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Synthesis and discovery of novel piperidone-grafted mono- and bis-spirooxindole-hexahydropyrrolizines as potent cholinesterase inhibitors

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ABSTRACT

Three-component reaction of a series of 1-acryloyl-3,5-bisbenzylidenepiperidin-4-ones with isatin and L-proline in 1:1:1 and 1:2:2 molar ratios in methanol afforded, respectively the piperidone-grafted novel mono- and bisspiro heterocyclic hybrids comprising functionalized piperidine, pyrrolizine and oxindole ring systems in good yields. The in vitro evaluation of cholinesterase enzymes inhibitory activity of these cycloadducts disclosed that monospiripyrrolizines (**8a–k**), are more active with IC_{50} ranging from 3.36 to 20.07 μM than either the dipolarophiles (**5a–k**) or bisspiropyrrolizines (**9a–k**). The compounds, **8i** and **8e** with IC_{50} values of 3.36 and 3.50 μM, respectively showed the maximum inhibition of acethylcholinesterase (AChE) and butrylylcholinestrase (BuChE). Molecular modeling simulation, disclosed the binding interactions of the most active compounds to the active site residues of their respective enzymes. The docking results were in accordance with the IC_{50} values obtained from in vitro cholinesterase assay.

1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia, in particular among old people. Based on world Alzheimer's reports, there were 36 million people living with dementia worldwide in 2010, predicted to increase to 66 million by 2030 and 115 million by 2050.[1] Its prevalence rises sharply with the age, the incidence of AD being substantial and is approximately 14 times higher among persons older than 85 years than those between 65 and 69 years of age.[2] Age related dementia is considered to arise from the steady loss of neurons that normally affects memory and thinking skills and ultimately the ability to perform even simple tasks in life.[3,4]

Neuropathology of AD is generally characterized by the presence of two microscopic features: (i) extracellular amyloid plaques, known as Aβ plaques and (ii) neurofibrillary tangles (NFT) comprising filaments of a phosphorylated form of microtubule associated protein, tau.[3,5,6] The secondary impact of these features are neurodegeneration and loss of cholinergic neurons in basal forebrain, which are believed to underlie the cognitive deficit and loss of short term memory seen in AD.[7,8] Consequently, one of the strategies for treating AD has been to enhance cholinergic function to sustain or prolong the action of remaining acetylcholine[9] using cholinesterase inhibitors.

The human brain contains two cholinesterase enzymes, (i) acetylcholinesterase (AChE) encoded by a gene on chromosome 7 and (ii) butyrylcholinesterase (BuChE) encoded by a gene on chromosome 3. Among these two, acetylcholinesterase has been studied more and it is the only one consistently associated with cholinergic transmission and cholinoceptive neurons.[10] The therapeutic role of cholinesterase inhibitors is mainly due to enhancement of cholinergic transmission at cholinergic autonomic synapses.[11] Acetylcholinesterase inhibitors such as galanthamine, donepezil, rivastigmine are used as drugs for correcting neurotransmitter disturbances or symptomatic treatment and many more drug candidates are under development at various stages.[12] All the known AChE inhibitor drugs suffer from shortcomings such as low bioavailability, short duration of biological action, narrow therapeutic effects and high toxicity. Hence discovery and development of new drug candidates as AChE inhibitors with enhanced potency and diminished toxicity is imperative.

Multi-component reactions with pot, atom and step economies offer a convenient tool for the assembly of highly functionalized

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organic molecules of structural complexity and diversity in a single procedural step resulting in convergence, atom economy, minimization of waste, flexibility and facile automation.[13] Multi-component 1,3-dipolar cycloaddition of azomethine ylides offers such a versatile protocol to build highly functionalized complex N-heterocycles.[14]

Pyrrolizines constitute an important class of heterocycles with highly pronounced biological activities.[15–17] Functionalized pyrrolizines with spirooxindole rings constitute prime sub-structures of numerous alkaloids and pharmacologically important compounds.[18] Isatin and its derivatives possess antibacterial,[19] antiprotozoal,[20] antifungal,[21] antiviral[22] and anti-HIV activities[23] and are widely used as precursors for the synthesis of natural products.[24]

AChE and BuChE have similar structures and adopt a very similar secondary structure topology with about 83.9% sequence similarity.[25] It is found that majority of the important functionality binding sites as well as the structurally important residues are conserved among AChE and BuChE. AChE and BuChE exhibit five important regions for binding, namely active gorge,[26] oxyanion hole,[27] anionic substrate site,[28] an acyl binding site[29] and peripheral anionic site[30] but there are some divergences between them. In AChE, more aromatic residues are found in the gorge, whilst in BuChE the gorge is lined with additional hydrophobic residues. Despite the different properties in the gorge region, Leu286 and Val288 are responsible for the acyl binding site in BuChE, instead of two Phe residues in AChE.[30] The active site gorge in both AChE and BuChE is located deep in the centre of the molecule with a narrow gorge made up of the catalytic triad Ser200, His440 and Glu327 involved in hydrolyzing acetylcholine. In the present study, we have introduced the multi-component 1,3-dipolar cycloaddition reactions for the synthesis of mono- and bispyrrolizines incorporating indolizine, piperidone and oxindole rings and reported their AChE and BuChE inhibitory activities. Molecular docking simulation was also employed to disclose the mechanism of inhibition for the most active compounds synthesized in the present work on AChE and BuChE receptors. The important structural aspects of AChE and BuChE receptors active sites were briefly discussed to enable understanding of their binding interactions with the most active compounds.

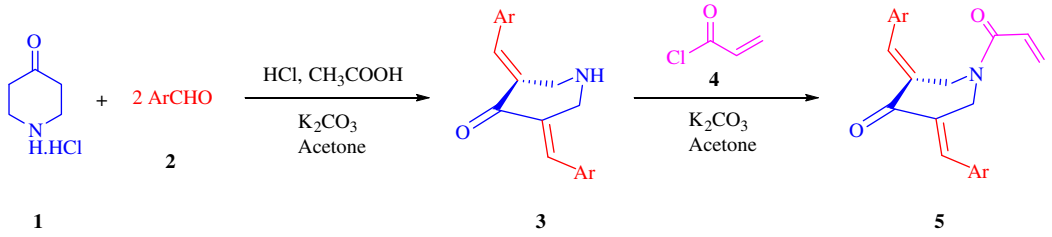
2. Results and discussion

2.1. Chemistry

The highly functionalized dipolarophiles viz. 1-acryloyl-3,5-diarylidenepiperidin-4-ones (**5**) required for the synthesis of spiroheterocycles were prepared by the Claisen–Schmidt condensation of 4-piperidone hydrochloride (**1**) with a series of aromatic aldehydes (Scheme 1) in the presence of HCl in acetic acid following the literature procedure.[31] Acylation of the N-unsubstituted 3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones (**3**) with acryloyl chloride furnished **5** in good yields. Among the synthesized dipolarophiles, **5b**, **5c**, **5e** and **5f** are new and their structure was derived by ^{1}H and ^{13}C NMR spectroscopic data and further confirmed by single crystal X-ray crystallographic study (Fig. 1).[32] Dipolarophiles **5** are versatile synthons for the assembly of spiro heterocycles, as they possess multi dipolarophilic functions, viz. three C=C and C=O groups. Hence the study of reactivity and product-selectivity of the cycloadditions of **5** are of considerable interest.

With a series of dipolarophiles (**5a–k**) in hand, we commenced our investigation on the three-component [3+2]-cycloaddition reaction of **5** with isatin (**6**) and L-proline (**7**). Initially, the cycloaddition reaction of an equimolar mixture of a series of 1-acryloyl-3,5-diarylidenepiperidin-4-ones (**5**), isatin (**6**) and L-proline (**7**) was performed in methanol under heating at reflux for 5 h. till the reaction went to completion (TLC). This reaction afforded the monospiropyrrolizines (**8**) (Scheme 2) as the sole product in 73–91% yields. When the reaction of **5**, **6** and **7** in a molar ratio 1:2:2 was performed for a longer duration of 15 h. the structurally more complex bisspiropyrrolizines (**9**) were obtained in moderate yields (53–74%). In both these reactions, the spiropyrrolizines (**8**) and (**9**) that precipitated out as colourless solid from the reaction mixture was filtered off and washed with cold methanol. The resulting samples **8** and **9** were found to be of high purity as disclosed by TLC and ^{1}H NMR spectroscopic data analysis. Further treatment of **8** or **9** with an excess of isatin and L-proline failed to afford the trispiroheterocycles (Scheme 2).

Structure of the mono and bispiropyrrolizines **8** and **9** is in accord with the combustion data as well as IR, 1D and 2D NMR spectroscopic data (vide infra). The elemental analysis results are within ±0.4% of the theoretical values. The ^{1}H NMR spectrum of **8a** shows a doublet of doublets at 3.96 ppm with J = 11.66 and 7.09 Hz for H-3 of the pyrrolizine ring due to coupling with CH_2-4. The C,H-COSY correlation of H-3 assigns the carbon signal at 51.2 ppm to C-3. Further, H-3 shows HMBCs (Fig. 2) with (i) C=O carbon linked to the N(1″) at 164.1 ppm, (ii) the oxindole C=O at 180.8 ppm, (iii) the spiro carbon C-2 at 77.4 ppm, besides showing correlation with the adjacent carbon C-4 at 35.3 ppm. The C,H-COSY correlation of C-4 assigns the multiplets at 2.20–2.26 and 2.43–2.56 ppm to 4-CH_2. The H,H-COSY correlation of 4-CH_2 assigns the multiplet at 4.01–4.12 ppm to H-4a. By similar considerations, the multiplets around 1.56–2.67 ppm is assigned to 5-, 6-, and 7-CH_2 hydrogens of the pyrrolizine ring. The multiplets around 4.24–4.66 ppm is due to 2″-CH_2 and 6″-CH_2 of the piperidone ring, which show HMBCs with the carbon signal at 184.2 ppm due to C-4″ besides showing correlation with the carbonyl at 164.1 ppm. From C,H-COSY correlation, the carbon signals at 42.3 and 46.5 ppm are assigned to C-6″ and C-2″, respectively. The singlets at 7.53, 7.56 and 8.70 ppm are due to the arylmethylidene hydrogens and NH hydrogen of the oxindole. The aromatic hydrogens appear as doublets and multiplets in region between 7.03 and 7.40 ppm. The ^{1}H and ^{13}C NMR chemical shifts of the bisspiropyrrolizines (**9**) were also assigned by similar straightforward considerations. Further the structure and stereochemistry of mono and bisspiropyrrolizines were confirmed by the single crystal X-ray crystallographic analysis of **8a**[36] (Fig. 3) and **9k**[33] (Fig. 4), respec-



Scheme 1. Synthesis of highly functionalized dipolarophiles **5**(**a–k**).

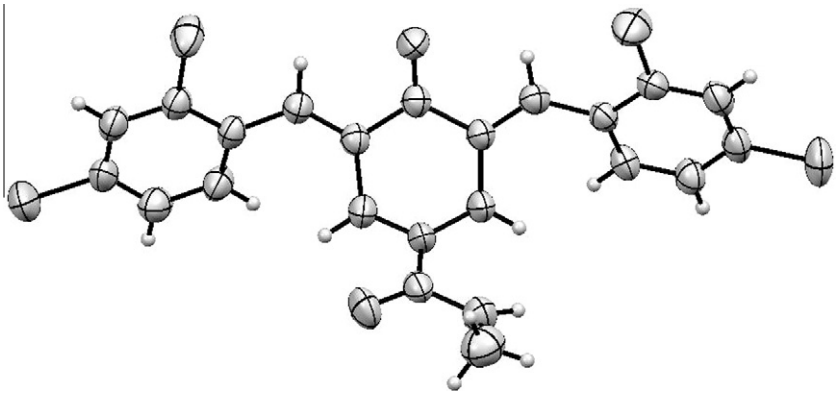
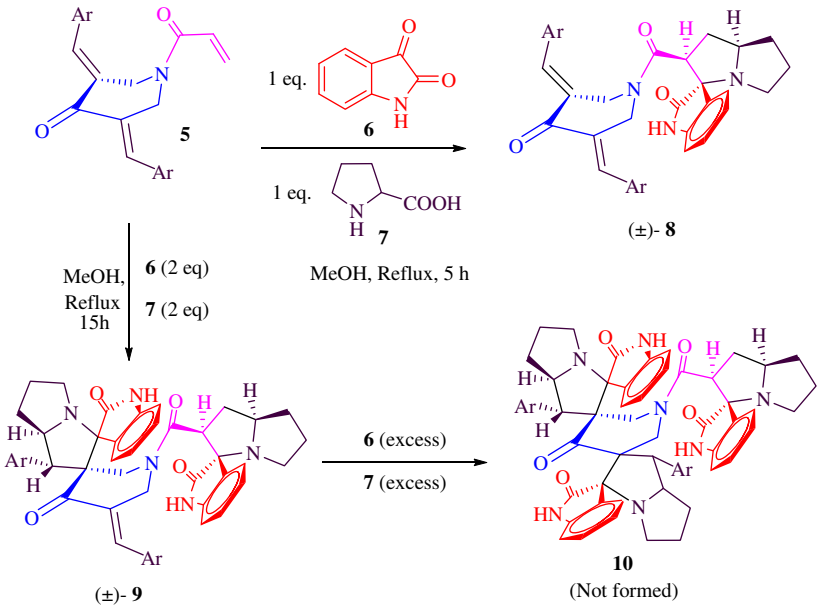


Figure 1. ORTEP diagram of **5g**.



Scheme 2. Synthesis of mono and bisspiropyrrolizines **8**(**a–k**) and **9**(**a–k**).

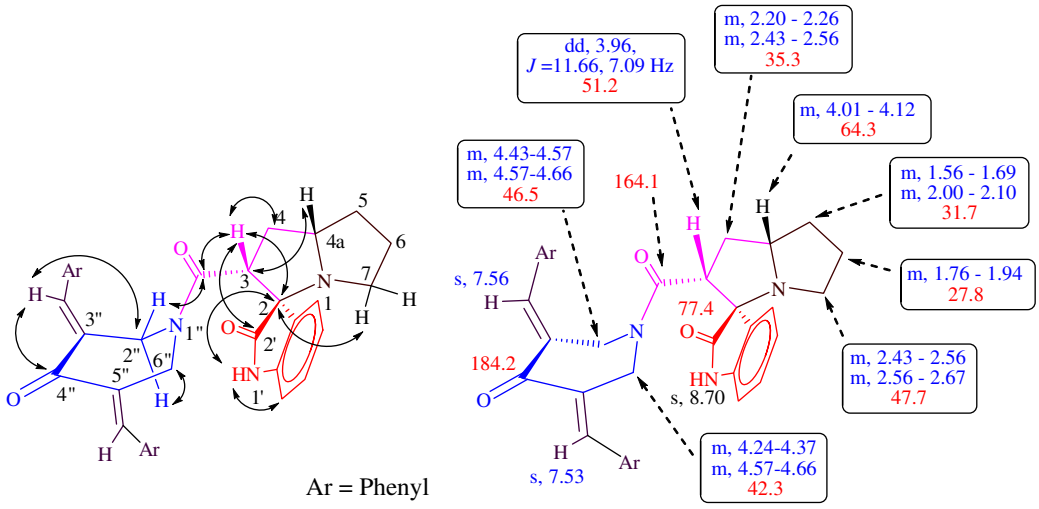


Figure 2. Selected HMBCs and ^{1}H and ^{13}C chemical shifts of **8a**.

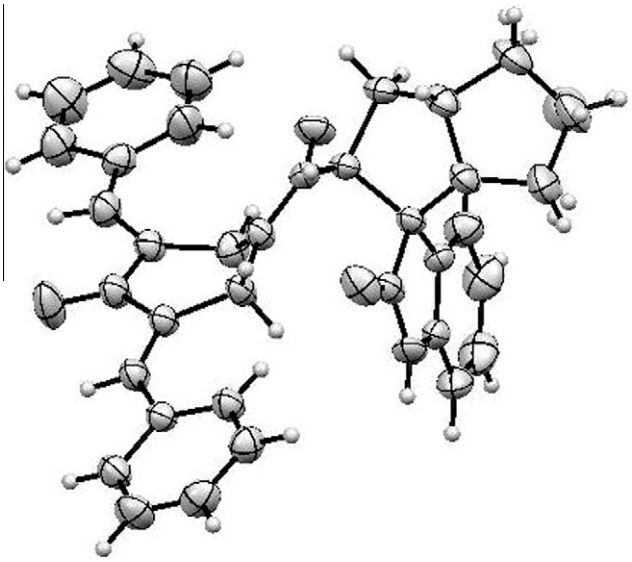


Figure 3. ORTEP diagram of **8a**.

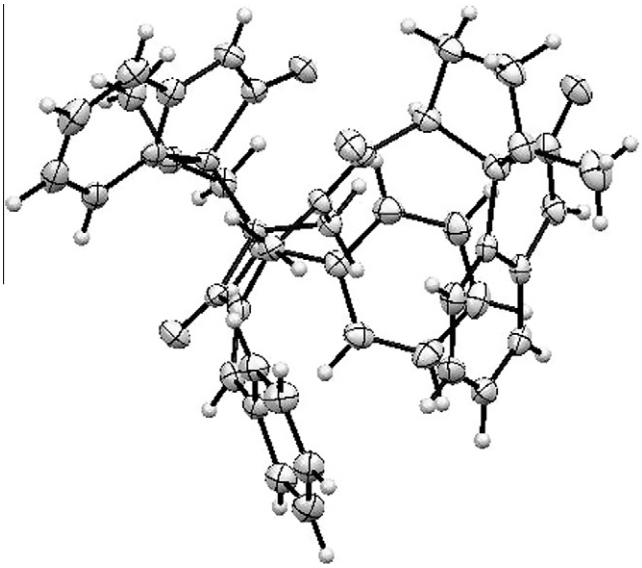


Figure 4. ORTEP diagram of **9k**.

tively. As the cycloadduct **9** has two oxindolo-pyrrolizine rings, to distinguish the ^{1}H and ^{13}C chemical shifts of the two rings, the ring attached to 1″-N–C=O is considered as ring A and the other ring attached to C-3″ of 4-piperidone moiety is considered as ring B.

The mechanism of the reaction involves the initial formation of azomethine ylide **11** from isatin and L-proline, which adds chemoselectively to C=C bond of acryloyl moiety of **5** to form the monospiropyrrolizine **8** (Scheme 3). This chemoselectivity is explicable by the relatively less hindrance encountered for the cycloaddition at the C=C bond of acryloyl moiety compared to the two arylidiene C=C functions. In case of reaction of **5** with an excess of **6** and **7** for a longer reaction time, the azomethine ylide further adds to one of the C=C bonds of initially generated **8** affording the dispiro cycloadduct (**9**). This is evident from the fact that (i) the hexahydrospiroindoline-3,3'-pyrrolizine ring formed by the reaction of the azomethine ylide to *N*-acryolyl group of **8** and **9** has the same relative configuration and (ii) the reaction of **8** with one mole equivalent of each isatin and L-proline, in a separate experiment, also furnished **9**. Further reaction of **9** with isatin and L-proline failed to afford the trispiro cycloadduct (**10**), ascribable to the steric hindrance arising from the cycloadduct (**9**) for further cycloaddition. The structure of **8** and **9** shows that the cycloaddition reactions proceed regioselectively with the nucleophilic carbon of azomethine ylide preferentially adding to the β-carbon of enone functions and stereoselectively affording only one stereoisomer of both the mono- and bis-cycloadducts **8** and **9**.

2.2. In vitro cholinesterase enzymes inhibitory activity

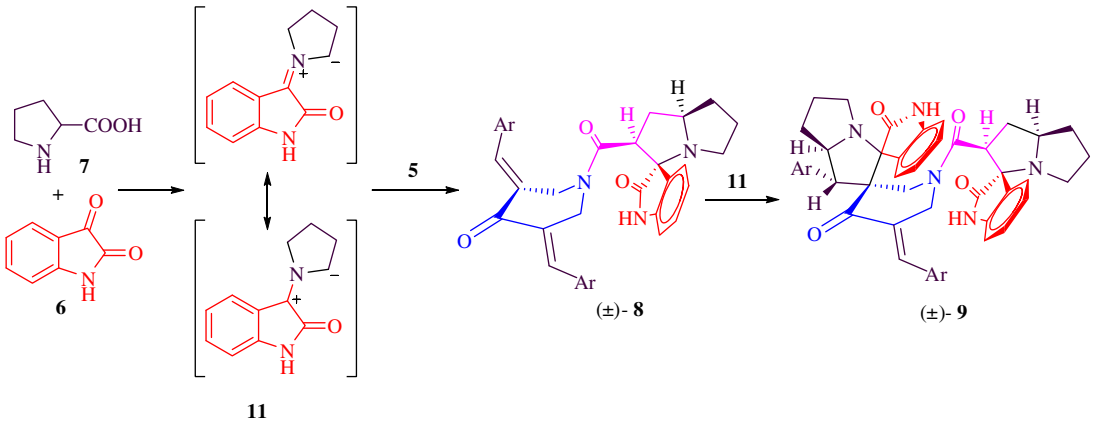
All the newly synthesized compounds were evaluated for their AChE and BuChE inhibitory activities using Ellman's method and the results are summarized in Table 1. The data in this table show that in the first series, **5**, only **5c** and **5f** bearing *ortho*-methoxy and *meta*-nitro moieties on aromatic ring, displayed good inhibitory activity against AChE enzyme with IC_{50} = 8.72 and 9.76 μM, respectively. The rest of the compounds in this series showed moderate inhibitory activity against AChE with IC_{50} ranging from 11.81 to 22.04 μM. Regarding BuChE, all the compounds, showed low inhibitory activity against this enzyme, with activities ranging from 22.06 to 54.04 μM. Compound **5f,** also showed the highest selectivity for AChE enzyme in this group with 5.24 times more selectivity toward AChE as compared to BuChE.

As for mono-spiropyrrolizine in series **8**, compounds **8i** carrying *para*-chloro, **8g** carrying *ortho* and *para* di-chloro, **8f** carrying *meta*-nitro and **8k** carrying naphthyl moiety on aromatic ring, showed noticeable inhibitory activity against AChE enzyme with IC_{50} = 3.36, 4.11, 5.99 and 6.76 μM, respectively. Compound **8i** also displayed highest inhibitory activity and selectivity for AChE among the compounds within this group and all the synthesized compounds. It is observable that presence of chloro substituent on aromatic ring has a significant influence on possessing AChE inhibitory activity within these mono-cycloadducts as observed for **8i** and **8g**. The rest of compounds in this series displayed moderate AChE inhibitory with activities ranging from 11.92 to 34.37 μM.

As for BuChE, compound **8e**, bearing fluoro substituent on *ortho* position of aromatic ring showed highest inhibitory activity and highest selectivity for this enzyme within mono-spiropyrrolizine and all synthesized compounds (IC_{50} = 3.51 μM). Compounds **8j** bearing fluoro substituent on *para* position of aromatic ring, also showed considerable BuChE inhibitory activity within this series with IC_{50} = 3.99 μM. It can be postulated that presence of fluoro substituent on aromatic ring of mono-spiropyrrolizine compounds, remarkably enhances the BuChE inhibitory activity presumably due to more appropriate interactions with active site residues of this enzyme. The remaining compounds in this series, showed moderate BuChE inhibitory activity ranging from 11.79 to 32.27 μM.

Considering the bis-spiropyrrolizines **9**, interestingly derivatives **9g** and **9i** with chlorine in the aromatic rings, again displayed the highest inhibitory activities with IC_{50} = 6.96 and 7.92 μM, respectively. However the remaining compounds of series **9** showed moderate inhibitory activity against AChE. Regarding BuChE, compounds **9k**, **9j**, **9e** and **9g** displayed considerable activities against BuChE with IC_{50} = 6.42, 8.29, 9.09 and 9.45 μM, respectively.

In conclusion, it can be justified that mono-spiropyrrolizines showed the best inhibitory activity in both AChE and BuChE compared to the starting precursors (**5**) and bis-spiropyrrolizines (**9**), probably owing to introduction of more functional groups into the molecule which seems necessary to bind the inhibitors to the active site residues. Moving from mono- to bis-spiropyrrolizines, due to increase in the size of molecule, AChE inhibitory activity



Scheme 3. Mechanism of formation of spiropyrrolizines **8** and **9**.

Table 1
Physical data, AChE and BuChE activities of **5**(**a–k**), **8**(**a–k**) and **9**(**a–k**)

Entry	Compound	Ar	Yield (%)	AChE inhibition IC_{50}		BuChE inhibition IC_{50}		Selectivity	
				μg/mL	μM	μg/mL	μM	AChE[a]	BuChE[b]
1	**5a**	C_6H_5	87	5.83 ± 0.29	**17.68**	7.26 ± 0.15	**22.06**	1.25	0.80
2	**5b**	2-$CH_3C_6H_4$	81	6.21 ± 0.22	**17.39**	10.72 ± 0.27	**30.02**	1.73	0.58
3	**5c**	2-$(OCH_3)C_6H_4$	83	3.44 ± 0.08	**8.72**	11.24 ± 0.32	**28.92**	3.32	0.30
4	**5d**	2-ClC_6H_4	74	6.49 ± 0.12	**16.32**	14.54 ± 0.22	**36.62**	2.24	0.45
5	**5e**	2-FC_6H_4	76	4.31 ± 0.15	**11.81**	19.73 ± 0.29	**54.04**	4.58	0.22
6	**5f**	3-$(O_2N)C_6H_4$	84	4.11 ± 0.24	**9.76**	21.44 ± 0.27	**51.16**	5.24	0.19
7	**5g**	2,4-$Cl_2C_6H_3$	83	7.31 ± 0.19	**15.75**	13.62 ± 0.21	**29.37**	1.86	0.54
8	**5h**	4-$CH_3C_6H_4$	85	6.81 ± 0.29	**18.06**	12.52 ± 0.17	**35.07**	1.94	0.51
9	**5i**	4-ClC_6H_4	82	8.72 ± 0.38	**22.04**	15.44 ± 0.31	**38.19**	1.73	0.58
10	**5j**	4-FC_6H_4	82	5.54 ± 0.17	**15.12**	11.16 ± 0.19	**30.58**	2.02	0.49
11	**5k**	1-Napthyl	91	5.94 ± 0.11	**13.84**	9.92 ± 0.24	**23.12**	1.67	0.60
12	**8a**	C_6H_5	83	9.22 ± 0.14	**17.49**	8.62 ± 0.19	**16.29**	0.93	1.07
13	**8b**	2-$CH_3C_6H_4$	74	7.22 ± 0.04	**12.47**	9.93 ± 0.23	**17.82**	1.43	0.70
14	**8c**	2-$(OCH_3)C_6H_4$	77	9.42 ± 0.28	**15.86**	5.49 ± 0.19	**9.41**	0.59	1.69
15	**8d**	2-ClC_6H_4	78	8.12 ± 0.06	**11.92**	11.21 ± 0.22	**19.73**	1.66	0.60
16	**8e**	2-FC_6H_4	73	19.42 ± 0.24	**34.37**	1.25 ± 0.02	**3.50**	0.10	9.82
17	**8f**	3-$(O_2N)C_6H_4$	81	3.99 ± 0.12	**5.99**	10.87 ± 0.32	**18.14**	3.03	0.33
18	**8g**	2,4-$Cl_2C_6H_3$	79	2.94 ± 0.21	**4.11**	12.93 ± 0.27	**21.16**	5.15	0.19
19	**8h**	4-$CH_3C_6H_4$	76	12.15 ± 0.32	**20.07**	14.04 ± 0.31	**25.2**	1.26	0.80
20	**8i**	4-ClC_6H_4	80	2.28 ± 0.07	**3.36**	19.26 ± 0.26	**32.27**	9.60	0.10
21	**8j**	4-FC_6H_4	78	13.52 ± 0.27	**19.84**	1.38 ± 0.04	**3.99**	0.20	4.97
22	**8k**	1-Naphthyl	84	4.58 ± 0.18	**6.76**	7.42 ± 0.17	**11.79**	1.74	0.57
23	**9a**	C_6H_5	72	15.23 ± 0.12	**19.65**	14.21 ± 0.23	**18.21**	0.93	1.08
24	**9b**	2-$CH_3C_6H_4$	61	21.15 ± 0.27	**27.45**	10.15 ± 0.10	**12.96**	0.47	2.12
25	**9c**	2-$(OCH_3)C_6H_4$	65	9.17 ± 0.27	**11.41**	15.43 ± 0.14	**19.21**	1.68	0.59
26	**9d**	2-ClC_6H_4	53	11.22 ± 0.12	**13.64**	9.67 ± 0.25	**11.74**	0.86	1.16
27	**9e**	2-FC_6H_4	53	19.42 ± 0.31	**24.58**	7.52 ± 0.17	**9.09**	0.37	2.70
28	**9f**	3-$(O_2N)C_6H_4$	59	22.43 ± 0.28	**26.6**	20.53 ± 0.28	**24.29**	0.91	1.10
29	**9g**	2,4-$Cl_2C_6H_3$	68	6.12 ± 0.16	**6.96**	8.42 ± 0.19	**9.45**	1.36	0.74
30	**9h**	4-$CH_3C_6H_4$	67	15.07 ± 0.15	**19.59**	13.75 ± 0.26	**17.88**	0.91	1.10
31	**9i**	4-ClC_6H_4	64	6.43 ± 0.10	**7.92**	8.94 ± 0.17	**10.89**	1.38	0.73
32	**9j**	4-FC_6H_4	62	18.73 ± 0.32	**23.67**	6.59 ± 0.16	**8.29**	0.35	2.86
33	**9k**	1-Naphthyl	74	17.64 ± 0.34	**20.63**	5.49 ± 0.15	**6.42**	0.31	3.21
34	Standard	Galanthamine	—	0.60 ± 0.01	**2.09**	5.55 ± 0.01	**19.34**	3.47	0.28

[a] Selectivity for AChE is defined as IC_{50}(BuChE)/IC_{50}(AChE).
[b] Selectivity for BuChE is defined as IC_{50}(AChE)/IC_{50}(BuChE).

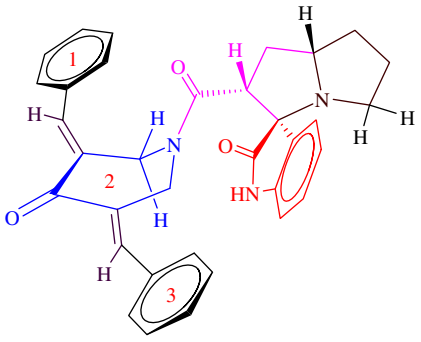
drops considerably, however BuChE inhibitory activity remains almost unchanged. This can be relevant to bigger active site gorge in the BuChE enzyme, in comparison to AChE that accommodates more bulky substrates/inhibitors. As mentioned earlier, presence of chloro substituent on aromatic ring in mono and bis-cycloadducts, significantly improves both inhibitory activities and selectivity for AChE enzyme. This effect is also noticeable for the mono and bis-spiropyrrolizines which carry fluoro substituent on aromatic ring, as their selectivity and inhibitory activity for BuChE are superior as compared to other derivatives.

2.3. Docking simulation

Docking simulation is a popular approach for the preliminary screening in structure based drug design. By performing docking simulation, information on feasible conformations of the ligand within the protein binding site can be obtained. This information can also reflect the nature and quality of the interaction. In our study, the grid box for docking simulation was built with enough size to enable probing into the binding with peripheral and gorge active sites. Docking simulations were carried out for the most ac-

Table 2
Binding interaction data for **8i** and **8e** to AChE and BuChE receptors

Entry	Ligand	Enzyme	Interacting site	Residue name	Bond type	Residue interacting moiety	Ligand interacting moiety
1	**8i**	*Tc*AChE	PAS	Tyr70	Hydrophobic	4-Methoxyphenyl	Pyrrolizine ring
2			PAS	Tyr121	Hydrophobic	Methoxy	Ring 1
3			PAS	Trp 279	Hydrophobic	Indole	Pyrrolizine ring, ring 2 and 3
4			PAS	Tyr334	H-bonding	C=O	NH of pyrrolizine ring
5			PAS	Tyr334	π,π-Stacking	4-Methoxyphenyl	Ring 1
6			Choline binding	Phe331	π,π-Stacking	Phenyl ring	Ring 1
7			Choline binding	Phe330	Hydrophobic	Phenyl ring	Ring 2
8			Acyl binding	Phe290	Hydrophobic	Phenyl ring	Ring3
9			Side chain	Arg289	H-bonding	N–H	C=O of ring 2
10	**8e**	BuChE	PAS	Trp 231	π,π-Stacking	Indole	Ring 1
11			PAS	Phe398	Hydrophobic	Phenyl ring	Ring 1
12			Acyl binding	Phe329	Hydrophobic	Phenyl ring	Pyrrolizine ring
13			Oxyanion hole	Gly116	Mild polar	N–H	Ring 1
14			Oxyanion hole	Ala199	Mild polar	N–H	Ring 2
15			Choline binding	Trp82	Hydrophobic	Indole ring	Oxo-indole and pyrrolizine ring
16			Catalytic triad	His 438	Mild polar	Imidazole ring	Ring 1
17			Side chain	Asp79	H-bonding	C=O	NH of pyrrolizine ring



tive inhibitors **8i** and **8e**, which displayed comparable potency to AChE and five times more potency to BuChE enzymes as compared to standard drug, galantamine. This strategy expected to disclose the binding affinity of these inhibitors to their respective receptors and their relative orientation inside the receptor and in contact with active site residues.

The docking results on AChE and BuChE receptors were collected using Schrodinger, Maestro 9.2 software for the most potent inhibitors, **8i** and **8e**, to their respective receptors. As summarized in Table 2, The most of the binding interactions within **8i** and the amino acid residues composing active site gorge in AChE receptor, possess hydrogen bonding, π,π-stacking and hydrophobic nature. Concisely, this compound displayed strong π,π-stacking interaction with Tyr334 at peripheral anionic site and Phe331 at choline binding site. Two strong hydrogen binding interaction with Tyr334 (2.18 Å) and Arg289 (2.05 Å) residues which are located at peripheral anionic site and side chain backbone, has been docked this compound to the active site gorge. Hydrophobic interactions with Tyr70, Tyr121 and Trp279 in peripheral anionic site together with Phe290 at acyl binding pocket and Phe330 at choline binding site are the other noticeable interactions which support the accommodation of **8i** inside the gorge. This compound via interaction with residues composing peripheral anionic site (PAS), presumably blocks the entrance of the gorge and prevents the insertion/accommodation of substrate inside the active site of the receptor (Fig. 5).

For **8e** which is docked into BuChE receptor, interactions have H-bonding, π,π-stacking, hydrophobic and mild polar nature (Table 2). This compound showed strong π,π-stacking interaction with Trp231 at peripheral anionic site and strong hydrogen bonding with Asp70 (2.03 Å) located at side chain. Val 288, Leu 286 and Phe 398 at peripheral anionic, Gly116, Gly117 and Ala199 at oxyanion hole, Trp82 and Phe 329 at choline binding site and His438 at catalytic triad are the other main residues which interact with **8e** within active site gorge. As depicted in Figure 6 it seems that this compound is completely accommodated inside the gorge owing to appropriate interactions with the key residues composing active site in BuChE receptor. This suitable embedding probably incur to prosperous prevention of substrates to insert into the gorge rising to its significant inhibitory property.

In conclusion, information gathered from docking simulation analysis for **8i** and **8e**, were in accordance with the IC_{50} values obtained from the cholinesterase inhibition assay. In general, better accommodation of inhibitor inside the active site gorge due to more appropriate interactions with its amino acid residues, ensue better inhibitory activities and lower IC_{50} values during in vitro assay.

3. Conclusion

A series of piperidone-grafted novel mono- and bisspiropyrrolizines has been synthesized by the three-component [3+2]-cycloaddition reactions of azomethine ylides to highly functionalized dipolarophiles. This method employs simple, readily available starting materials and facile work up procedure without requiring column chromatographic purification. Mono-spiripyrrolizines **8i** and **8e**, synthesized in the present study are found to be significantly active in cholinesterase inhibitory assay with IC_{50} = 3.36 and 3.50 μM displayed respectively comparable potency to AChE and five times more potent than BuChE enzymes as compared to standard drug. The molecular modeling simulation for the most active compounds completely coincided with IC_{50} data obtained from in vitro assay. The synthesis and screening for biological activity of further series of spirocycloadducts derived from different cyclic and acyclic amino acids are currently under investigation in our research group.

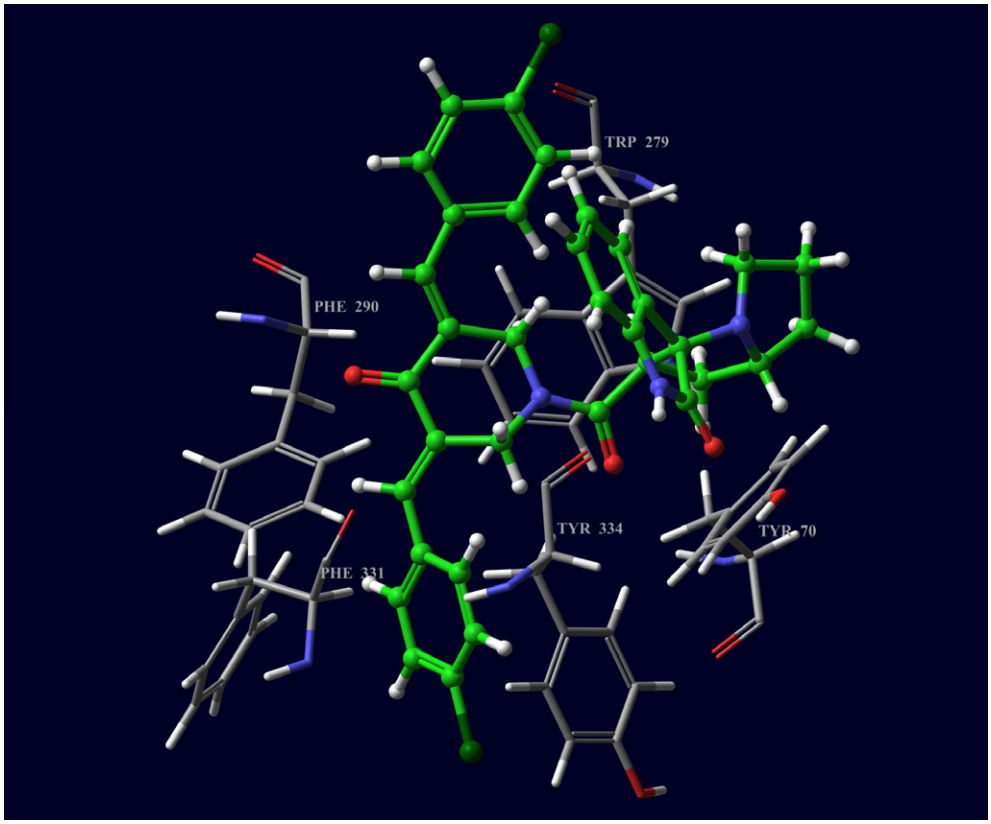


Figure 5. Orientations and interaction of **8i** with main residues in AChE receptor.

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 ^{1}H, ^{13}C and the 2D NMR spectra were recorded on a Bruker (Avance) 500 MHz NMR instrument using TMS as internal standard, DMSO-d_6 and $CDCl_3$ as solvents. Standard Bruker software was used throughout. 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 on a Perkin Elmer 2400 Series II Elemental CHNS analyzer.

4.2. General procedure for the synthesis of 5

To a mixture of 3,5-diarylidenepiperidin-4-ones (4.82 mmol) and K_2CO_3 (4.82 mmol) in acetone (10 ml) in an ice bath, acryloyl chloride (7.24 mmol) was added dropwise under stirring. The reaction was continued for 24 h. at ambient temperature. After completion of the reaction, by monitoring with TLC, the reaction mixture was poured into ice, the resulting precipitate was filtered off and washed with water to obtain pure **5** as yellow solid.

4.2.1. (3*E*,5*E*)-1-Acryloyl-3,5-bis(2-methylphenylmethylidene) piperidin-4-one (5b)

Yellow solid; (1.52 g, 89%); mp 100–102 °C; IR (KBr) $\nu_{max:}$ 3451, 1650, 1463 cm^{-1}; Anal. Calcd for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92. Found: C, 80.59; H, 6.43; N, 3.87. NMR (500 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 4.70–4.99 (m, 4H, CH_2), 5.50–5.61 (m, 1H, CH), 6.12–6.30 (m, 2H, CH_2), 7.16–7.32 (m, 8H, H-aromatic), 7.74 (s, 1H, H-arylmethylidene), 7.85 (s, 1H, H-arylmethylidene). ^{13}C NMR (125 MHz, $CDCl_3$): 21.83, 21.89, 48.57, 127.17, 129.14, 129.72, 130.00, 131.25, 134.69, 137.61, 140.45, 165.94, 187.05.

4.2.2. (3*E*,5*E*)-1-Acryloyl-3,5-bis(2-methoxyphenylmethylidene) piperidin-4-one (5c)

Yellow solid; (1.79 g, 91%); mp 166–168 °C; IR (KBr) $\nu_{max:}$ 3447, 1652, 1471 cm^{-1}; Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.11; H, 6.05; N, 3.69. NMR (500 MHz, $CDCl_3$): δ 3.8 9 (s, 6H, OCH_3), 4.90–5.08 (m, 4H, CH_2), 5.61–5.69 (m, 1H, CH), 6.09–6.18 (m, 1H, CH), 6.49–6.54 (m, 1H, CH), 7.05–7.54 (m, 8H, H-aromatic), 7.78 (s, 1H, H-arylmethylidene), 8.06 (s, 1H, H-arylmethylidene). ^{13}C NMR (125 MHz, $CDCl_3$): 47.06, 47.47, 55.45, 127.63, 128.22, 128.54, 130.58, 132.88, 133.01, 135.56, 161.03, 186.47.

4.2.3. (3*E*,5*E*)-1-Acryloyl-3,5-bis(2-fluorophenylmethylidene) piperidin-4-one (5e)

Yellow solid; (1.59 g, 81%); mp 120–122 °C; IR (KBr) $\nu_{max:}$ 3444, 1651, 1463 cm^{-1}; Anal. Calcd for $C_{22}H_{17}F_2NO_2$: C, 72.32; H, 4.69; N, 3.83. Found: C, 72.31; H, 4.67; N, 3.81. NMR (500 MHz, $CDCl_3$): δ 4.67−4.91 (m, 4H, CH_2), 5.52–5.61 (m, 1H, CH), 6.12–6.31 (m, 2H, CH_2), 7.09–7.48 (m, 8H, H-aromatic), 7.87 (s, 1H, H-arylmethylidene), 7.91 (s, 1H, H-arylmethylidene). ^{13}C NMR (125 MHz, $CDCl_3$): 48.39, 124.34, 124.39, 124.71, 124.76, 126.94, 129.24, 131.13, 131.16, 131.28, 131.32, 131.45, 131.89, 165.93, 186.30.

4.2.4. (3*E*,5*E*)-1-Acryloyl-3,5-bis(3-nitrophenylmethylidene) piperidin-4-one (5f)

Yellow solid; (1.42 g, 79%); mp 147–149 °C; IR (KBr) $\nu_{max:}$ 3449, 1654, 1470 cm^{-1}; Anal. Calcd for $C_{22}H_{17}N_3O_6$: C, 63.01; H, 4.09; N, 10.02. Found: C, 63.09; H, 4.14; N, 10.07. ^{1}H NMR (500 MHz, $CDCl_3$):

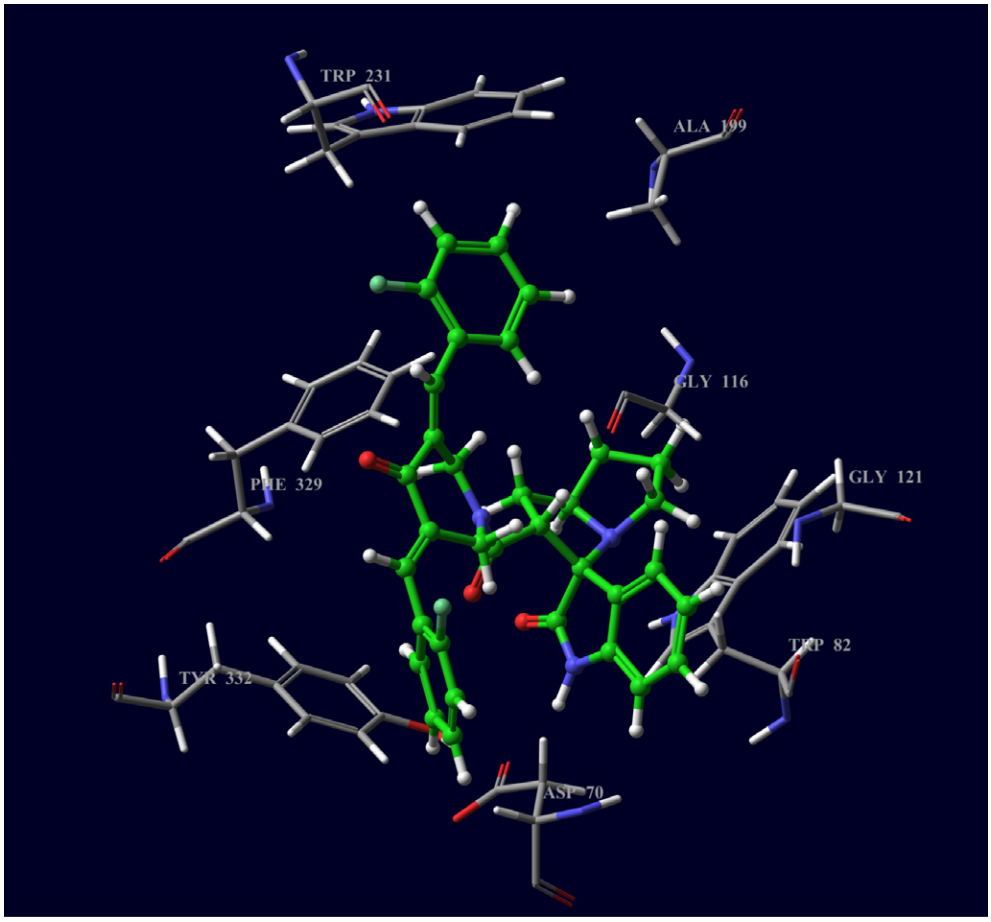


Figure 6. Orientations and interaction of **8e** with main residues in BuChE receptor.

δ 4.98–5.25 (m, 4H, CH_2), 5.57–5.64 (m, 1H, CH), 6.04–6.12 (m, 1H, CH), 6.41–6.53 (m, 1H, CH), 7.76–8.34 (m, 10H, H-aromatic, H-arylmethylidene). ^{13}C NMR (125 MHz, $CDCl_3$): 39.32, 124.25, 124.95, 127.31, 128.88, 130.55, 134.62, 136.53, 143.93, 166.52, 186.00.

4.3. General procedure for the synthesis of (8) and (9)

A mixture of 1-acryloyl-3,5-diarylidenepiperidin-4-ones (**5**, 0.364 mmol), isatin (**6**, 0.364 mmol) and L-proline (**7**, 0.364 mmol) was dissolved in methanol (5 mL) and heated under reflux for 5 h. The reaction progress was monitored by TLC analysis. After completion of the reaction, the precipitate obtained was filtered and washed with cold methanol (25 ml) to afford the monospiropyrrolizines (**8**) as white solid. The same reaction employing (**5**, 0.364 mmol), (**6**, 0.728 mmol) and (**7**, 0.728 mmol) in refluxing methanol (5 ml) for 15 h afforded the bisspiropyrrolizines (**9**) as white solid. The purity of both (**8**) and (**9**) were checked using TLC and ^{1}H NMR techniques.

4.3.1. 1″-Carbonyl (spiro[2.3′]oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis[(*E*)-phenylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8a)

White solid; (0.134 g, 83%); mp 228–230 °C; IR (KBr) $\nu_{max:}$ 3432, 1719, 1611 cm^{-1}; Anal. Calcd for $C_{34}H_{31}N_3O_3$: C, 77.10; H, 5.90; N, 7.93. Found: C, 77.02; H, 5.95; N, 7.97. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.56–1.69 (m, 1H, H-5), 1.76–1.94 (m, 2H, H-6), 2.00–2.10 (m, 1H, H-5), 2.20–2.26 (m, 1H, H-4), 2.43–2.56 (m, 2H, H-4, H-7), 2.56–2.67 (m, 1H, H-7), 3.96 (td, J = 11.66, 7.09 Hz, 1H, H-3), 4.01–4.12 (m, 1H, H-4a), 4.24–4.37 (m, 1H, H-6″), 4.43–4.57 (m, 1H, H-2″), 4.57–4.66 (m, 2H, H-2″, H-6″), 7.03–7.40 (m, 14H, H-aromatic), 7.53 (s, 1H, H-phenylmethylidene), 7.56 (s, 1H, H-phenylmethylidene), 8.70 (s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): 27.75, 31.73, 35.26, 42.30, 46.54, 47.74, 51.20, 64.28, 77.38, 110.29, 115.95, 116.24, 122.15, 125.49, 127.64, 128.96, 129.21, 131.35, 131.88, 132.65, 132.77, 136.68, 140.74, 164.10, 180.83, 184.20.

4.3.2. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis[(*E*)-2-methylpheylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8b)

White solid; (0.125 g, 74%); mp 160–162 °C; IR (KBr) $\nu_{max:}$ 3439, 1729, 1615 cm$^{-1;}$ Anal. Calcd for $C_{36}H_{35}N_3O_3$: C, 77.53; H, 6.33; N, 7.53. Found: C, 77.41; H, 6.39; N, 7.56. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.59–1.68 (m, 1H, H-5), 1.79–1.98 (m, 2H, H-6), 1.83–2.10 (m, 1H, H-5), 2.12–2.26 (m, 1H, H-4), 2.28 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.42–2.56 (m, 2H, H- 4, H-7), 2.59–2.73 (m, 1H, H-7), 3.94–3.98 (m, 1H, H-3), 3.99–4.10 (m, 1H, H-4a), 4.34–4.56 (m, 3H, H-2″, H-6″), 4.71 (d, J = 16.23 Hz, 1H, H-6″), 6.79–7.38 (m, 12H, H-aromatic), 7.51–7.65 (m, 2H, H-arylmethylidene), 8.71 (br s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.55, 27.51, 31.47, 35.41, 42.78, 46.62, 47.71, 51.07, 64.16, 73.31, 110.29, 122.03, 125.67, 127.59, 129.47, 130.41, 130.82, 131.79, 137.48, 137.69, 140.21, 141.11, 167.02, 181.05, 186.62.

4.3.3. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis[(*E*)-2-methoxy phenylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8c)

White solid; (0.127 g, 77%); mp 252–254 °C; IR (KBr) $\nu_{max:}$ 3442, 1719, 1614 cm^{-1}; Anal. Calcd for $C_{36}H_{35}N_3O_5$: C, 73.33; H, 5.98; N,

7.13. Found: C, 73.31; H, 5.62; N, 7.24. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.59–1.68 (m, 1H, H-5), 1.76–1.84 (m, 1H, H-6), 1.84–1.92 (m, 1H, H-6), 2.01–2.09 (m, 1H, H-5), 2.23–2.35 (m, 1H, H-4), 2.43–2.54 (m, 2H, H-7, H-4), 2.63–2.71 (m, 1H, H-7), 3.71 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.96 (dd, J = 10.48, 7.33 Hz, 1H, H-3), 4.03–4.10 (m, 1H, H-4a), 4.32 (d, J = 16.55 Hz, 1H, H-6″), 4.43 (d, J = 15.76 Hz, 1H, H-2″), 4.53 (d, J = 15.76 Hz, 1H, H-2″), 4.65 (d, J = 16.39 Hz, 1H, H-6″), 6.80–7.35 (m, 12H, H-aromatic), 7.53 (s, 1H, H-arylmethylidene), 7.57 (s, 1H, H-arylmethylidene), 8.71 (br s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.36, 31.37, 35.37, 42.66, 46.62, 47.81, 50.94, 55.33, 64.13, 73.29, 110.27, 114.21, 114.48, 122.01, 125.68, 127.08, 127.28, 127.58, 128.86, 129.34, 129.40, 132.27, 132.75, 136.98, 137.29, 141.04, 160.52, 160.73, 169.07, 180.77, 186.44.

4.3.4. 1″-Carbonyl (spiro[2.3′]oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis[(*E*)-2-chlorophenylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8d)

White solid; (0.132 g, 78%); mp 248–250 °C; IR (KBr) $\nu_{max:}$ 3442, 1716, 1621 cm^{-1}; Anal. Calcd for $C_{34}H_{29}Cl_2N_3O_3$: C, 68.23; H, 4.88; N, 7.02. Found: C, 68.29; H, 4.83; N, 7.06. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.48–1.67 (m, 2H, H-5, H-6), 1.71–1.82 (m, 1H, H-6), 1.87–1.96 (m, 1H, H-5), 2.00–2.10 (m, 1H, H-4), 2.17–2.26 (m, 2H, H-4, H-7), 2.52–2.57 (m, 1H, H-7), 3.60 (t, J = 8.67 Hz, 1H, H-3), 3.73–3.83 (m, 1H, H-4a), 4.09 (d, J = 16.87 Hz, 1H, H-6″), 4.27 (d, J = 15.61 Hz, 1H, H-2″), 4.49 (d, J = 15.61 Hz, 1H, H-2″), 4.59 (d, J = 16.87 Hz, 1H, H-6″), 6.74–7.70 (m, 12H, H-aromatic), 7.70–7.75 (m, 2H, H-arylmethylidene), 10.17 (s, 1H, N–H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 26.50, 30.351, 48.56, 71.99, 109.69, 126.36, 126.92, 127.03, 127.32, 127.56, 128.79, 129.73, 129.92, 130.44, 130.86, 130.96, 131.73, 131.81, 132.28, 133.20, 133.85, 134.18, 142.11, 170.12, 181.15, 188.60.

4.3.5. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis [(*E*)-2-fluoro phenylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8e)

White solid; (0.115 g, 73%); mp 232–234 °C; IR (KBr) $\nu_{max:}$ 3431, 1717, 1614 cm^{-1}; Anal. Calcd for $C_{34}H_{29}F_2N_3O_3$: C, 72.20; H, 5.17; N, 7.43. Found: C, 72.27; H, 5.11; N, 7.40. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.56–1.67 (m, 1H, H-5), 1.83–1.93 (m, 2H, H-6), 2.00–2.08 (m, 1H, H-4), 2.39–2.53 (m, 2H, H-4, H-7), 2.65–2.79 (m, 1H, H-7), 3.84–3.95 (m, 1H, H-3), 3.98–4.10 (m, 2H, H-4a, H-6″), 4.42 (d, J = 15.25 Hz, 1H, H-2″), 4.52 (d, J = 15.25 Hz, 1H, H-2″), 4.82 (d, J = 15.61 Hz, 1H, H-6″), 6.73–7.45 (m, 12H, H-aromatic), 7.56 (s, 1H, N–H), 7.69 (s, 1H, H-arylmethylidene), 7.73 (s, 1H, H-arylmethylidene). ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.26, 31.25, 35.30, 42.77, 46.62, 47.83, 50.98, 64.06, 73.07, 110.17, 115.79, 115.96, 116.14, 122.17, 122.40, 122.46, 124.20, 124.58, 125.27, 127.56, 127.69, 129.56, 129.66, 130.54, 130.70, 131.25, 131.32, 131.78, 131.82, 132.55, 132.94, 140.41, 159.93, 161.92, 168.92, 180.06, 185.86.

4.3.6. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis [(*E*)-3-nitro phenylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8f)

White solid; (0.125 g, 81%); mp 200–202 °C; IR (KBr) $\nu_{max:}$ 3442, 1721, 1615 cm^{-1}; Anal. Calcd for $C_{34}H_{29}N_5O_7$: C, 65.91; H, 4.72; N, 11.30. Found: C, 65.85; H, 4.77; N, 11.38. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.59–1.69 (m, 1H, H-5), 1.81–1.97 (m, 2H, H-6), 2.05–2.14 (m, 1H, H-5), 2.24–2.31 (m, 1H. H-4), 2.42–2.57 (m, 2H, H-4, H-7), 2.58–2.64 (m, 1H, H-7), 4.02 (dd, J = 10.92, 7.12 Hz, 1H, H-3), 4.05–4.16 (m, 1H, H-4a), 4.29 (d, J = 15.76 Hz, 1H, H-6″), 4.52–4.69 (m, 3H, H-2″, H-6″), 6.78–7.28 (m, 12H, H-aromatic), 7.57 (s, 2H, H-arylmethylidene), 8.71 (br s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): 27.82, 31.82, 35.28, 42.33, 46.72, 47.68, 51.39, 64.32, 73.31, 110.14, 122.31, 125.59, 127.77, 129.02, 129.30, 129.53, 131.38, 131.47, 131.54, 131.69, 132.11, 132.86, 1323.11, 135.89, 136.25, 136.42, 137.09, 140.51, 168.92, 180.49, 186.71.

4.3.7. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis[(*E*)-2,4-dichloro phenylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8g)

White solid; (0.114 g, 79%); mp 210–212 °C; IR (KBr) $\nu_{max:}$ 3434, 1701, 1642 cm^{-1}; Anal. Calcd for $C_{34}H_{27}C_{l4}N_3O_3$: C, 61.19; H, 4.08; N, 6.30. Found: C, 61.11; H, 4.02; N, 6.37. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53–1.64 (m, 1H, H-5), 1.80–1.95 (m, 2H, H-6), 2.00–2.11 (m, 1H, H-5), 2.17–2.26 (m, 1H, H-4), 2.41–2.49 (m, 1H, H-7), 2.49–2.57 (m, 2H, H-4, H-7), 3.76 (d, J = 15.61 Hz, 1H, H-3), 3.93 (d, J = 15.61 Hz, 1H, H-6″), 3.98–4.07 (m, 1H, H-4a), 4.34 (d, J = 17.18 Hz, 1H, H-2″), 4.75 (d, J = 17.18 Hz, 1H, H-2″), 4.86 (d, J = 15.61 Hz, 1H, H-6″), 6.75–7.51 (m, 10H, H-aromatic), 7.66 (s, 1H, H-arylmethylidene), 7.74 (br s, 2H, H-arylmethylidene and N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.18, 32.08, 35.15, 42.04, 46.44, 47.51, 51.84, 64.45, 73.40, 110.24, 122.19, 125.28, 127.23, 127.62, 127.74, 129.64, 129.69, 129.97, 130.99, 131.15, 131.17, 132.37, 133.28, 133.57, 134.26, 135.59, 135.68, 135.74, 136.13, 140.09, 168.16, 180.44, 186.28.

4.3.8. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis[(*E*)-4-methylpheylmethylidene] tetrahydro-4″(1H)-pyridinones (8h)

White solid; (0.112 g, 76%); mp 233–235 °C; IR (KBr) $\nu_{max:}$ 3447, 1714, 1614 cm$^{-1;}$ Anal. Calcd for $C_{36}H_{35}N_3O_3$: C, 77.53; H, 6.33; N, 7.53. Found: C, 77.38; H, 6.41; N, 7.59. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.56–1.65 (m, 1H, H-5), 1.74–1.92 (m, 2H, H-6), 1.80–2.08 (m, 1H, H-5), 2.10–2.24 (m, 1H, H-4), 2.27 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.39–2.54 (m, 2H, H-4, H-7), 2.56–2.69 (m, 1H, H-7), 3.89–3.94 (m, 1H, H-3), 3.97–4.07 (m, 1H, H-4a), 4.33–4.55 (m, 3H, H-2″, H-6″), 4.69 (d, J = 16.08 Hz, 1H, H-6″), 6.75–7.34 (m, 12H, H-aromatic), 7.53–7.63 (m, 2H, H-arylmethylidene), 8.72 (br s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.45, 27.45, 31.43, 35.37, 42.74, 46.62, 47.73, 51.04, 64.14, 73.31, 110.37, 122.04, 125.66, 127.56, 129.41, 130.39, 130.79, 131.76, 137.44, 137.63, 140.22, 140.97, 166.92, 180.84, 186.55.

4.3.9. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis[(*E*)-4-chloro phenylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8i)

White solid; (0.117 g, 80%); mp 166–168 °C; IR (KBr) $\nu_{max:}$ 3442, 1729, 1616 cm^{-1}; Anal. Calcd for $C_{34}H_{29}Cl_2N_3O_3$: C, 68.23; H, 4.88; N, 7.02. Found: C, 68.28; H, 4.85; N, 7.07. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.55–1.68 (m, 1H, H-5), 1.76–1.93 (m, 2H, H-6), 2.00–2.10 (m, 1H, H-5), 2.20–2.28 (m, 1H. H-4), 2.41–2.55 (m, 2H, H-4, H-7), 2.55–2.62 (m, 1H, H-7), 3.97 (dd, J = 10.80, 7.01 Hz, 1H, H-3), 4.00–4.10 (m, 1H, H-4a), 4.25 (d, J = 15.76 Hz, 1H, H-6″), 4.51–4.67 (m, 3H, H-2″, H-6″), 6.76–7.39 (m, 12H, H-aromatic), 7.53 (s, 2H, H-arylmethylidene), 8.62 (br s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): 27.80, 31.77,35.26, 42.35, 46.69, 47.64, 51.38, 64.28, 73.30, 110.11, 122.25, 125.39, 127.73, 128.99, 129.30, 129.46, 131.31, 131.41, 131.55, 131.72, 131.83, 132.72, 132.78, 135.46, 136.01, 136.10, 136.72, 140.32, 168.59, 180.41, 186.62.

4.3.10. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis [(*E*)-4-fluoro phenylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8j)

White solid; (0.129 g, 78%); mp 230–232 °C; IR (KBr) $\nu_{max:}$ 3442, 1729, 1610 cm^{-1}; Anal. Calcd for $C_{34}H_{29}F_2N_3O_3$: C, 72.20; H, 5.17; N, 7.43. Found: C, 72.29; H, 5.14; N, 7.38. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.56–1.68 (m, 1H, H-5), 1.72–1.80 (m, 1H, H-6), 1.80–1.95 (m, 2H, H-5, H-6), 1.95–2.05 (m, 1H, H-4), 2.13–2.20 (m, 1H, H-7), 2.33–2.40 (m, 1H, H-4), 2.42–2.56 (m, 1H, H-7), 3.95 (dd, J = 10.96,

7.01 Hz, 1H, H-4a), 4.00–4.10 (m, 1H, H-6″), 4.53 (d, *J* = 17.25 Hz, 1H, H-2″), 4.67 (d, *J* = 17.25 Hz, 1H, H-2″), 5.05 (d, *J* = 15.76 Hz, 1H, H-6″), 6.71–7.45 (m, 12H, H-aromatic), 7.56 (s, 1H, H-arylmethylidene), 7.63 (s, 1H, H-arylmethylidene), 8.05–8.12 (br s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.69, 31.67, 35.29, 45.59, 47.68, 51.26, 64.23, 73.34, 110.15, 115.78, 115.96, 116.28, 122.16, 125.48, 127.66, 129.43, 130.54, 130.78, 130.96, 132.11, 132.18, 132.65, 132.71, 136.24, 136.78, 140.54, 162.14, 162.29, 164.13, 164.30, 168.74, 180.59, 186.72.

4.3.11. 1″-Carbonyl (spiro[2.3']oxindole-hexahydro-1*H*-pyrrolizine)-3″,5″-bis[(*E*)-naphthylmethylidene] tetrahydro-4″(1*H*)-pyridinones (8k)

White solid; (0.154 g, 84%); mp 210–212 °C; IR (KBr) $\nu_{max:}$ 3431, 1716, 1617 cm^{-1}; Anal. Calcd for $C_{42}H_{35}N_3O_3$: C, 80.10; H, 5.60; N, 6.67. Found: C, 80.02; H, 5.53; N, 6.60. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.51–1.61 (m, 1H, H-5), 1.73–1.81 (m, 1H, H-6), 1.81–1.91 (m, 1H, H-6), 1.91–2.03 (m, 1H, H-5), 2.09 (m, 1H, H-4), 2.30–2.40 (m, 1H, H-7), 2.43 (m, 1H, H-4), 2.61 (m, 1H, H-7), 3.66–3.75 (m, 1H, H-3), 3.89–4.02 (m, 1H, H-4a), 4.30 (d, *J* = 15.92 Hz, 1H, H-6″), 4.36–4.45 (m, 2 H, H-2″), 4.60 (d, *J* = 15.92 Hz, 1H, H-6″), 6.60–8.00 (m, 21H, H-aromatic and N–H), 8.31 (s, 1H, H-arylmethylidene), 8.34 (s, 1H, H-arylmethylidene). ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.44, 27.18, 31.04, 35.12, 42.70, 46.31, 47.76, 51.13, 58.48, 63.96, 72.90, 109.93, 122.02, 124.32, 124.49, 125.31, 125.56, 125.64, 126.42, 126.60, 126.88, 127.32, 127.45, 127.56, 128.70, 129.00, 129.43, 129.82, 130.18, 131.19, 131.28, 131.77, 131.94, 132.64, 133.55, 133.62, 136.06, 136.12, 140.45, 159.38, 168.94, 179.84, 187.05.

4.3.12. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(phenylmethylidene) tetrahydro-4″-(1*H*)-pyridinone-4-(phenylmethylidene) hexahydro-1*H*-pyrrolizine (9a)

White solid; (0.110 g, 72%); mp 160–162 °C; IR (KBr) $\nu_{max:}$ 3442, 1712, 1616 cm^{-1}; Anal. Calcd for $C_{46}H_{43}N_5O_4$: C, 75.70; H, 5.94; N, 9.60. Found: C, 75.65; H, 5.98; N, 9.64.^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (d, *J* = 14.15 Hz, 1H, H-2″), 1.68–1.95 (m, 4H, H-6 of ring A, H-6 of ring B, H-5 of ring B), 2.00–2.60 (m, 6H, H-5 of ring A, H-6 of ring B, H-5 of ring B, H-4 of ring A), 2.84 (d, *J* = 16.71 Hz, 1H, H-6″), 2.90–3.00 (m, 1H, H-7 of ring A), 3.38–3.42 (m, 1H, H-7 of ring B), 3.72 (d, *J* = 16.87 Hz, 1H, H-6″), 3.95 (d, *J* = 14.19 Hz, 1H, H-2″), 4.00–4.10 (m, 1H, H-3 of ring A), 4.22–4.32 (m, 1H, H-7 of ring B), 4.40–4.45 (m, 1H, H-3 of ring A), 4.60–4.70 (m, 2H, H-4, H-4a of ring B), 5.93–7.45(m, 18H, H-aromatic), 7.58 (s, 1H, H-phenylmethylidene), 8.60 (s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.25, 25.91, 28.30, 29.38, 34.38, 43.95, 45.23, 47.94, 48.53, 51.64, 53.04, 64.31, 65.95, 68.05, 71.86, 72.67, 109.38, 110.71, 121.34, 122.27, 125.22, 126.14, 126.75, 126.92, 128.53, 128.84, 129.21, 129.53, 129.82, 130.21, 130.49, 130.68, 134.43, 137.96, 140.29, 141.23, 142.41, 173.09, 177.91, 179.97, 198.71.

4.3.13. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(2-methylphenylmethylidene)tetrahydro-4″-(1*H*)-pyridinone-4-(2-methylphenylmethylidene) hexahydro-1*H*-pyrrolizine (9b)

White solid; (0.109 g, 61%); mp 158–160 °C; IR (KBr) $\nu_{max:}$ 3432, 1714, 1619 cm^{-1}; Anal. Calcd for $C_{48}H_{47}N_5O_4$: C, 76.07; H, 6.25; N, 9.24. Found: C, 76.17; H, 6.06; N, 9.14. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.02–1.12 (m, 1H, H-2″), 1.35–1.70 (m, 4H, H-6 of ring A, H-6 of ring B, H-5 of ring B), 1.80–2.7 (m, 16H, H-6″, H-7 of ring B, H-6 of ring B, H-5 of ring B, H-4a of ring A, H-5 of ring A, H-7 of ring A, 2 × CH_3), 3.40–3.55 (m, 2H, H-2″, H-6″), 3.60–4.25 (m, 3H, H-7 of ring B, H-3 of ring A, H-4a of ring A), 3.66–4.99 (m, 2H, H-4a of ring B, H-4 of ring B), 5.76–7.96 (m, 15H, H-aromatic, H-arylmethylidene, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.98, 26.05, 30.74, 31.99, 44.67, 45.07, 45.23, 45.50, 45.89, 53.75, 54.15, 64.33, 66.24, 68.58, 113.56, 114.23, 126.12, 126.55, 132.71, 132.87, 133.84, 133.97, 134.15, 134.23, 135.49, 135.76, 135.91, 135.99, 141.73, 142.25, 147.67, 147.84, 177.78, 184.06, 184.22, 204.19.

4.3.14. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(2-methoxyphenylmethylidene) tetrahydro-4″-(1*H*)-pyridinone-4-(2-methoxyphenylmethylidene) hexahydro-1*H*-pyrrolizine (9c)

White solid; (0.131 g, 65%); mp 216–218 °C; IR (KBr) $\nu_{max:}$ 3430, 1717, 1612 cm^{-1}; Anal. Calcd for $C_{48}H_{47}N_5O_6$: C, 72.98; H, 6.00; N, 8.87. Found: C, 72.91; H, 5.92; N, 8.80. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.75–0.88 (m, 1H, H-2″), 1.10–1.90 (m, 4H, H-6 of ring A, H-6 of ring B, H-5 of ring B), 1.87–2.66 (m, 7H, H-5 of ring A, H-6 of ring B, H-5 of ring B, H-4 of ring A, H-6″), 2.78–3.74 (m, 8H, H-7 of ring A, H-7 of ring B, H-2″, H-6″, H-3 of ring A, H-4a of ring A), 3.84 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 4.96–4.99 (m, 1H, H-4a of ring B), 6.50–7.40 (m, 18H, H-aromatic, N–H), 7.58 (s, 1H, H-arylmethylidene). ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.41, 27.83, 31.37, 31.92 35.27, 42.61, 43.26, 46.52, 46.81, 47.73, 50.94, 55.33, 64.15, 66.29, 72.12, 73.29, 110.27, 110.52, 114.23, 114.57, 122.09, 125.74, 127.12, 127.31, 127.58, 128.77, 129.37, 129.44, 132.27, 132.78, 137.01, 137.35, 141.08, 160.50, 160.81, 169.07, 179.21, 180.77, 186.44.

4.3.15. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(2-chlorophenylmethylidene)tetrahydro-4″-(1*H*)-pyridinone-4-(2-chlorophenylmethylidene) hexahydro-1*H*-pyrrolizine (9d)

White solid; (0.117 g, 53%); mp 150–152 °C; IR (KBr) $\nu_{max:}$ 3428, 1721, 1611 cm^{-1}, Anal. Calcd for $C_{46}H_{41}Cl_2N_5O_4$: C, 69.17; H, 5.17; N, 8.77. Found: C, 69.10; H, 5.18; N, 8.75. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (d, *J* = 13.60 Hz, 1H, H-2″), 1.60–1.90 (m, 4H, H-6 of ring A, H-6 of ring B, H-5 of ring B), 1.95–2.05 (m, 1H, H-5 of ring A), 2.10–2.40 (m, 5H, H-6 of ring B, H-5 of ring B, H-4 of ring A, H-5 of ring A), 2.42–2.53 (m, 1H, H-6″), 2.60–2.80 (m, 2H, H-7 of ring A, H-7 of ring B), 3.35 (d, *J* = 14 Hz, H-6″), 3.51–3.64 (m, 2H, H-2″, H-7 of ring B), 3.80–3.90 (m, 1H, H-3 of ring A), 4.01–4.09 (m, 1H, H-4a of ring A), 4.90–5.00 (m, 1H, H-4a of ring B), 5.82–7.50 (m, 12H, H-aromatic), 7.65 (s, 1H, H-arylmethylidene), 8.07 (br s, 2H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.58, 25.76, 29.77, 29.91, 34.22, 43.80, 45.34, 47.50, 48.49, 50.01, 52.66, 64.11, 66.55, 67.58, 72.13, 109.44, 110.91, 120.71, 121.76, 126.38, 126.81, 127.98, 128.20, 129.50, 129.63, 129.72, 130.04, 130.58, 131.10, 131.46, 132.90, 133.29, 136.36, 136.44, 141.39, 143.00, 172.01, 178.50, 179.65, 201.95.

4.3.16. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(2-fluorophenylmethylidene)tetrahydro-4″-(1*H*)-pyridinone-4-(2-fluorobenzylidene) hexahydro-1*H*-pyrrolizine (9e)

White solid; (0.110 g, 53%); mp 158–160 °C; IR (KBr) $\nu_{max:}$ 3431, 1715, 1612 cm^{-1}; Anal. Calcd for $C_{46}H_{41}F_2N_5O_4$: C, 72.14; H, 5.40; N, 9.14. Found: C, 72.11; H, 5.45; N, 9.17. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33–1.42 (m, 1H, H-2″), 1.67–2.24 (m, 5H, H-6 of ring A, H-6 of ring B, H-5 of ring A, H-5 of ring B), 2.41–2.96 (m, 9H, H-6″, H-7 of ring B, H-6 of ring B, H-5 of ring B, H-4 of ring A, H-5 of ring A, H-7 of ring A), 2.98–3.32 (m, 3H, H-2″, H-6″, H-7 of ring B), 3.82–3.94 (m, 1H, H-3 of ring A), 4.22–4.33 (m, 1H, H-4a of ring A), 4.51–4.74 (m, 2H, H-4a of ring B, H-4 of ring B), 5.05–7.15 (m, 16H, H-aromatic), 7.39 (s, 1H, H-arylmethylidene), 7.31–7.47 (br s, 2H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.31, 25.92, 27.56, 28.09, 35.89, 46.51, 47.23, 47.69, 57.35, 58.04, 59.76, 61.29, 63.84, 71.44, 73.67, 73.34, 75.05, 110.10,

110.58, 115.27, 115.39, 115.59, 115.67, 122.39, 123.19, 125.64, 128.19, 129.28, 129.82, 132.58, 132.74, 132.88, 132.92, 142.28, 172.96, 180.17, 180.59, 211.21.

4.3.17. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(3-nitrophenylmethylidene)tetrahydro-4″-(1*H*)-pyridinone-4-(3-nitrophenylmethylidene) hexahydro-1*H*-pyrrolizine (9f)

White solid; (0.108 g, 59%); mp 160–162 °C; IR (KBr) $\nu_{max:}$ 3434, 1715, 1612 cm^{-1}; Anal. Calcd for $C_{46}H_{41}N_7O_8$: C, 67.39; H, 5.04; N, 11.96. Found: C, 67.45; H, 5.09; N, 12.02. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.67–0.73 (m, 1H, H-2″), 1.45–2.25 (m, 4H, H-6 of ring B, H-5 of ring B, H-6 of ring A), 2.26–2.77 (m, 10H, H-6 of ring B, H-5 of ring B, H-7 of ring B, H-6″, H-4 of ring A, H-5 of ring A, H-7 of ring A), 3.97–4.15 (m, 5H, H-2″, H-6″, H-7 of ring B, H-3 of ring A, H-4a of ring A), 4.50–5.17 (m, 5H, H-4a of ring B, H-4 of ring B), 6.02–7.73 (m, 16H, H-aromatic), 7.87 (s, 1H, H-arylmethylidene), 8.11 (s, 1H, N–H), 8.19 (s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.10, 29.25, 30.23, 30.64, 31.52, 39.45, 61.42, 63.36, 64.88, 67.20, 74.86, 76.63, 76.75, 113.16, 113.95, 125.96, 126.30, 126.48, 129.08, 129.49, 129.64, 129.90, 131.56, 133.14, 133.54, 139.35, 139.51, 140.15, 140.37, 145.37, 145.64, 145.73, 151.43, 151.51, 176.36, 183.11, 184.01, 209.05.

4.3.18. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(2,4-dichlorophenylmethylidene) tetrahydro-4″-(1H)-pyridinone-4-(2,4-dichloro phenylmethylidene) hexahydro-1*H*-pyrrolizine (9g)

White solid; (0.132 g, 68%); mp 140–142 °C; IR (KBr) $\nu_{max:}$ 3429, 1717, 1611 cm^{-1}, Anal. Calcd for $C_{46}H_{39}C_{l4}N_5O_4$: C, 63.68; H, 4.53; N, 8.07. Found: C, 63.61; H, 4.54; N, 8.11. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03–1.12 (m, 1H, H-2″), 1.26–2.12 (m, 5H, H-6 of ring A, H-6 of ring B, H-5 of ring B, H-5 of ring A), 2.22–3.23 (m, 9H, H-6″, H-7 of ring B, H-6 of ring B, H-5 of ring B, H-4 of ring A, H-5 of ring A, H-7 of ring A), 3.27–3.86 (m, 4H, H-6″, H-2″, H-7 of ring B, H-3 of ring A), 3.93–4.08 (m, 1H, H-4a of ring A), 4.10–4.81 (m, 2H, H-4 of ring B, H-4a of ring B), 5.47–7.67 (m, 15H, H-aromatic, H-arylmethylidene), 7.76 (s, 1H, N–H), 8.03 (s, 1H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.32, 28.53, 32.41, 32.77, 35.15, 42.04, 43.09, 46.51, 46.84, 47.25, 49.11, 64.45, 66.24, 66.91, 71.15, 73.40, 110.11, 110.93, 122.24, 125.33, 127.42, 127.75, 127.89, 129.72, 129.83, 130.12, 131.25, 131.42, 131.48, 132.56, 133.43, 133.67, 134.35, 135.71, 135.92, 136.08, 136.31, 140.19, 168.15, 179.11, 180.51, 187.03.

4.3.19. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(4-methylphenylmethylidene)tetrahydro-4″-(1*H*)-pyridinone-4-(4-methylphenylmethylidene) hexahydro-1*H*-pyrrolizine (9h)

White solid; (0.124 g, 67%); mp 184–186 °C; IR (KBr) $\nu_{max:}$ 3434, 1712, 1613 cm^{-1}; Anal. Calcd for $C_{48}H_{47}N_5O_4$: C, 76.07; H, 6.25; N, 9.24. Found:, C, 76.16; H, 6.07; N, 9.11. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (d, J = 14.17 Hz, 1H, H-2″), 1.10–1.80 (m, 4H, H-6 of ring A, H-6 of ring B, H-5 of ring B), 1.83 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.10-2.70 (m, 7H, H-5 of ring A, H-6 of ring B, H-5 of ring B, H-4 of ring A, H-6″), 2.90–3.00 (m, 1H, H-7 of ring A), 3.30–3.45 (m, 1H, H-7 of ring B), 3.72 (d, J = 16.87 Hz, 1H, H-6″), 3.95 (d, J = 14.17 Hz, 1H, H-2″), 4.05–4.15 (m, 1H, H-3 of ring A), 4.20–4.30 (m, 1H, H-7 of ring B), 4.40–4.45 (m, 1H, H-4a of ring B), 4.50–4.60 (m, 1H, H-4 of ring B), 6.30–7.30 (m, 12H, H-aromatic), 9.32 (s, 1H, H-arylmethylidene), 9.56 (s, 1H, N–H), 9.66 (s, 1H, N–H).^{13}C NMR (125 MHz, $CDCl_3$): δ 26.02, 26.13, 30.63, 32.25, 44.65, 44.93, 45.21, 45.48, 45.76, 53.64, 54.05, 64.54, 66.18, 68.49, 114.86, 115.23, 126.36, 126.50, 132.79, 132.84, 133.76, 133.93, 134.07, 134.17, 135.49, 135.67, 135.91, 135.99, 141.88, 142.05, 147.52, 147.84, 177.78, 185.17, 185.55, 205.15.

4.3.20. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(4-chlorophenylmethylidene)tetrahydro-4″-(1*H*)-pyridinone-4-(4-chlorophenylmethylidene) hexahydro-1*H*-pyrrolizine (9i)

White solid; (0.124 g, 64%); mp 170–172 °C; IR (KBr) $\nu_{max:}$ 3434, 1721, 1611 cm^{-1}; Anal. Calcd for $C_{46}H_{41}Cl_2N_5O_4$: C, 69.17; H, 5.17; N, 8.77. Found: C, 69.13; H, 5.17; N, 8.79. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30–1.40 (m, 1H, H-2″), 1.70–2.30 (m, 5H, H-6 of ring A, H-6 of ring B, H-5 of ring B, H-5 of ring A), 2.35–3.00 (m, 9H, H-6″, H-7 of ring B, H-6 of ring B, H-5 of ring B, H-4 of ring A, H-5 of ring A, H-7 of ring A), 3.00–3.5 (m, 4H, H-2″, H-6″, H-7 of ring B, H-3 of ring A), 3.90–4.00 (m, 1H, H-4a of ring A), 4.55–4.65 (m, 1H, H-4 of ring B), 4.73–4.80 (m, 1H, H-4a of ring B), 5.10–7.52 (m, 14H, H-aromatic, H-arylmethylidene, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.69, 25.81, 27.55, 27.75, 30.00, 35.73, 46.30, 47.32, 47.68, 57.33, 58.11, 61.36, 61.87, 64.30, 71.42, 73.29, 74.68, 75.35, 110.01, 110.35, 121.54, 122.32, 125.60, 126.27, 126.91, 127.23, 128.17, 128.70, 129.45, 129.74, 129.85, 129.91, 130.41, 131.43, 132.53, 134.04, 142.18, 142.37, 172.82, 180.01, 180.87, 204.32.

4.3.21. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(4-fluorophenylmethylidene)tetrahydro-4″-(1*H*)-pyridinone-4-(4-fluorophenylmethylidene) hexahydro-1*H*-pyrrolizine (9j)

White solid; (0.118 g, 62%); mp 170–172 °C; IR (KBr) $\nu_{max:}$ 3430, 1716, 1616 cm^{-1}; Anal. Calcd for $C_{46}H_{41}F_2N_5O_4$: C, 72.14; H, 5.40; N, 9.14. Found: C, 72.10; H, 5.43; N, 9.11. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30–1.40 (m, 1H, H-2″), 1.65–2.20 (m, 5H, H-6 of ring A, H-6 of ring B, H-5 of ring B, H-5 of ring A), 2.35–2.91 (m, 9H, H-6″, H-7 of ring B, H-6 of ring B, H-5 of ring B, H-4 of ring A, H-5 of ring A, H-7 of ring A), 3.00–3.40 (m, 3H, H-2″, H-6″, H-7 of ring B), 3.80–3.90 (m, 1H, H-3 of ring A), 4.20–4.30 (m, 1H, H-4a of ring A), 4.52–4.72 (m, 2H, H-4a of ring B, H-4 of ring B), 5.05–7.12 (m, 16H, H-aromatic), 7.36 (s, 1H, H-arylmethylidene), 7.35–7.45 (br s, 2H, N–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.61, 25.89, 27.52, 27.71, 35.78, 46.30, 47.32, 47.61, 57.31, 57.97, 59.70, 61.24, 63.79, 71.35, 73.09, 73.34, 74.84, 110.09, 110.56, 115.27, 115.35, 115.55, 115.63, 122.32, 122.94, 125.63, 128.19, 129.21, 129.75, 132.58, 132.69, 132.82, 132.92, 142.20, 172.93, 180.11, 180.50, 212.38.

4.3.22. Spiro-[2.3']-oxindole-spiro[3.3″]-1″-carbonyl(spiro[2.3'] oxindole-hexahydro-1*H*-pyrrolizine)-5″-(naphthalen-1-ylphenylmethylidene)tetrahydro-4″-(1*H*)-pyridinone-4-(naphthalen-1-ylphenylmethyliden) hexahydro-1*H*-pyrrolizine (9k)

White solid; (0.142 g, 74%); mp 220–222 °C; IR (KBr) $\nu_{max:}$ 3428, 1718, 1619 cm^{-1}; Anal. Calcd for $C_{54}H_{47}N_5O_4$: C, 78.14; H, 5.71; N, 8.44. Found: C, 78.10; H, 5.65; N, 8.48. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80 (d, J = 13.87 Hz, 1H, H-2″), 1.54–1.65 (m, 1H, H-6 of ring A), 1.71 - 1.82 (m, 2H, H-5 of ring B, H-6 of ring A), 1.84–1.92 (m, 1H, H-6 of ring B), 2.00–2.09 (m, 1H, H-5 of ring A), 2.10–2.20 (m, 3H, H-5 of ring A, H-6 of ring B, H-5 of ring B), 2.25–2.40 (m, 2 H, H-4 of ring A), 2.48 (d, J = 16.71 Hz, 1H, H-6″), 2.67–2.75 (m, 2H, H-7 of ring A, H-7 of ring B), 3.41 (d, J = 16.87 Hz, 1H, H-6″), 3.63 (d, J = 13.87 Hz, 1H, H-2″), 3.70–3.78 (m, 1H, H-7 of ring B), 3.82 (t, J = 8.20 Hz, 1H, H-3 of ring A), 4.12–4.20 (m, 1H, H-4a of ring A), 5.05 (d, J = 9.62 Hz, 1H, H-4 of ring B), 5.24–5.32 (m, 1H, H-4a of ring B), 5.58–8.08 (m, 24H, H-aromatic, N–H), 8.11 (s, 1H, H-naphthylmethylidene). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.03, 24.24, 28.40, 28.66, 33.10, 43.47, 44.42, 47.45, 47.80, 48.81, 49.80, 63.34, 66.30, 67.05, 71.59, 73.52, 108.28, 110.33, 120.10, 121.63, 123.38, 123.86, 124.06, 124.09, 124.57, 125.01, 125.47, 126.06, 126.13, 126.24, 126.53, 127.05, 127.33, 127.69,

128.39, 128.63, 129.63, 129.84, 130.29, 131.19, 132.56, 132.90, 133.33, 133.49, 133.74, 138.24, 139.91, 142.26, 171.95, 177.13, 178.66, 201.64.

4.4. In vitro cholinesterase enzymes inhibitory activity

The test samples for cholinesterase enzymes inhibitory potential was evaluated using modified Ellman's method as described by Ahmed and Gilani.[34] Galantamine was used as positive control. Solutions of test samples and galantamine were prepared in DMSO at an initial concentration of 1 mg/mL (1000 ppm). The concentration of DMSO in final reaction mixture was 1%. At this concentration, DMSO has no inhibitory effect on both acetylcholi-nesterase and butyrylcholinesterase enzymes.[35]

For acetylcholinesterase (AChE) inhibitory assay, 140 μL of 0.1 M sodium phosphate buffer of pH 8 was first added to a 96-wells microplate followed by 20 μL of test samples and 20 μL of 0.09 units/mL acetylcholinesterase enzyme. 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. Thirty minutes after the initiation of enzymatic reaction, absorbance of the colored end-product was measured using BioTek PowerWave X 340 Microplate Spectrophotometer at 412 nm. For butyrylcholinesterase (BuChE) inhibitory assay, the same procedure described above was followed, except for the use of enzyme and substrate, instead of which, butyrylcholine esterase from equine serum and *S*-butyrylthiocholine chloride were used.

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

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

4.5. Molecular modeling study

To date, there are a total of 145 AChE and 29 BuChE co-crystal structures and NMR structures available in Protein Data Bank (PDB).[36]

Using Glide, (version 5.7, Schrödinger, LLC, New York, NY, 2011), compounds **8i** and **8e** were docked onto 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 to BuChE derived from complex of the enzyme with Tabun analogue (PDB code: 2WIJ).

Water molecules and hetero groups were deleted from receptor beyond the radius of 5 Å of reference ligand (E2020 or Tabun), 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 BuChE 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 bioactive compounds, handed in 5 poses per ligand, in which the best pose with highest score was displayed for each ligand.

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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.bmc.2013.01.066.

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