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Synthesis and discovery of highly functionalized mono- and bis-spiro-pyrrolidines as potent cholinesterase enzyme inhibitors

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ABSTRACT

Novel mono and bis spiropyrrolidine derivatives were synthesized via an efficient ionic liquid mediated, 1,3-dipolar cycloaddition methodology and evaluated in vitro for their AChE and BChE inhibitory activities in search for potent cholinesterase enzyme inhibitors. Most of the synthesized compounds displayed remarkable AChE inhibitory activities with IC_{50} values ranging from 1.68 to 21.85 μM, wherein compounds **8d** and **8j** were found to be most active inhibitors against AChE and BChE with IC_{50} values of 1.68 and 2.75 μM, respectively. Molecular modeling simulation on *Torpedo californica* AChE and human BChE receptors, showed good correlation between IC_{50} values and binding interaction template of the most active inhibitors docked into the active site of their relevant enzymes.

Alzheimer's disease (AD) as a neurodegenerative disorder is manifested by progressive deterioration of intellectual and cognitive functions, memory loss and personality changes.[1,2] Pathogenesis of AD is characterized by two major hallmarks. First, accumulation of extracellular plaques composed of beta-amyloid peptides (Aβ) due to overexpressed cleavage of amyloid precursor protein (APP) and second, appearance of intracellular neurofibrillary tangles composed of phosphorylated tau proteins, which together severely damage cholinergic neurons comprising basal forebrain.[3] The loss of cholinergic neurons reduces synaptic availability of acetylcholine (ACh) neurotransmitter that lead to the cognitive impairments in AD.[4]

Two major enzymes hydrolyze and regulate acetylcholine in vertebrates; acetylcholinesterase (AChE) and butyrylcholinesterase. AChE is abundant in brain, muscle and erythrocyte membrane, whereas BChE has highest activity in liver, intestine, heart, kidney and lung.[5,6] BChE has been supposed to be a naturally developed protecting enzyme against ChE toxicants.[7] According to the so-called cholinergic hypothesis, the decreased levels of acetylcholine in the brain eventuates memory loss and other cognitive dysfunctions in AD.[8] Thus, to increase ACh levels by the aid of acetylcholinesterase inhibitors (AChEI) is a promising approach to symptomatic treatment of AD patients.

Presently, there are two classes of drugs being used for the treatment of AD, namely the cholinesterase inhibitors and glutamate receptor antagonist. These agents are mainly for symptomatic treatment of AD and are widely prescribed to ameliorate cognitive impairments in these patients.[9] Despite the tremendous efforts in search for novel disease modifying agents working via β-amyloid or tau pathways, none are clinically available due to their adverse effects.

The overall architecture of the AChE and BChE enzymes is quite similar. Their active site is located at the bottom of a 20 Å deep cavity named as 'aromatic gorge'. Substrate and inhibitor guidance down the aromatic gorge is facilitated by hydrophobic interactions with aromatic residues lining the gorge wall such as phenylalanine (Phe), tryptophan (Trp) and tyrosine (Tyr).[10] In the active site of BChE, aromatic residues such tryptophan and phenylalanine, are mostly replaced with hydrophobic ones including leucine (Leu) and valine (Val), making BChE more appropriate to accommodate bulkier substrates and inhibitors.[11]

Multi-component reactions offer a wide range of possibilities for the efficient synthesis of highly complex molecules in a single operational step. These reactions eliminate the need for several workups and purification steps, enabling a great saving of both solvents and reagents.[12]

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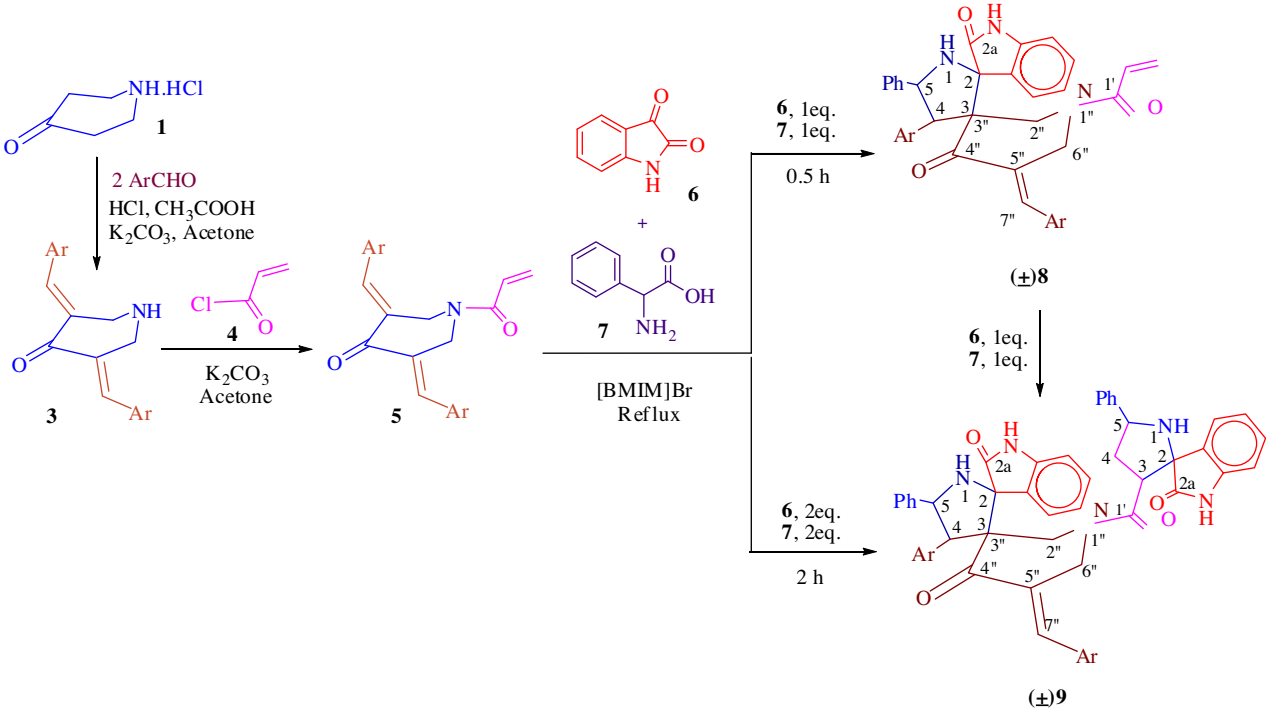
The spiro-oxindoles can be obtained from the cycloaddition reaction of azomethine ylides generated in situ from isatin and α-amino acids, to dipolarophiles bearing exocyclic double bonds. This heterocyclic system is the core structure of many pharmacological agents and natural alkaloids.[13]

In our earlier study,[14] we have reported the synthesis and cholinesterase inhibitory activities of spiropyrrolidines, which some of them possessed good inhibition against AChE and BChE enzymes. Herein we wish to report the ionic liquid mediated synthesis and cholinesterase inhibitory activities of another class of novel mono and bis-cycloadducts comprising spiropyrrolidines, piperidine and oxindole rings. In addition, molecular docking analysis was also performed to disclose the binding interaction template of the most active inhibitors to the amino acid residues composing active site of the AChE and BChE enzymes and the findings are represented in this manuscript.

The highly functionalized dipolarophiles viz. 1-acryloyl-3,5-bis-arylmethylidenepiperidin-4-ones (**5**) were prepared by the aldol condensation of 4-piperidone hydrochloride (**1**) with a series of aromatic aldehydes, according to the literature procedure[15] followed by acylation of the resulting N-unsubstituted 3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones (**3**) with acryloyl chloride (**4**). The dipolarophiles (**5**) are appropriate synthons for the construction of more complex spiro-heterocycles as they possess diverse dipolarophilic functions such as three C=C and two C=O groups. Three-component [3+2]-cycloaddition reaction of a series of **5** with azomethine ylide generated from isatin (**6**) and phenylglycine (**7**) was investigated in 1-butyl-3-methylimidazolium bromide ([BMIM]Br), due to its unrivaled catalytic properties to enhance the rate and the yield of the reactions as well as its recyclability.[16,17] Refluxing equimolar mixture of **5**, **6** and **7** in 1 molar equivalent of [BMIM]Br for 0.5 h afforded the mono-spiropyrrolidines **8**(**a**–**k**) in good yields. The above reaction in 1:2:2 molar ratio of **5**, **6** and **7** in 2 molar equivalent of [BMIM]Br for longer period of time (2 h) also furnished to more complex bis-spiropyrrolidine **9**(**a**–**k**) in moderate yields (Scheme 1). In both the reactions, spiropyrrolidines (**8**) and (**9**) were obtained with good purity, as evident from TLC and ^{1}H NMR spectroscopic analysis.

The structure of the spiropyrrolidine **8** was in accordance with its combustion data, 1D and 2D NMR spectroscopic analysis (vide infra). The ^{1}H NMR spectrum of **8j**[18] showed a doublet at 4.78 ppm (J = 9.7 Hz) for H-4 and a doublet at 5.56 ppm (J = 9.7 Hz) for the H-5 of the pyrrolidine ring. HMQC correlations of H-4 and H-5 assigned the carbon signal at 56.1 and 64.9 ppm to C-4 and C-5, respectively. Further, H-4 shows HMBCs with (i) the 4″-C=O at 197.2 ppm and (ii) the spiro carbon C-3 at 71.0 ppm. 2″-CH_2 of piperidone ring appeared as two doublets in 2.61 and 4.34 ppm with J = 14.1. 6″-CH_2 also showed two doublets at 3.36 and 4.20 ppm with J = 17.9 Hz. HMQC correlated carbon signals at 46.2 and 44.0 ppm to C-2″ and C-6″, respectively. The protons of acryloyl moiety showed up as two doublets at 5.72 and 6.27 ppm with J = 10.7 and 16.7 Hz for 3′-CH_2 and a doublet of doublets at 6.64 ppm with J = 16.7 and 10.7 Hz for 2′-CH. From the HMQC correlation, the carbon signals at 132.6 and 132.9 ppm were assigned to C-3′ and C-2′, respectively. The singlets at 7.54, 7.55 and 7.72 ppm were due to the arylmethylidene hydrogens and NH hydrogen of the oxindole. The aromatic hydrogens appeared as multiplets around 6.85–7.30 ppm (Fig. 1). The ^{1}H and ^{13}C NMR chemical shifts of the other spiropyrrolidines were also assigned by similar straightforward considerations.

A plausible mechanism to rationalize the formation of the spiropyrrolidines **8** is depicted in Scheme 2. The electron-deficient hydrogen atom of [BMIM]Br, forms hydrogen bonding interaction with the carbonyl moiety of isatin, facilitating the generation of reactive azomethine ylide via decarboxylative condensation of isatin and phenylglycine.[17,19] The newly formed ylide, attacks the C=C bond of piperidone ring of (**5**) chemo-selectively, furnishing the mono-spiropyrrolidine (**8**). Bis-spiropyrrolidines (**9**) were obtained due to addition of two moles of azomethine ylide to the C=C bonds of piperidone ring and acryloyl entity of (**5**). In both the reactions, nucleophilic carbon of azomethine ylide adds regio-selectively to the enone moiety in piperidone ring/acryloyl entity.



Scheme 1. Synthesis of spiropyrrolidines **8**(**a**–**k**) and **9**(**a**–**k**).

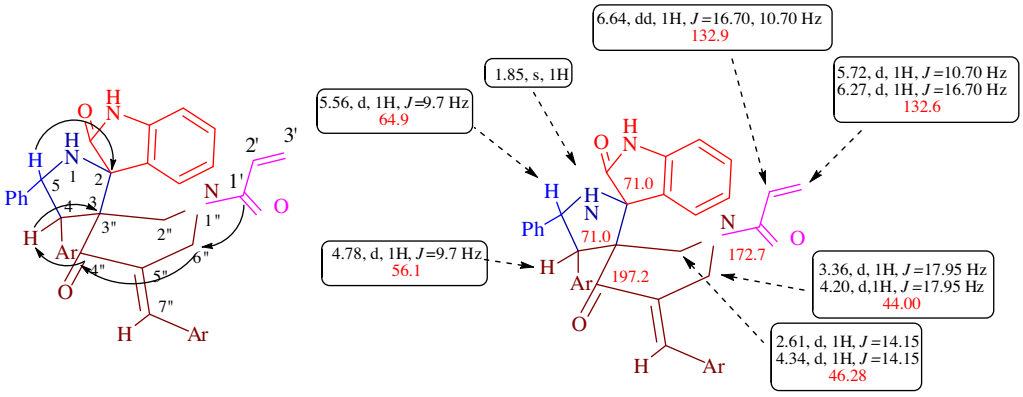
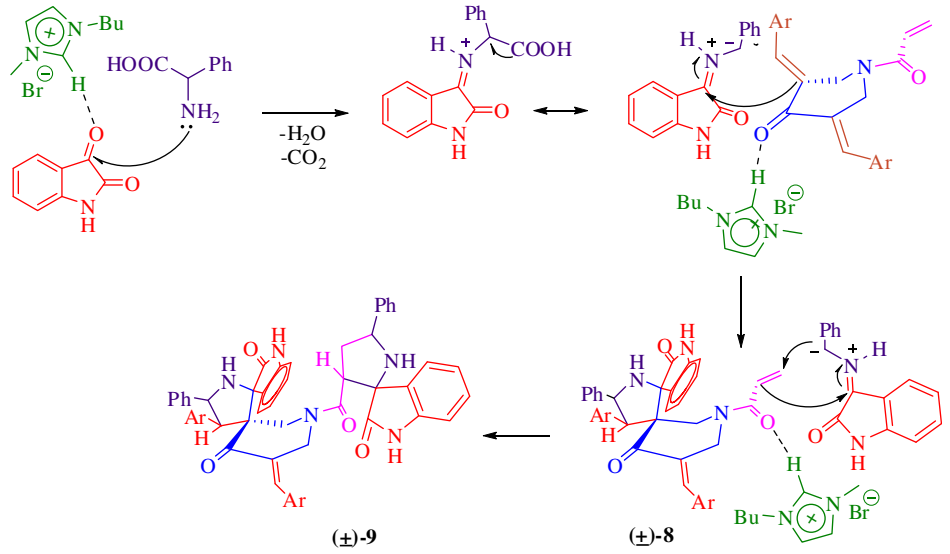


Figure 1. Selected HMBCs and ^{1}H and ^{13}C chemical shifts of **8j**.



Scheme 2. Plausible mechanism for the formation of **8** and **9**.

All the newly synthesized spiropyrrolidines **8**/**9** were evaluated in vitro for their AChE and BChE inhibitory activities against AChE enzyme from electric eel and BChE enzyme from equine serum (Table 1). In general, mono-spiropyrrolidines **8**(**a**–**k**) displayed remarkable AChE inhibitory activities with seven compounds possessing IC_{50} values of less than 10 μM; therein compounds **8d** (Ar=2-ClC_6H_4), **8e** (2-FC_6H_4) and **8k** (Ar = 1-naphthyl) with IC_{50} values of 1.68, 2.27 and 2.07 μM displayed comparable or even more AChE inhibition than standard drug. *ortho* Substituted derivatives in this series, mostly displayed better activities than their *para*-substituted analogs. It is worth to mention that presence of chloro and fluoro atoms on either *ortho* or *para* position of phenyl ring, showed notable influence on the inhibitory activities. On the other hand, bis-cycloadducts in series **9**, displayed lower AChE inhibition than mono-cycloadducts with moderate activities ranging from 7.45 to 30.51 μM. In this series also, *ortho*-substituted derivatives displayed better inhibitory activities than *para*-substituted ones and the effect of chloro and fluoro atoms was still obvious on the activities, whereby compound **9e** with (Ar = 2-FC_6H_4) displayed the highest activity in this series.

Regarding BChE, compounds **8j** bearing fluoro substituent at *para* position of the aromatic ring remarkably displayed the highest inhibition with IC_{50} value of 2.75 μM, almost 7 times stronger than standard drug. *para*-Substituted **8h** and **8i** also showed good activities with IC_{50} <10 μM. Rest of the compounds in this series displayed moderate activities with IC_{50} values ranging from 11.76 to 27.12 μM. However compounds **8b**, **8d** and **8g** showed better BChE inhibition potencies than galanthamine. Bis-spiropyrrolidines in series **9**, had lower BChE inhibitory activities than their mono-analogs with moderate IC_{50} values of 14.42–35.86 μM.

Compounds **8d** and **8j**, as the most active inhibitors, were docked into the active site of their AChE and BChE enzymes, to disclose their binding interaction profile to the relevant receptors. This analysis for compound **8d** as the most active AChE inhibitor showed that it has efficiently bound to the peripheral anionic site of the enzyme, at entrance of the active site channel, through a network of hydrophobic interactions with Trp279, Tyr70 and Tyr334 in addition to strong hydrogen bonding to Ser286 (1.90 Å) and Arg289 (2.09 Å) (Fig. 2). According to this template, compound **8d** can act as a peripheral anionic binding site inhibitor. It is suggested that deposition of amyloid plaque in AD could be accelerated or even triggered by interaction of β-amyloid with the peripheral anionic site of AChE enzyme. Thus, inhibitors binding at the peripheral anionic site of the enzyme not only symptomatically improve the AD, but also can have disease modifying effects.[20]

Docking simulation study for compound **8j**, with the highest BChE inhibitory activity, disclosed its complete accommodation inside the enzyme active site channel. This compound showed π,π-stacking interaction with Tyr332 and hydrophobic interactions with Phe398 at peripheral anionic site along with hydrophobic interactions with Trp82 and Phe329 at choline binding site of the

Table 1
Physical data, AChE and BChE inhibitory activities of **8**(**a**–**k**) and **9**(**a**–**k**)

Entry	Compound	Ar	Yield (%)	AChE IC_{50} ± SD (μM)	BChE IC_{50} ± SD (μM)	AChE selectivity	BChE selectivity
1	**8a**	C_6H_5	75	4.57 ± 0.11	12.32 ± 0.27	2.69	0.37
2	**8b**	2-$CH_3C_6H_4$	69	10.22 ± 0.17	21.78 ± 0.19	2.13	0.47
3	**8c**	2-$(CH_3O)C_6H_4$	72	5.57 ± 0.14	17.52 ± 0.17	3.14	0.32
4	**8d**	2-ClC_6H_4	67	1.68 ± 0.09	22.19 ± 0.22	13.22	0.08
5	**8e**	2-FC_6H_4	70	2.27 ± 0.12	11.76 ± 0.18	5.18	0.19
6	**8f**	3-$(NO_2)C_6H_4$	65	15.15 ± 0.22	10.61 ± 0.13	0.70	1.43
7	**8g**	2,4-$Cl_2C_6H_3$	74	6.16 ± 0.18	27.12 ± 0.21	4.40	0.23
8	**8h**	4-$CH_3C_6H_4$	77	14.58 ± 0.21	5.87 ± 0.16	0.40	2.48
9	**8i**	4-ClC_6H_4	73	4.61 ± 0.15	8.75 ± 0.21	1.90	0.53
10	**8j**	4-FC_6H_4	75	5.98 ± 0.19	2.75 ± 0.12	0.46	2.17
11	**8k**	1-Naphthyl	81	2.07 ± 0.11	13.77 ± 0.15	6.65	0.15
12	**9a**	C_6H_5	62	20.78 ± 0.17	26.30 ± 0.21	1.27	0.79
13	**9b**	2-$CH_3C_6H_4$	59	30.5 ± 0.23	29.07 ± 0.24	0.95	1.05
14	**9c**	2-$(CH_3O)C_6H_4$	65	9.15 ± 0.16	32.12 ± 0.20	3.51	0.28
15	**9d**	2-ClC_6H_4	55	11.28 ± 0.21	17.63 ± 0.18	1.56	0.64
16	**9e**	2-FC_6H_4	57	7.45 ± 0.15	35.86 ± 0.25	4.81	0.21
17	**9f**	3-$(NO_2)C_6H_4$	52	16.79 ± 0.22	17.63 ± 0.22	1.05	0.95
18	**9g**	2,4-$Cl_2C_6H_3$	67	8.61 ± 0.18	26.30 ± 0.17	3.06	0.33
19	**9h**	4-$CH_3C_6H_4$	71	20.19 ± 0.14	19.81 ± 0.19	0.98	1.02
20	**9i**	4-ClC_6H_4	64	12.34 ± 0.16	27.81 ± 0.27	2.25	0.44
21	**9j**	4-FC_6H_4	69	21.85 ± 0.20	14.42 ± 0.18	0.66	1.51
22	**9k**	1-Naphthyl	74	9.24 ± 0.19	19.22 ± 0.23	2.08	0.48
23	—	Galanthamine	—	2.09 ± 0.11	19.34 ± 0.17	3.47	0.28

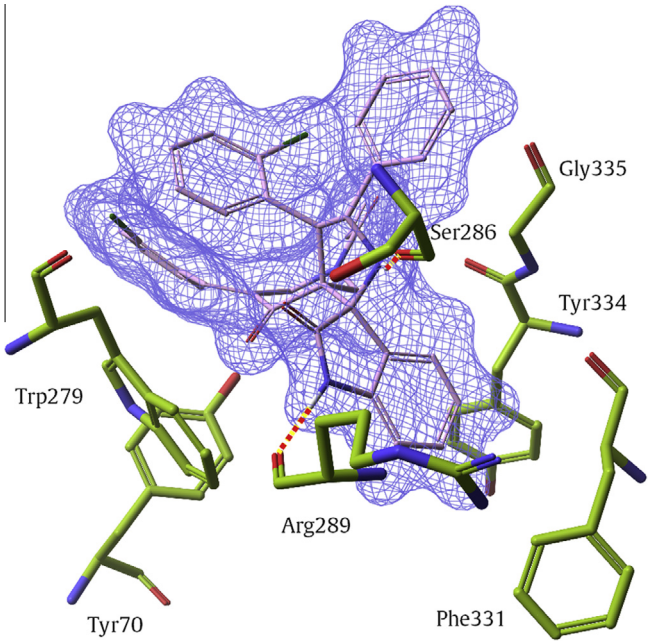


Figure 2. Orientation and binding interaction of **8d** to the active site of AChE receptor.

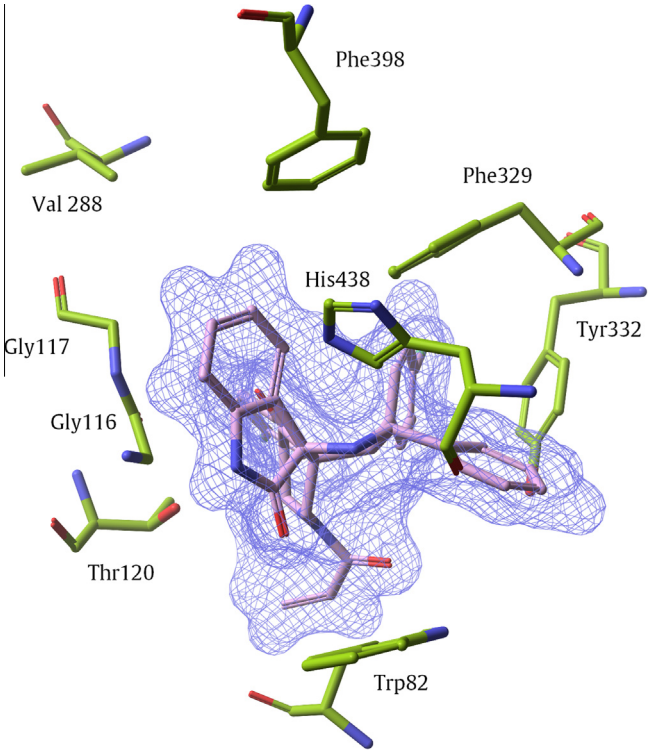


Figure 3. Orientation and binding interaction of **8j** to the active site of BChE receptor.

BChE enzyme. Mild polar interaction with His438 at catalytic triad as well as Gly116 and Gly117 at oxyanion hole of the enzyme, were other major interactions observed for this compound. This extensive hydrophobic and mild polar interaction pattern suggested that **8j** is effectively bound to the BChE active site, thus efficiently avoided insertion of substrate into the channel and its subsequent hydrolysis, which obviously coincides with the significant in vitro activity observed for this compound (Fig. 3).

In conclusion, a series of piperidone-grafted spiropyrrolidines has been synthesized by the three-component [3+2]-cycloaddition reaction of azomethine ylides to highly functionalized dipolarophiles in search for novel potent AChE and BChE inhibitors. Most of the synthesized compounds were found to be significantly active with IC_{50} values ranging from 1.68 to 21.85 μM. Compounds, **8d** and **8j**, showed the maximum inhibitory activity against AChE and BChE with IC_{50} values of 1.68 and 2.75 μM, respectively. Molecular docking analysis for the most active derivative completely coincides with their in vitro results.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.019.

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18. *Synthesis of 4,5-(diphenyl)pyrrolo-(spiro[2.3′]oxindole)-spiro[3.3″]-5″-(phenylmethylidene)-1″-N-acrolylpiperidin-4″-one* ***8*****(*****a*****–*****k*****)**: A mixture of 1-acryloyl-3, 5-diarylidenepiperidin-4-ones (**5**, 0.364 mmol), isatin (**6**, 0.364 mmol) and phenylglycine (**7**, 0.364 mmol) was refluxed in 1 molar equivalent of [BMIM]Br for 0.5 h. The reaction progress was monitored by TLC analysis. After completion of the reaction, the product was obtained through flash column chromatography and its purity of were checked using TLC and ^{1}H NMR techniques.
4-(4-Fluoro)-5-(phenyl) pyrrolo-(spiro[2.3′]oxindole)-spiro[3.3]-5-(4-fluorophenylmethylidene)-1-N-acrolylpiperidin-4-one (***8j***): White solid; (0.139 g, 75%); mp 173–175 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.85 (s, 1H, NH), 2.61 (d, 1H, J = 14.15 H-2″), 3.36 (d, 1H, J = 17.95, H-6″), 4.20 (d, 1H, J = 17.95 Hz, H-6″), 4.34 (d, 1H, J = 14.15 Hz, H-2″), 4.78 (d, 1H, J = 9.70 Hz, H-4), 5.56 (d, 1H, J = 9.70 Hz, H-5), 5.72 (d, 1H, J = 10.70 Hz, H-3′), 6.27 (d, 1H, J = 16.70 Hz, H-3′), 6.64 (dd, 1H, J = 16.70, 10.70 Hz, H-2′), 6.85–7.30 (m, 17H, H-aromatic), 7.54 (s, 1H, H-arylmethylidene), 7.55 (s, 1H, H-arylmethylidene), 7.72 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 44.00, 46.28, 56.13, 63.54, 64.92, 71.06, 110.36, 115.50, 115.66, 115.86, 116.03, 116.23, 112.65, 126.29, 126.68, 127.51, 127.85, 128.49, 128.87, 129.57, 129.97, 130.21, 131.61, 132.51, 132.67, 132.73, 139.21, 140.79, 141.46, 162.27, 163.12, 164.28, 166.92, 178.80, 197.25.
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