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PAPER

Heteropoly acid-catalyzed microwave-assisted three-component aza-Diels–Alder cyclizations: diastereoselective synthesis of potential drug candidates for Alzheimer's disease

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A highly diastereoselective microwave-assisted three component synthesis of azabicyclo[2.2.2]octan-5-ones by a silicotungstic acid-catalyzed aza-Diels–Alder cyclization is described. The one-pot process involves the formation of the *in situ* generated Schiff base and its immediate cyclization with cyclohex-2-enone. The short reaction times, good yields and excellent diastereoselectivity make this annulation a practical and environmentally attractive method for the synthesis of the target compounds. Preliminary assays were carried out to determine the activity of the products in AChE as well as in amyloid β fibrillogenesis inhibition.

Introduction

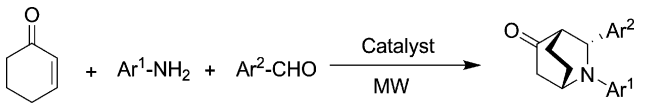
Nitrogen-containing heterocyclic compounds are of utmost importance in synthetic and medicinal chemistry, and materials science[1] as such compounds show widespread biological effects. They are, for example, orexin receptor antagonists,[2] monoamine neurotransmitter reuptake inhibitors,[3] nicotinic acetylcholine receptor ligands,[4] and human macrophage metalloelastase inhibitors.[5] The aza-Diels–Alder reaction is an important contributor to the synthesis of such compounds.[6] The use of imines as dienophile precursors with either preformed dienes or other diene substitutes in these reactions opened up new synthetic pathways for the synthesis of aza-bicyclooctane derivatives.[7]

In several recent studies, the preformed diene has been substituted with α,β-unsaturated ketones, cyclohexenone derivatives, in particular.[8] Due to the relatively low reactivity of both of these diene equivalents and the dienophile imines, the reaction is usually activated by acid catalysts.[8] These catalysts include several common Lewis and Brønsted acids, such as BF_3–OEt_2, $InCl_3$, transition metal carbonyls, lanthanide triflates, $BiCl_3$, as well as trifluoroacetic acid, *p*-TsOH, or triphenylphosphonium perchlorate.[9] Enantioselective alternatives of the aza-Diels–Alder reaction of imines with cyclohexenone derivatives have also been explored by the application of proline or BINOL-based chiral phosphoric acid esters.[10]

The available procedures are heterogeneous in terms of their suitability for applications in sustainable chemistry. While, except for a few, most procedures use a three component domino approach, the yields are mostly moderate. Although the more recent[8] approaches apply aqueous medium, and water-tolerant catalysts, the majority of the catalysts are not green and, in many cases, are moisture-sensitive.

Therefore, the development of new contemporary synthetic methods that comply with recent environmental standards is highly desirable.

Continuing our efforts in developing sustainable synthetic methods for organic synthesis, the above problems prompted us to conduct the aza-Diels–Alder reaction in a three-component one-pot domino design with catalysts that overcome the above obstacles. The process is illustrated in Scheme 1.



Scheme 1 Synthesis of azabicyclo[2.2.2]octan-5-ones by a microwave-assisted acid-catalyzed three component reaction.

The reaction was activated by microwave irradiation, a technique that was once peculiar but emerged as one of the most useful technical improvements in organic synthesis.[11] Aside from the broad agreement in the literature, recent applications of microwave-assisted reactions resulted in useful procedures for the synthesis of diverse organic scaffolds[12] and has prompted us to use microwaves as a choice of activation.

In the current study, the emphasis is placed on the sustainable synthesis of the target compounds. Due to the bioactivity of such core structures,[2–5] the products have been pre-evaluated in two Alzheimer's disease related *in vitro* assays and molecular docking has been used to validate the experimental data.

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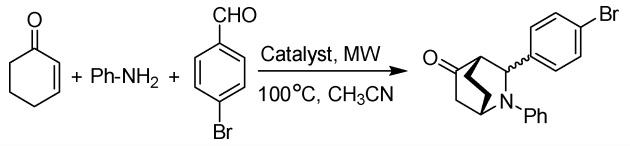
Table 1 Effect of catalysts on the yield and selectivity of the microwave-assisted test aza-Diels–Alder reaction[a]



Entry	Catalyst	Time (min)	Yield[b] (%)	*endo*/*exo*[c]
1	$H_3PW_{12}O_{40}$	5	62	67 : 33
2	$H_4SiMo_{12}O_{40}$	5	74	62 : 38
3	$H_4SiW_{12}O_{40}$	5	87	77 : 23
4	$H_3PMo_{12}O_{40}$	5	51	49 : 51
5	K-10[d]	20	8	79 : 21
6	Nafion-H[e]	5	traces	—

[a] Reaction conditions: 5 mol% catalyst loading, 2.5 mmol mL^{-1} aniline, 1.2 eq. aldehyde, 2 eq. ketone. [b] GC yields. [c] Determined by GC and NMR. [d] 200 mg catalyst. [e] 150 mg catalyst, in toluene.

Results and discussion

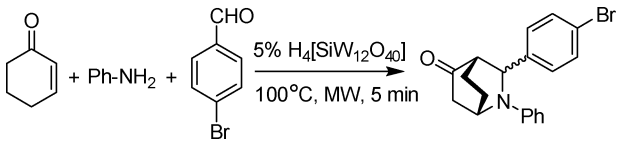
Synthesis of azabicyclo[2.2.2]octan-5-ones

As the Diels–Alder reactions of unsaturated carbonyl compounds can be catalyzed by common acid catalysts we have decided to explore several catalysts in the reaction. The focus was on testing catalysts that are considered to be environmentally sustainable, and chemically stable under the reaction conditions. Thus, we have chosen a group of heteropolyacids (HPAs),[13] K-10 montmorrillonite[14] and Nafion-H.[15] All of these materials/catalysts are commercially available and stable under usual organic reaction conditions. The reaction of aniline, cyclohexene-2-one and 4-bromobenzaldehyde was chosen to serve as a test reaction to find the best performing catalyst and to later determine the optimum reaction conditions (Table 1).

The data clearly show that while all heteropolyacids showed reasonable performance the traditionally excellent solid acids, K-10 montmorillonite and Nafion-H failed in the reaction. Although the heteropolyacids are soluble in the solvent, they remain stable and do not decompose under the experimental conditions. The formation of water in the condensation step, which rules out the use of many moisture sensitive Lewis acids in the domino style reaction, does not pose any problem as these acids do not undergo hydrolysis and can be regenerated from the product mixture.[16] Based on these preliminary data we have decided to select $H_4SiW_{12}O_{40}$ for further investigations. This catalyst provided the best yield and selectivity combination among the catalysts studied. As the next step in the optimization process, we tested the effect of reagent concentration and solvents on the yield and stereoselectivity of the above test reaction (Table 2).

The effects of the concentration and solvent (Table 2), do not appear to be as obvious as those of the catalyst (Table 1). The best yield was obtained in dimethylformamide (entry 1), the selectivity, however, is only moderate. In contrast, moderate yields and excellent diastereoselectivity were obtained in acetonitrile. Considering the fact that increasing the reaction time would result in improved overall yields, the higher selectivity was favored. Thus, we selected acetonitrile as the solvent of choice for further investigation with 0.5 mmol mL^{-1} aniline concentration.

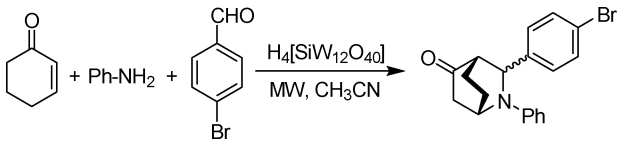
Table 2 Effect of aniline concentration, solvent and reagent concentration on the yield and selectivity of the $H_4SiW_{12}O_{40}$-catalyzed microwave-assisted test aza-Diels–Alder reaction[a]



Entry	$C_{Aniline}$ (mmol mL^{-1})	Solvent	Yield[b] (%)	*endo*/*exo*[c]
1	0.5	DMF	62	79 : 21
2	0.5	DMSO	46	80 : 20
3	0.5	EtOH	42	75 : 25
4	1.25	CH_3CN	62	83 : 17
5	0.83	CH_3CN	62	83 : 17
6	0.625	CH_3CN	36	89 : 11
7	0.5	CH_3CN	42	92 : 8

[a] Reaction conditions: 1.2 eq. aldehyde, 2 eq. ketone. [b] GC yields. [c] Determined by GC and NMR.

Table 3 Effect of reaction time and temperature on the yield and selectivity of the $H_4SiW_{12}O_{40}$-catalyzed microwave-assisted test aza-Diels–Alder reaction[a]



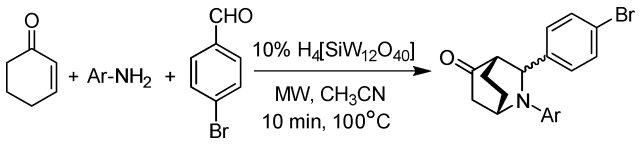
Entry	Catalyst loading (%)	*T*/°C	Time (min)	Yield[b] (%)	*endo*/*exo*[c]
1	5	100	5	42	92 : 8
2	5	80	5	16	84 : 16
3	5	120	5	19	82 : 18
4	2	100	5	13	90 : 10
5	10	100	5	57	79 : 21
6	5	100	10	31	93 : 7
7[d]	10	100	10	36	68 : 32
8	10	100	10	59	93 : 7

[a] Reaction conditions: 0.5 mmol mL^{-1} aniline, 1.2 eq. aldehyde, 2 eq. ketone. [b] GC yields. [c] Determined by GC and NMR. [d] The reaction was carried out with conventional (oil bath) heating.

As a final step in the optimization process, we tested the effect of catalyst loading, reaction temperature and irradiation time on the test reaction, applying the above set of experimental conditions (Table 3).

The results in Table 3 indicate that in order to improve the yield and the diastereoselectivity, increased catalyst loading and longer reaction times were necessary. 10 mol% catalyst gave higher yields by 2% or 5%. Using 10 mol% catalyst and increasing the reaction time from 5 min to 10 min resulted in a slight increase in the yield. The stereoselectivity of the reaction under these conditions (entry 8) remained virtually the same as in the previous Tables. To observe the effect of the microwave irradiation a reaction was also conducted by conventional heating (Table 3 entry 7). The data clearly show that the microwave reaction resulted in higher yield (59% *vs.* 36%) and significantly higher selectivity (97 : 3 *vs.* 68 : 32). It is worth mentioning that an open amine formed during conventional heating as the major byproduct; a precursor that was not observed under microwave conditions.

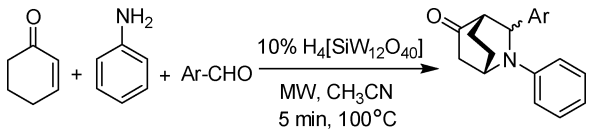
Table 4 Synthesis of azabicyclo[2.2.2]octan-5-ones from anilines, 4-Br-benzaldehyde and 2-cyclohexenone *via* $H_4SiW_{12}O_{40}$-catalyzed microwave-assisted aza-Diels–Alder reaction[a]



Product	Ar	Yield[b] (%)	*endo*/*exo*[c]
1	4-Cl-C_6H_4-	63	95 : 5
2	4-CH_3-C_6H_4-	57	80 : 20
3	4-CH_3O–C_6H_4-	20	96 : 4
4	4-Br-C_6H_4-	65	91 : 9
5	4-F-C_6H_4-	61	92 : 8
6	3-CF_3-C_6H_4-	61	83 : 17
7	3-Cl-C_6H_4-	75	86 : 14

[a] Reaction conditions: 0.5 mmol mL^{-1} aniline, 1.2 eq. aldehyde, 2 eq. ketone. [b] GC yields. [c] Determined by GC and NMR.

Table 5 Synthesis of azabicyclo[2.2.2]octan-5-ones from aniline, substituted benzaldehydes and 2-cyclohexenone *via* $H_4SiW_{12}O_{40}$-catalyzed microwave-assisted aza-Diels–Alder reaction[a]



Product	Ar	Yield[b] (%)	*endo*/*exo*[c]
8	4-NO_2-C_6H_4-	65	89 : 11
9	3,4-di(CH_3O)-C_6H_3-	7	>99 : 1
10	3,4-di(Cl)-C_6H_3-	48	90 : 10
11	4-HO-C_6H_4-	13	85 : 15
12	C_6H_5-	49	90 : 10
13	4-F-C_6H_4-	58	90 : 10
14	4-Me-C_6H_4-	43	94 : 6
15	4-Br-C_6H_4-	59	93 : 7
16	2-Br-C_6H_4-	62	14 : 86
17	4-Cl-C_6H_4-	56	83 : 17
18	2-naphthyl-	42	92 : 8
19	4-NC-C_6H_4-	64	93 : 7

[a] Reaction conditions: 0.5 mmol mL^{-1} aniline, 1.2 eq. aldehyde, 2 eq. ketone. [b] GC yields. [c] Determined by GC and NMR.

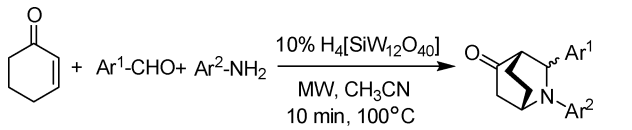
Based on the above studies we determined the optimum reaction conditions and decided to explore the scope of the reaction by carrying out three sets of reactions. First we selected a number of aniline derivatives, while the other reactants remained the same, thus enabling us to assess the effect of substituents on the aniline used. The results are shown in Table 4.

Tha data show that the reaction provided the products in good yields with moderate substituent effects. 4-Methoxy-aniline gave poor yields while the other substituted anilines showed satisfactory performance.

After testing anilines, several substituted benzaldehydes were applied in the reaction using aniline and cyclohexenone (Table 5).

Based on the data summarized in Table 5, it appears that the outcome of the reaction is significantly more sensitive to the structure of the aldehyde unit than it was to that of the aniline component. In most cases, the yields are moderate to good, with a few exceptions (product 9, 11). The stereoselectivity also shows

Table 6 Synthesis of azabicyclo[2.2.2]octan-5-ones from 2-cyclohexenone and substituted anilines and benzaldehydes *via* $H_4SiW_{12}O_{40}$-catalyzed microwave-assisted aza-Diels–Alder reaction[a]



Product	Ar^1	Ar^2	Yield[b] (%)	*endo*/*exo*[c]
20	4-CN-C_6H_4-	4-Cl-C_6H_4-	71	89 : 11
21	4-CN-C_6H_3-	4-F-C_6H_4-	48	94 : 6
22	4-CN-C_6H_3-	3-Cl-C_6H_4-	46	82 : 18
23	4-F-C_6H_4-	3-Cl-C_6H_4-	74	92 : 8
24	3,4-di(Cl)-C_6H_3-	4-F-C_6H_4-	50	92 : 8
25	3,4-di(Cl)-C_6H_3-	3-Cl-C_6H_4-	59	83 : 17
26	4-F-C_6H_4-	4-Cl-C_6H_4-	70	92 : 8
27	4-F-C_6H_4-	4-F-C_6H_4-	56	95 : 5
28	3,4-di(Cl)-C_6H_3-	4-Cl-C_6H_4-	75	89 : 11
29	4-O_2N–C_6H_4-	3-Cl-C_6H_4-	57	85 : 15
30	4-O_2N–C_6H_4-	4-F-C_6H_4-	45	90 : 10
31	4-O_2N–C_6H_4-	4-Cl-C_6H_4-	59	88 : 12

[a] Reaction conditions: 0.5 mmol mL^{-1} aniline, 1.2 eq. aldehyde, 2 eq. ketone. [b] GC yields. [c] Determined by GC and NMR.

a reasonable variation as a function of the substituents. In some cases the *endo* selectivity is excellent (product 10, 12–15, 18, 19), and virtually exclusive in the case of product 9.

Interestingly, the *endo-exo* selectivity changed completely giving close to 90% *exo* product when 2-bromo-benzaldehyde was used. This is most likely due to steric reasons, and the presence of the adjacent bromine atom on the aldehydes phenyl ring. It also appears that oxygen-containing substituents (OH, OCH_3, product 9, 11) consistently give poor yields. This most likely occurs due to the strong complex forming ability of oxygen with the catalyst, rather than the electron donating character of these substituents, as tolualdehyde (product 14) with an electron donor CH_3- group gave acceptable yield.

As a further attempt to extend the scope of the process we selected several substituted anilines and benzaldehydes and carried out the reaction with these, so far untested, combinations. Our goal was to determine whether the expected products could be obtained in virtually any combination of these two reactants. The data are summarized in Table 6.

The mechanism of the reaction involves multiple steps and follows the traditional Diels–Alder pathway, except the first step that, in the current multicomponent reaction, is the formation of the imine from the aldehyde and aniline.

Our synthetic efforts clearly show that heteropolyacids are good catalysts for the multicomponent reaction of anilines, benzaldehydes and 2-cyclohexenone. The best performance was exhibited by silicotungstic acid ($H_4SiW_{12}O_{40}$), which catalyzed the reaction efficiently and with high diastereoselectivity. Compared to other currently available methods, the microwave-assisted $H_4SiW_{12}O_{40}$-catalyzed process provides a generally applicable method that is able to accommodate a broad range of substituted anilines and benzaldehydes providing the products in good yields. The major advantages of our method are: (i) only a very short reaction time (10 min) is necessary compared to 24–48 h reported by others; and (ii) the diastereoselectivities are excellent and significantly exceed the usual 60 : 40 ratio that is common in earlier reports.

Furthermore, silicotungstic acid is an environmentally benign catalyst that does not decompose under the initial condensation reaction or the regular aqueous workup conditions and can thus be recovered after the reactions.

Preliminary assays in human acetylcholinesterase (AChE) inhibition and inhibition of amyloid β (Aβ) self-assembly

Among several factors responsible for Alzheimer's disease, self-assembly of amyloid-β (Aβ) peptide to oligomers and fibrils is considered to be of primary importance (amyloid cascade hypothesis).[17] In addition, according to the cholinergic theory, reduction of the acetylcholine (ACh) in specific areas of the brain may also aggravate the disease.[18] Acetylcholine is hydrolyzed by its metabolic enzyme, the acetylcholinesterase (AChE). As recent studies have shown a complex formation between AChE and Aβ peptide that may induce amyloid fibrillogenesis, it appears that the two processes are at least partially linked together. The need for better therapeutic drugs has initiated efforts to synthesize multi-target-directed small molecule ligands. For this purpose, we evaluated the above synthesized azabicyclo[2.2.2]octan-5-ones as possible dual inhibitors of both processes. While the primary goal of the current work was synthetic, these experiments serve as simple preliminary assays to determine the possible potential of the azabicyclo[2.2.2]octan-one skeleton in the design of possible drug candidates. First, we determined the potency of several azabicyclo[2.2.2]octan-5-ones in the inhibition of Aβ fibril formation. The efficacy of the compounds was evaluated by the commonly applied Thioflavin-T (THT) fluorimetric assay, as described earlier.[19] The calculated fluorescence intensity (I_{THT}) values were based on maximum fluorescence intensities in the 480-490 nm region (emission spectra) after subtracting the background fluorescence of the starting solutions (0 h). The samples were incubated for up to 140 h, and the increase in the fluorescence intensities was periodically measured. The data were compared to the fluorescence intensity of the inhibitor-free samples (control). The comparative results are shown in Fig. 1.

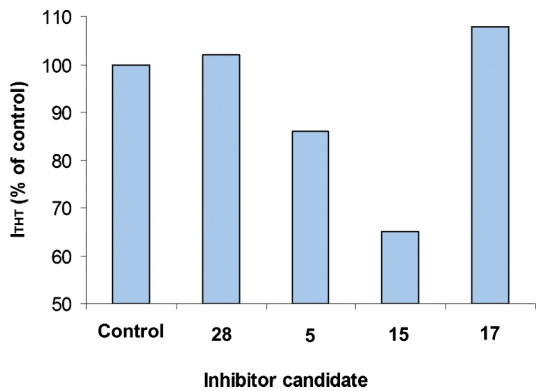


Fig. 1 Inhibition of $A\beta_{1-40}$ fibrillogenesis by azabicyclo[2.2.2]octan-5-ones at $mol_{inhibitor}/mol_{peptide}$ = 10 stoichiometry. THT fluorescence intensities (I_{THT}) are normalized to that of the inhibitor-free $A\beta_{1-40}$ sample (control).

The data show that some of the compounds (*e.g.* **15**) inhibited the fibril formation, however, due to solubility problems, the number of compounds that we were able to test was limited. The data clearly point toward the need to synthesize additional derivatives with enhanced water solubility.

In the next assay we applied the azabicyclo[2.2.2]octan-5-ones to inhibit the hydrolytic activity of AChE. The AChE inhibition was evaluated by Ellman's spectrophotometric method.[20] Due to the exploratory nature of these investigations, we have selected galanthamine,[18] a well-known AChE inhibitor, to serve as a reference compound. Thus, the results were compared to that of galanthamine (100%) to determine the initial activity of our products. The enzyme inhibition assays require significantly lower inhibitor concentration, thus we were able to test a broader variety of compounds. The comparative enzyme inhibition activities are illustrated in Fig. 2.

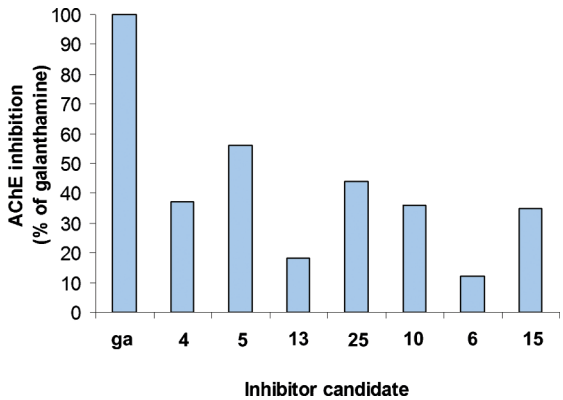


Fig. 2 Inhibition of the hydrolytic activity of human AChE by azabicyclo[2.2.2]octan-5-ones. The obtained inhibitory activities are normalized to that of galathamine (ga).

The data show that all compounds tested exhibited moderate to significant inhibition of AChE. While some molecules are relatively weak inhibitors (6, 13), others (4, 5, 10, 15, 25) exhibited activity comparable to that of galanthamine (40–60%). This is promising for future inhibitor development. The validation of the binding of the compounds was carried out by molecular docking using Autodock 4.[21] The human acetylcholinesterase crystal structure was obtained from the Protein Data Base (1B41) and after some modifications it was subjected to docking studies. The details of the docking investigation and protein preparation has been described elsewhere.[22] The structure of hAChE, its binding site with the flexible residues and a representative example of a docked inhibitor are shown in Fig. 3.

The close-out of the docked structure indicates that the ligand binds to the active site in a "rod"-like conformation, when the two phenyl rings stretch out, while the azabicyclo system occupies a central position in the binding pocket. It appears that the aniline-based phenyl ring is deeply buried in the binding pocket, while the aldehyde based phenyl points toward the opening of the pocket, and the proximity to the adjacent hydroxyl group suggests hydrogen bonding with the fluorine. This arrangement highlights that the size and electronic nature of the substituent on the aniline-based phenyl group is very important, while the similar position on the aldehyde based phenyl can stabilize the binding.

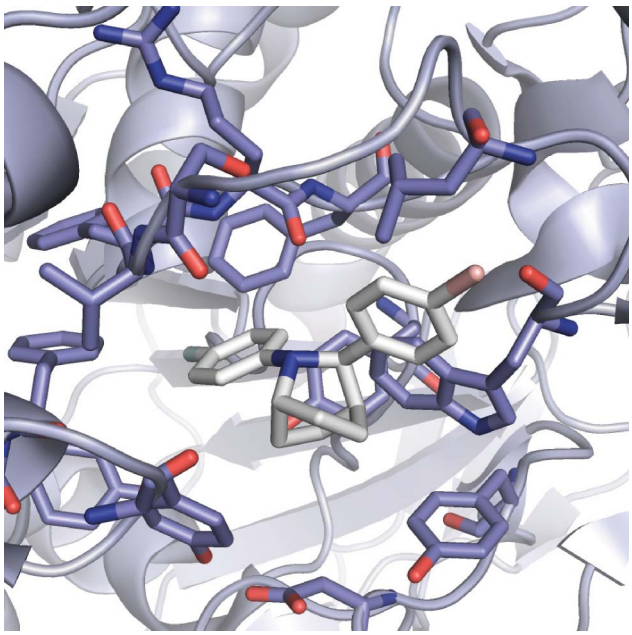


Fig. 3 The close-out of the structure of human acetylcholinesterase (code1b41) with docked compounds of (*R*)-*exo*-5 in the binding pocket.

Conclusions

In conclusion, we have developed an environmentally sustainable method for the synthesis of azabicyclo[2.2.2]-octan-5-ones. The three component two step condensation–aza-Diels–Alder reaction domino sequence was carried out by a microwave-assisted silico-tungstic acid-catalyzed one-pot reaction. The process has been evaluated using a broad range of starting materials (anilines and benzaldehydes). It was found to be generally applicable providing good yields and excellent diastereoselectivities compared to earlier methods. The major advantages of the procedure, such as the very short reaction times, the excellent diastereoselectivities, and the environmentally benign nature and stability of the catalyst, make this process a viable alternative for the synthesis of the target compounds. The products have been pre-evaluated in the inhibition of amyloid β fibrillogenesis, as well as the inhibition of AChE activity—processes that are thought to be important in the development of Alzheimer's disease. Some compounds showed promising activity in these assays raising the possibility of using them as lead scaffolds for the synthesis of dual target inhibitors.

Experimental

General Information

Cyclohex-2-enone, aldehydes and anilines were purchased from Aldrich and were used without further purification. Catalysts such as montmorillonite K-10, Nafion NR50 and heteropoly acids were purchased from Aldrich. Solvents used in synthesis were of minimum purity 99.5% and were Aldrich products. $CDCl_3$, used as a solvent (99.8%) for the NMR studies, and $CFCl_3$, used as reference for ^{19}F NMR, were obtained from Aldrich. The mass spectrometric identification of the products have been carried out by an Agilent 6850 gas chromatograph-5973 mass spectrometer system (70 eV electron impact ionization) using a 30 m long DB-5 type column (J&W Scientific). The determination of the diastereomeric ratios was based on the total ion chromatograms and verified by NMR spectroscopy. The ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a 300 MHz superconducting Varian NMR spectrometer, in $CDCl_3$ solvent with tetramethylsilane and $CFCl_3$ as internal standards. The temperature was 25 °C (accuracy ±1 °C) and controlled by the Varian control unit. For thin-layer chromatography (TLC), silica gel plates EMD 60 F254 were used and compounds were visualized under UV light. Preparative thin layer chromatography was applied for the purification of the products (silica gel Davisil®, Grade 635, pore size 60 Å (60-100 mesh)). Melting points are uncorrected and were recorded on MEL-TEMP apparatus.

General procedure for the synthesis of azabicyclo[2.2.2]octan-5-ones

0.25 mmol of aniline, 0.3 mmol of aldehyde, 0.5 mmol of cyclohex-2-enone were dissolved in 0.5 ml of acetonitrile in a microwave test tube followed by the addition of 0.025 mmol of the tungstosilicilic acid. The reaction mixture was irradiated in a microwave reactor for 10 min at 100 °C, then quenched with a saturated solution of Na_2CO_3 and extracted with ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate and analyzed by GC-MS and/or evaporated and the product was isolated using preparative TLC.

Endo-4-[2-(4-chlorophenyl)-5-oxo-2-azabicyclo[2.2.2]oct-3-yl]benzonitrile (20)

Colorless oil. Isolated yield 43%. **MS** (70 eV) *m*/*z*: 336 (100%, M^+), 293 (95%), 295 (41%), 338 (35%, M^+), 294 (21%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.70 (d, J = 7.5 Hz, 2H), 7.52 (d, J = 7.5 Hz, 2H), 7.10 (d, J = 8.7 Hz, 2H), 6.45 (d, J = 8.7 Hz, 2H), 4.76 (bd, J = 2.1 Hz, 1H), 4.51 (m, 1H), 2.77-2.69 (m, 2H), 2.44 (d, J = 18.9 Hz, 1H), 2.28-2.17 (m, 1H), 2.01–1.90 (m, 1H), 1.75–1.56 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 211.8, 146.0, 145.3, 132.8, 129.3, 126.9, 123.2, 118.5, 114.2, 111.6, 62.1, 50.3, 48.6, 42.2, 25.8, 16.1.

Endo-3-(4-chlorophenyl)-2-phenyl-2-azabicyclo[2.2.2]octan-5-one (17)

Colorless oil. Isolated yield 47%. **MS** (70 eV) *m*/*z*: 311 (100%, M^+), 268 (88%), 270 (38%), 313 (36%, M^+), 312 (22%), 269(19%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.35 (m, 4H), 7.16 (t, J = 7.2 Hz, 2H), 6.74 (t, J = 7.2 Hz, 1H), 6.57 (d, J = 7.8 Hz, 2H), 4.74 (bd, J = 3 Hz, 1H), 4.55 (m, 1H), 2.75 (dt, ^{1}J = 18.6 Hz, ^{2}J = 3 Hz, 1H), 2.67 (dd, J = 3 Hz, 1H), 2.41 (d, J = 18.6 Hz, 1H), 2.27–2.16 (m, 1H), 1.97–1.86 (m, 1H), 1.69–1.63 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 213.1, 147.8, 135.6, 133.1, 129.4, 129.0, 127.5, 118.0, 113.0, 61.8, 50.7, 48.1, 42.2, 25.9, 16.2.

Endo-3-(4-bromophenyl)-2-phenyl-2-azabicyclo[2.2.2]octan-5-one (15)

Colorless oil. Isolated yield 44%. **MS** (70 eV) *m*/*z*: 312 (100%), 314 (95%), 357 (95%, M^+), 355 (82%, M^+), 315 (26%), 158 (25%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.43 (d, J = 8.7 Hz, 2H), 7.21–7.14 (m, 4H), 6.76 (t, J = 10.2 Hz, 1H), 6.63 (d, J = 7.8 Hz,

2H), 4.60 (bd, J = 2.7 Hz, 1H), 4.54 (m, 1H), 2.78–2.67 (m, 2H), 2.48 (d, J = 18.6 Hz, 1H), 2.28–2.20 (m, 1H), 2.14–1.97 (m, 2H), 1.80–1.68 (m, 1H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 211.3, 147.7, 140.8, 132.1, 129.3, 127.4, 121.4, 118.0, 113.3, 65.3, 52.0, 48.5, 45.8, 22.6, 22.4.

***Endo*-2-(3-chlorophenyl)-3-(3,4-dichlorophenyl)-2-azabicyclo[2.2.2]octan-5-one (25)**

Colorless oil. Isolated yield 45%. **MS** (70 eV) m/z: (100%), 336 (98%), 295 (41%), 379 (89%, M^+), 381 (82%, M^+), 340 (34%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.47 (m, 2H), 7.23 (m, 1H), 7.06 (t, J = 8.4 Hz, 1H), 6.73 (d, J = 7.5 Hz, 1H), 6.60 (m, 1H), 6.37 (d, J = 8.7 Hz, 1H), 4.69 (bd, J = 2.7 Hz, 1H), 4.50 (m, 1H), 2.76-2.66 (m, 2H), 2.43 (d, J = 18.6 Hz, 1H), 2.27–2.15 (m, 1H), 1.99–1.88 (m, 2H), 1.71–1.65 (m, 1H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 211.9, 148.7, 139.9, 135.3, 133.3, 131.7, 131.0, 130.4, 128.0, 125.4, 118.3, 113.0, 111.4, 61.4, 50.3, 48.5, 42.2, 25.7, 16.1.

***Endo*-2-(4-chlorophenyl)-3-(3,4-dichlorophenyl)-2-azabicyclo[2.2.2]octan-5-one (28)**

Colorless oil. Isolated yield 55%. **MS** (70 eV) m/z: 381 (100%, M^+), 379 (98%), 336 (94%), 338 (89%), 340 (42%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.47 (m, 2H), 7.22 (m, 1H), 7.11 (d, J = 9.3 Hz, 2H), 6.48 (d, J = 9.3 Hz, 1H), 4.66 (bd, J = 2.7 Hz, 1H), 4.48 (m, 1H), 2.75–2.65 (m, 2H), 2.45–2.33 (m, 1H), 2.24–2.17 (m, 1H), 2.05–1.92 (m, 1H), 1.71–1.65 (m, 2H).; **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 212.1, 146.2, 140.1, 133.3, 131.7, 131.7, 131.0, 129.8, 129.3, 128.0, 125.4, 123.2, 116.7, 114.3, 61.6, 50.4, 48.5, 42.1, 25.9, 16.1.

***Endo*-2-(4-chlorophenyl)-3-(4-fluorophenyl)-2-azabicyclo[2.2.2]octan-5-one (26)**

Colorless oil. Isolated yield 58%. **MS** (70 eV) m/z: 329 (100%, M^+), 286 (60%), 331 (38%, M^+), 288 (24%), 330 (23%, M^+), 287 (14%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm).34 (m, 2H), 7.07 (m, 4H), 6.49 (d, J = 9.3 Hz, 2H), 4.70 (bd, J = 2.1 Hz, 1H), 4.49 (m, 1H), 2.75–2.65 (m, 2H), 2.41 (d, J = 18.6 Hz, 1H), 2.28–2.18 (m, 1H), 1.96–1.86 (m, 1H), 1.71–1.63 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 212.9, 146.5, 135.0, 129.1, 127.6, 122.8, 116.0, 115.7, 114.2, 61.7, 50.9, 48.6, 42.2, 25.9, 16.1.

***Endo*-2-(4-chlorophenyl)-3-(4-nitrophenyl)-2-azabicyclo[2.2.2]octan-5-one (31)**

Colorless oil. Isolated yield 49%. **MS** (70 eV) m/z: 313 (100%), 356 (93%, M^+), 315 (45%), 358 (36%, M^+), 357 (24%, M^+), 314 (22%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 8.26 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H), 7.10 (d, J = 9 Hz, 2H), 6.46 (d, J = 9 Hz, 2H), 4.81 (bd, J = 2.4 Hz, 1H), 4.53 (m, 1H), 2.79–2.70 (m, 2H), 2.46 (d, J = 18.6 Hz, 1H), 2.28–2.17 (m, 1H), 2.02–1.92 (m, 1H), 1.79–1.59 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 211.7, 147.3, 146.0, 129.3, 129.2, 127.1, 124.2, 123.3, 114.3, 62.0, 50.2, 48.6, 42.2, 25.9, 16.1.

***Endo*-2-(3-chlorophenyl)-3-(4-nitrophenyl)-2-azabicyclo[2.2.2]octan-5-one (29)**

Colorless oil. Isolated yield 45%. **MS** (70 eV) m/z: 313 (100%), 356 (83%, M^+), 315 (45%), 358 (30%, M^+), 192 (20%), 314 (20%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 8.26 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 8.7 Hz, 2H), 7.06 (m, 1H), 6.72 (m, 1H), 6.58 (m, 1H), 6.35 (m, 1H), 4.85 (bd, J = 2.4 Hz, 1H), 4.55 (m, 1H), 2.80–2.71 (m, 2H), 2.47 (d, J = 18.9 Hz, 1H), 2.30–2.17 (m, 1H), 2.03–1.93 (m, 1H), 1.77–1.58 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 211.5, 148.5, 147.1, 135.3, 130.4, 127.0, 124.2, 118.4, 117.5, 113.0, 111.3, 61.8, 50.2, 48.6, 42.3, 25.7, 16.1.

***Endo*-3-(4-bromophenyl)-2-(4-fluorophenyl)-2-azabicyclo[2.2.2]octan-5-one (5)**

Colorless oil. Isolated yield 52%. **MS** (70 eV) m/z: 375 100%, M^+), 373 (96%, M^+), 332 (91%), 330 (83%), 176 (28%), 95 (27%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.51 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 8.7 Hz, 2H), 6.86 (m, 2H), 6.49 (m, 2H), 4.65 (bd, J = 2.7 Hz, 1H), 4.45 (m, 1H), 2.73 (dt, ^1J = 18.9 Hz, ^2J = 2.7 Hz, 1H), 2.65 (dd, J = 2.7 Hz, 1H), 2.41 (d, J = 18.9 Hz, 1H),, 2.28–2.17 (m, 1H), 1.96–1.86 (m, 1H), 1.70–1.62 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 213.0, 157.4, 133.0, 132.1, 127.9, 115.9, 115.6, 114.0, 113.9, 62.2, 50.7, 48.8, 42.0, 26.1, 16.1.

***Endo*-3-(4-bromophenyl)-2-[3-(trifluoromethyl)phenyl]-2-aza–bicyclo[2.2.2]octan-5-one (6)**

Colorless oil. Isolated yield 45%. **MS** (70 eV) m/z: 382 (100%), 380 (95%), 425 (82%, M^+), 423 (81%, M^+), 226 (39%), 145 (35%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.52 (d, J = 8.1 Hz, 2H), 7.25 (m, 3H), 6.98-6.87 (m, 2H), 6.60 (m, 1H), 4.74 (bd, J = 2.7 Hz, 1H), 4.58 (m, 1H), 2.77–2.67 (m, 2H), 2.47 (d, J = 18.9 Hz, 1H), 2.30–2.19 (m, 1H), 2.03–1.90 (m, 1H), 1.73–1.62 (m, 2H).; **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 212.3, 148.5, 147.1, 135.3, 130.4, 127.0, 124.2, 118.4, 117.5, 113.0, 111.3, 61.8, 50.6, 48.5, 42.3, 25.7, 16.1.

***Endo*-3-(4-bromophenyl)-2-(4-methylphenyl)-2-azabicyclo[2.2.2]octan-5-one (2)**

Colorless oil. Isolated yield 45%. **MS** (70 eV) m/z: 369 (100%, M^+), 371 (94%, M^+), 328 (82%), 326 (77%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.50 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 8.7 Hz, 2H), 6.97 (d, J = 9 Hz, 2H), 6.47 (d, J = 9 Hz, 2H), 4.68 (bd, J = 2.7 Hz, 1H), 4.50 (m, 1H), 2.75 (dt, ^1J = 18.9 Hz, ^2J = 3 Hz, 1H), 2.65 (dd, J = 2.7 Hz, 1H), 2.39 (d, J = 18.9 Hz, 1H), 2.27–2.15 (m, 1H), 2.2 (s, 3H), 1.95–1.85 (m, 1H), 1.70–1.62 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 213.3, 145.7, 139.4, 131.9, 129.9, 128.0, 127.3, 121.1, 113.1, 61.9, 50.7, 48.3, 42.1, 26.1, 20.1, 16.3.

***Endo*-3-(4-methylphenyl)-2-phenyl-2-azabicyclo[2.2.2]octan-5-one (14)**

Colorless oil. Isolated yield 39%. **MS** (70 eV) m/z: 291 (100%, M^+), 248 (66%), 194 (19%), 158 (14%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.29 (d, J = 7.8 Hz, 2H), 7.14 (m, 4H), 6.70 (t, J = 7.2 Hz, 1H), 6.60 (d, J = 9 Hz, 2H), 4.73 (bd, J = 2.4 Hz, 1H), 4.53 (m, 1H), 2.78–2.66 (m, 2H), 2.43–2.19 (m, 2H), 2.35 (s, 3H), 1.94–1.83 (m, 1H), 1.78–1.60 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm)

213.8, 148.2, 137.0, 136.9, 129.5, 129.2, 126.0, 117.5, 113.0, 62.1, 51.0, 48.0, 42.2, 26.0, 21.0, 16.3.

***Endo*-3-(4-fluorophenyl)-2-phenyl-2-azabicyclo[2.2.2]octan-5-one (13)**

Colorless oil. Isolated yield 39%. MS (70 eV) *m/z*: 295 (M^+, 100%), 222 (63%), 243 (45%), 134 (15%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.37 (m, 2H), 7.18–7.03 (m, 5H), 6.73 (t, J = 7.5 Hz, 1H), 6.58 (d, J = 8.4 Hz, 2H), 4.75 (bd, J = 2.4 Hz, 1H), 4.55 (m, 1H), 2.75 (dt, ^1J = 19.2 Hz, ^2J = 3 Hz, 1H), 2.65 (dd, J = 3 Hz, 1H), 2.41 (d, J = 19.2 Hz, 1H), 2.29–2.17 (m, 1H), 2.2 (s, 3H), 1.96–1.86 (m, 1H), 1.70–1.62 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 213.3, 147.9, 129.3, 127.7, 127.6, 117.9, 115.8, 115.6, 113.1, 61.7, 50.9, 48.1, 42.2, 25.9, 16.2.

***Endo*-2,3-diphenyl-2-azabicyclo[2.2.2]octan-5-one (12)**

Colorless oil. Isolated yield 42%. MS (70 eV) *m/z*: 277 (100%, M^+), 234 (79%), 77 (25%), 180 (24%), 158 (23%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.37 (m, 4H), 7.31 (m, 1H), 7.15 (m, 2H), 6,71 (t, J = 7.2 Hz, 1H), 6.60 (d, J = 7.8 Hz, 2H), 4.77 (bd, J = 2.4 Hz, 1H), 4.56 (m, 1H), 2.80–2.69 (m, 2H), 2.41 (d, J = 18.9 Hz, 1H), 2.31–2.20 (m, 1H), 1.96–1.85 (m, 1H), 1.72–1.61 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 213.7, 148.1, 140.0, 129.3, 128.8, 127.4, 126.1, 117.7, 113.0, 62.3, 50.9, 48.0, 42.2, 25.9, 16.3.

***Endo*-3-(4-nitrophenyl)-2-phenyl-2-azabicyclo[2.2.2]octan-5-one (8)**

Colorless oil. Isolated yield 57%. MS (70 eV) *m/z*: 322 (100%, M^+), 279 (25%), 158 (17%), 172 (11%), 266 (9%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 8.19 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.20 (t, J = 7.5 Hz, 2H), 6.79 (t, J = 7.5 Hz, 1H), 6.61 (d, J = 7.5 Hz, 2H), 4.75 (bd, J = 2.4 Hz, 1H), 4.60 (m, 1H), 2.81–2.70 (m, 2H), 2.52 (d, J = 18.9 Hz, 1H), 2.31–2.05 (m, 3H), 1.86–1.76 (m, 1H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 210.6, 149.2, 147.3, 129.5, 126.7, 124.4, 123.8, 118.6, 113.5, 65.3, 51.9, 48.8, 45.9, 22.6, 22.4.

***Endo*-2-(3-chlorophenyl)-3-(4-fluorophenyl)-2-azabicyclo[2.2.2]octan-5-one (23)**

Colorless oil. Isolated yield 54%. MS (70 eV) *m/z*: 329 (100%, M^+), 331 (43%, M^+), 286 (23%), 149 (19%), 232 (18%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.33 (m, 2H), 7.06 (m, 3H), 6.69 (m, 1H), 6.60 (t, J = 2.1 Hz, 1H), 6.38 (d, J = 8.4 Hz, 1H), 4.73 (bd, J = 2.4 Hz, 1H), 4.50 (m, 1H), 2.65–2.76 (m, 2H), 2.42 (d, J = 18.9 Hz, 1H), 2.27–2.17 (m, 1H), 1.97–1.86 (m, 1H), 1.71–1.63 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 212.7, 149.0, 135.1, 130.3, 127.6, 127.5, 117.8, 116.0, 115,7, 112.9, 111.3, 61.5, 50.8, 48.5, 42.3, 25.7, 16.0.

***Endo*-2,3-bis(4-bromophenyl)-2-azabicyclo[2.2.2]octan-5-one (4)**

Colorless oil. Isolated yield 58%. MS (70 eV) *m/z*: 435 (M^+, 100%), 433 (M^+, 51%), 437 (M^+, 50%), 436 (M^+, 21%), 434 (M^+, 11%), 438 (M^+, 10%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.50 (d, J = 8.1 Hz, 2H), 7.23 (m, 4H), 6.43 (d, J = 8.7 Hz, 2H), 4.66 (bd, J = 2.7 Hz, 1H), 4.48 (m, 1H), 2.73–2.64 (m, 2H), 2.41 (d, J = 18.6 Hz, 1H), 2.24–2.15 (m, 1H), 1.94–1.86 (m, 1H), 1.70–1.62 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 212.5, 146.7, 138.4, 132.0, 131.9, 127.8, 121.4, 114.7, 109.9, 61.7, 50.5, 48.4, 42.1, 25.8, 16.0.

***Endo*-4-(5-oxo-2-phenyl-2-azabicyclo[2.2.2]oct-3-yl)benzonitrile (19)**

Colorless oil. Isolated yield 58%. MS (70 eV) *m/z*: 259 (100%), 77 (78%), 302 (73%, M^+), 104 (47%), 158 (46%), 205 (35%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.69 (d, J = 7.8 Hz, 2H), 7.55 (d, J = 7.8 Hz, 2H), 7.17 (m, 2H), 6.76 (t, J = 7.2 Hz, 1H), 6.54 (d, J = 8.7 Hz, 2H), 4.81 (bd, J = 2.4 Hz, 1H), 4.58 (m, 1H), 2.81–2.68 (m, 2H), 2.44 (d, J = 18.9 Hz, 1H), 2.28–2.19 (m, 1H), 2.00–1.89 (m, 1H), 1.70–1.57 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 212.2, 147.5, 145.9, 132.7, 129.4, 129.3, 127.0, 118.6, 118.3, 113.2, 113.0, 111.4, 62.0, 50.3, 48.1, 42.2, 25.9, 16.2.

***Endo*-3-(3,4-dichlorophenyl)-2-phenyl-2-azabicyclo[2.2.2]octan-5-one (10)**

Colorless oil. Isolated yield 58%. MS (70 eV) *m/z*: 345 (100%, M^+), 302 (93%), 304 (68%), 347 (65%, M^+), 346 (24%, M^+), 303 (20%); **^{1}H NMR** (300.12 MHz, $CDCl_3$), δ (ppm) 7.52–7.45 (m, 2H), 7.27–7.15 (m, 3H), 6.77 (t, J = 7.5 Hz, 1H), 6.57 (d, J = 8.7 Hz, 1H), 4.71 (bd, J = 3 Hz, 1H), 4.55 (m, 1H), 2.80–2.65 (m, 2H), 2.42 (d, J = 18.9 Hz, 1H), 2.27–2.16 (m, 1H), 1.99–1.88 (m, 1H), 1,71–1.65 (m, 2H); **^{13}C NMR** (75.46 MHz, $CDCl_3$), δ (ppm) 212.6, 147.7, 140.7, 133.2, 131.4, 131.0, 129.5, 128.1, 125.5, 118.3, 113.1, 61.6, 50.5, 48.1, 42.1, 26.0, 16.2.

Thioflavin-T fluorometric assay for the determination of inhibitor activity in Aβ fibrillogenesis[19]

The synthetic lyophilized $A\beta_{1-40}$ peptide was dissolved in 100 mM NaOH to a concentration of 40 mg ml^{-1} and diluted in 10 mM HEPES, 100 mM NaCl, 0.02% NaN_3 (pH = 7.4) buffer to a final peptide concentration of 100 μM. The inhibitors were dissolved in DMSO and added to the Aβ samples (inhibitor/Aβ = 10). After 30 s of vigorous vortexing the solutions were incubated at 37 °C with gentle shaking (77 rpm) and the increase in fibril amount in each sample was followed by Thioflavin-T fluorescence, and atomic force microscopy (AFM). The fluorescence measurements have been carried out using a Hitachi F-2500 fluorescence spectrophotometer. The incubated peptide solutions were briefly vortexed before each measurement, and then 3.5 μl aliquots of the suspended fibrils were withdrawn and added into 700 μl of 5 μM Thioflavin-T prepared freshly in 50 mM glycine-NaOH (pH = 8.5) buffer. The fluorescence spectra of these mixtures have been measured at 430 nm (excitation) and 484 nm (emission) wavelengths, respectively. None of the inhibitor compounds showed fluorescence intensity in this region.

AChE inhibition assay

The Ellman method was applied to test the compounds in AChE inhibition.[20] Galanthamine, used for comparison, and other components used in the assay were obtained from Sigma-Aldrich. AChE (0.5 IU mg^{-1}) derived from human erythrocytes was also purchased from Sigma Chemical. The assay solution consisted of a 0.1 M phosphate buffer, pH 8.0, along with of

340 μM 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent), 0.02 unit mL^{-1} AChE and 550 μM acetylthiocholine iodide. The concentration of the inhibitors was kept constant at the IC_{50} value of galanthamine for the screening. The final assay volume was 3 mL. Test compounds were added to the assay solution and preincubated at 37 °C for 20 min following the addition of the substrate. Initial rate assays were performed at 37 °C using an Agilent 8453 spectrophotometer. The rate of increase of the absorbance at 412 nm was followed for 20 min. Assays were also carried out with a control solution containing all components except AChE, to account for nonenzymatic reaction. The reaction rates were compared and the percent inhibition due to the presence of test compounds was calculated. Five parallel assays of each compound were performed. The percent inhibition of the enzyme activity due to the presence of test compound with respect to galanthamine was calculated by the following expression: $[(V_0 - V_i)/(V_0 - V_g)] \times 100$, where V_i is the rate calculated in the presence of inhibitor and V_0 is the enzyme activity and V_g is the rate calculated in the presence of galathamine.

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