, H-6"), 4.04 (d, J = 13.55, 1H, H-2"), 4.12–4.19 (m, 1H, H-3 of ring A), 4.25–4.28 (m, 1H, H-5 of ring B), 4.79–4.84 (m, 1H, H-4 of ring B), 6.11–7.56 (m, 16H, H-aromatic), 7.54 (s, 1H, H-arylmethylidene), 8.19 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 24.25, 29.32, 38.19, 38.76, 41.29, 48.24, 48.92, 51.77, 57.12, 60.21, 64.92, 66.25, 76.77, 79.31, 112.20, 113.25, 117.90, 119.25, 119.41, 119.72, 125.81, 126.41, 127.83, 127.87, 128.42, 128.64, 129.12, 129.89, 130.52, 132.76, 132.56, 133.19, 134.12, 134.79, 135.56, 135.91, 135.99, 136.79, 144.82, 146.58, 174.26, 181.31, 182.94, 198.15.

4.1.1.22. Spiro-[2.3']-oxindole-spiro[3.3"]-1"-carbonyl(spiro [2.3']oxindole-N-methyl pyrrolo)-5"-(1-naphthyl)tetrahydro-4"-(1H)-pyridinone-1-methyl-4-(1-naphthyl) pyrrolidine (9k). White solid; (0.172 g, 72%); mp 195–197 °C; IR (KBr) v_{max} : 3406, 1711, 1618 cm⁻¹; Anal. Calcd for C₅₀H₄₂N₅O₄: C, 77.30; H, 5.45; N, 9.01. Found: C, 77.81; H, 5.92; N, 9.28. ¹H NMR (500 MHz, CDCl₃): 1.89 (s, 3H, CH₃), 2.19 (s, 3H, CH₃), 2.20-2.25 (m, 1H, H-4 of ring A), 2.50-2.57 (m, 2H, H-6", H-4 of ring A), 3.15-3.22 (m, 2H, CH₂-5 of ring A), 3.38-3.44 (m, 1H, H-5 of ring A), 3.59 (d, *J* = 13.84 Hz, 1H, H-2"), 3.92 (d, *J* = 17.35 Hz, 1H, H-6"), 4.13 (d, J = 13.84, 1H, H-2"), 4.24-4.27 (m, 1H, H-3 of ring A), 4.80-4.86 (m, 1H, H-4 of ring B), 5.60-5.64 (m, 1H, H-5 of ring B), 6.13-7.47 (m, 24H, H-aromatic) 7.66 (s, 1H, H-arylmethylidene), 8.02 (s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 25.12, 30.22, 38.19, 38.79, 44.09, 46.51, 46.92, 53.24, 64.12, 67.03, 75.91, 112.19, 125.72, 127.52, 128.39, 129.64, 129.88, 129.96, 130.12, 130.74, 130.99,131.44, 136.17, 138.54, 142.44, 145.32, 176.46, 179.83, 182.25, 198.35.

4.2. AChE and BChE inhibitory assay

The cholinesterase enzymes inhibitory potential was evaluated using modified Ellman's method as described by Ahmed and Gilani.²³ Galanthamine was used as reference standard. Solutions of test samples and galanthamine were prepared in DMSO at an initial concentration of 1 mg/mL (100% DMSO). The concentration of DMSO in final reaction mixture was 1%. At this concentration, DMSO has no inhibitory effect on both acetylcholinesterase and butyrylcholinesterase enzymes.²⁴

For acetylcholinesterase (AChE) inhibitory assay, 140 µL of 0.1 M sodium phosphate buffer of pH 8 was first added to a 96-well microplate followed by 20 uL of test samples and 20 uL of 0.09 units/mL acetylcholinesterase. After 15 min of incubation at 25 °C, 10 µL of 10 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was added into each well followed by 10 µL of 14 mM acetylthiocholine iodide. Absorbance of the colored end-product was measured using BioTek **PowerWave** Х 340 microplate spectrophotometer at 412 nm for thirty min after the initiation of enzymatic reaction. For butyrylcholinesterase (BChE) inhibitory assay, the same procedure described above was followed, except for the use of enzyme and substrate, instead of which, butyrylcholinesterase from equine serum and S-butyrylthiocholine chloride were used.

Each test was conducted in triplicate. Absorbance of the test samples were corrected by subtracting the absorbance of their respective blank. Percentage inhibition was calculated using the following formula:

Percentage of inhibition

$$=\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

4.2.1. Enzyme kinetics and mode of inhibition

Lineweaver-Burk plot analysis was performed to determine the mode of enzyme inhibition and enzyme kinetics of compounds 9j and **8e** on AChE and BChE, respectively. The enzyme inhibition kinetics was carried out in the absence and presence of 9j (0, 0.70 and 1.40 μ M) and **8e** (0, 0.90 and 1.81 μ M) at various concentrations of substrates (1, 2, 4 and 8 mM). Kinetic parameters including Michaelis–Menten (K_m) constant and maximum velocity (V_{max}) were derived from Lineweaver–Burk plot while inhibition constant (*K*_i) was derived from Dixon plot.

4.3. Molecular docking study

Molecular docking was performed using Glide software (version 5.7, Schrödinger, LLC, New York, NY, 2011). All the synthesized compounds were docked into the active site of *Tc*AChE derived from three-dimensional structure of the enzyme complex with anti-Alzheimer's drug, E2020 (Aricept[™]) (PDB ID: 1EVE) and BChE derived from complex of the enzyme with its substrate BCh (PDB code: 1POP). Water molecules and hetero groups were deleted from active site beyond the radius of 5 Å of reference ligand (E2020 or BCh), resulting protein structure refined and minimized by Protein Preparation Wizard using OPLS-2005 force field. Receptor Grid Generation program were used to prepare AChE and BChE grid and all the ligands were optimized by LigPrep program by using OPLS-2005 force field to generate lowest energy state of respective ligands. Docking stimulations were carried out on all compounds, handed in 5 poses per ligand, in which the best pose with highest score was displayed for each ligand.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.002.

References and notes

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