European Journal of Medicinal Chemistry 44 (2009) 4057–4062

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Active site directed docking studies: Synthesis and pharmacological evaluation of *cis*-2,6-dimethyl piperidine sulfonamides as inhibitors of acetylcholinesterase

H.R. Girisha, J.N. Narendra Sharath Chandra, Sriramamurthy Boppana, Manish Malviya, C.T. Sadashiva, Kanchugarakoppal S. Rangappa*

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore - 570006, Karnataka, India

ARTICLE INFO

Article history:
Received 1 May 2007
Received in revised form
10 April 2009
Accepted 17 April 2009
Available online 8 May 2009

Keywords:
Alzheimer's diseases
AChE
Docking

ABSTRACT

Hypocholinergic function associated with Alzheimer's disease (AD) is well-accepted hypothesis, in this regard, many research attempts have been made to elevate the reduced cholinergic neurotransmission, among them two main treatment strategies were widely explored, namely stimulation of muscarinic receptor 1 and/or reversible inhibition of acetylcholinesterase (AChE) enzyme. In an attempt to improve the efficacy and to minimize general side effects of these AChE inhibitors, many lead molecules are developed in research; one among them is piperidine derivative. Donazepil is a widely prescribed AChE inhibitor which displays a piperidine ring in its structure. In the present study, we have docked *cis*-2,6-dimethyl piperidine sulfonamides (**3a**–**i**) on AChE enzyme and synthesized by nucleophilic substitution reaction between *cis*-2,6-dimethyl piperidine and alkyl/aryl sulfonyl chlorides in the presence of triethylamine. These piperidine sulfonamides were subjected to in vitro AChE enzyme inhibition studies and in vivo antiamnesic study to reverse scopolamine induced memory loss in rats. Two derivatives (**3a** and **f**) in this class of piperidines (**3a**–**i**) showed considerable inhibition against different sources of AChE in vitro and reduced average number of mistakes done by wistar rats as compared to scopolamine treated group in vivo (rodent memory evaluation).

1. Introduction

Modern methods for computer-assisted drug design fall into two major categories generally known as ligand-based and target-based methods. The former, which include conventional quantitative structure–activity relationships (QSARs) [1], active analog approach [2], and recently comparative molecular field analysis (CoMFA) [3], are based entirely on experimental structure–activity relationship for receptor ligands or enzyme inhibitors, and their application from past 30 years has led to several drugs now in clinical practice [4]. The latter methods which include docking and advanced molecular simulations require the structural information about target as provided by X-ray crystallography, NMR or protein homology model building. This strategy has become available only recently, with rapid advances in structure elucidation methods yielding several promising drug candidates in clinical practice [5].

The cholinergic hypothesis of AD has provided the rationale for the current major therapeutic approach to the disease, which is aimed to correct the cognitive decline by enhancing cholinergic neurotransmission [6,7]. In this regard, multiple possible strategies (muscarinic 1 receptor activation, muscarinic 2 receptor blocking, nicotinic receptor activation and AChE inhibition) were used. Among them, cholinesterase inhibition has been so for the most extensively used approach for the treatment of Alzheimer's disease. In our present study, we have investigated the interaction of alkyl and aryl *cis*-2,6-dimethyl piperidine sulfonamides (**3a**–**i**) with mouse AChE by docking study, along with their in vitro inhibition of AChE and in vivo evaluation of memory and learning in male wistar rats (rodent memory evaluation study).

2. Materials and methods

2.1. Experimental section

2.1.1. Protein–ligand docking

The ligands including all hydrogen atoms were built and optimized with Chemsketch software suite (Advanced Chemistry Development, Inc). Extremely Fast Rigid Exhaustive Docking (FRED) version 2.1 was used for docking studies (Open Eye Scientific

* Corresponding author. Tel.: +91 821 2419661; fax: +91 821 2412191.
E-mail addresses: rangappaks@gmail.com, rangappaks@chemistry.uni-mysore.ac.in (K.S. Rangappa).

0223-5234/$ – see front matter © 2009 Published by Elsevier Masson SAS.
doi:10.1016/j.ejmech.2009.04.042

Software, Santa Fe, NM). This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site of 4 Å. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used to dock were converted in 3-dimension with Omega (Open Eye Scientific Software, Santa Fe, NM). To this set, the substrate (generation of multiconformer with Omega) corresponding to the modeled protein was added. It is an implementation of multiconformer docking, meaning that a conformational search of the ligand is first carried out and all relevant low-energy conformations are then rigidly placed in the binding site. This two-step process allows only the remaining six rotational and translational degrees of freedom for the rigid conformer to be considered. The FRED (Fast Rigid Exhaustive Docking) process uses a series of shape-based filters and the default scoring function is based on Gaussian shape fitting.

2.2. Chemistry

2.2.1. General procedure for the synthesis of cis-2,6-dimethyl piperidine sulfonamides derivatives (3a–i)

To a solution of *cis*-2,6-dimethyl piperidine **1** (1 eq) and triethylamine (3 eq) in dry dichloromethane at 0 °C awas dded alkyl/aryl sulfonyl chlorides (1.2 eq). The reaction mixture was stirred at 0 °C for about 2 h and stirring was continued at room temperature for about 4–5 h (completion of the reaction was monitored by TLC). After the completion of the reaction, the reaction mass was quenched with distilled water and extracted with dichloromethane (3 × 15 mL). Finally, the combined organic layer was washed with distilled water again and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuum, the residue was purified by recrystallisation.

The melting point was recorded on a SELACO-650 hot stage apparatus and is uncorrected. IR (KBr) spectra recorded on a Jusco FTIR-4100 Fourier transform infrared spectrometer, ^{1}H NMR were recorded on a Shimadzu AMX, spectrometer by using $CDCl_3$ as solvent and TMS as an internal standard (Chemical shift in ppm). TLC was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck). Visualization was made in ultraviolet light. All extracted solvents were dried over anhydrous Na_2SO_4 and evaporated with a BUCHI rotary evaporator. Reagents were obtained commercially and used as received.

2.2.1.1. Synthesis of cis-*2,6-dimethyl-1-methyl sulfonyl piperidine (**3a**).* The general synthetic method described above affords **3a** as a colorless crystalline solid from piperidine **1** (1 g, 8.85 mmol) and methane sulfonyl chloride **2a** (1.215 g, 10.62 mmol). IR ν_{max} 1370, 1465, 1150 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2), 2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, –CH_3), 2.9 (s, 3H, –SO_2, –CH_3). mp 155–157 °C (Yield = 82%). Anal. ($C_8H_{17}N_1O_2S_1$) C, H, N.

*2.2.1.2. Synthesis of 1-benzene sulfonyl-*cis-*2,6-dimethyl piperidine (**3b**).* The general synthetic method described above affords **3b** as a colorless crystalline solid from piperidine **1** (1 g, 8.85 mmol) and benzene sulfonyl chloride **2b** (1.874 g, 10.62 mmol). IR ν_{max} 1370, 1465, 1350, 1150, 1600, 1480 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2), 2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, –CH_3), 7.8–7.9 (d, 2H, –ArH), 7.5–7.6 (t, 2H, –ArH), 7.2–7.3 (t, 1H, –ArH). mp 163–165 °C (Yield = 83%). Anal. ($C_{13}H_{19}N_1O_2S_1$) C, H, N.

*2.2.1.3. Synthesis of 1-(4-methyl benzene)-sulfonyl-*cis-*2,6-dimethyl piperidine (**3c**).* The general synthetic method described above affords **3c** as a colorless crystalline solid from piperidine **1** (1 g, 8.85 mmol) and toluene-4-sulfonyl chloride **2c** (2.023 g, 10.62 mmol). IR ν_{max} 1370, 1465, 1350, 1150, 1600, 1480 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2), 2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, –CH_3), 7.7–8.0 (d, 2H, –ArH), 7.2–7.4 (d, 2H, –ArH), 7.2–7.4 (d, 2H, –ArH), 2.3 (s, 3H, –$ArCH_3$). mp 162–164 °C (Yield = 85%). Anal. ($C_{14}H_{21}N_1O_2S_1$) C, H, N.

*2.2.1.4. Synthesis of 1-(4-*tert*-butyl benzene)-sulfonyl-*cis*-2,6-dimethyl piperidine (**3d**).* The general synthetic method described above affords **3d** as a colorless crystalline solid from piperidine **1** (1 g, 8.85 mmol) and 4-*tert*-butylbenzene sulfonyl chloride **2d** (2.46 g, 10.62 mmol). IR ν_{max} 1370, 1465, 1350, 1150, 1600, 1480 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2), 2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, –CH_3), 7.7–8.0 (d, 2H, –ArH), 7.3–7.4 (d, 2H, –ArH), 1.4 (s, 9H, –$(CH_3)_3$). mp 138–141 °C (Yield = 78%). Anal. ($C_{17}H_{27}N_1O_2S_1$) C, H, N.

*2.2.1.5. Synthesis of 1-(4-chloro benzene)-sulfonyl-*cis-*2,6-dimethyl piperidine (**3e**).* The general synthetic method described above affords **3e** as a colorless crystalline solid from piperidine **1** (1 g, 8.85 mmol) and 4-chloro benzene sulfonyl chloride **2e** (2.240, 10.62 mmol). IR ν_{max} 1370, 1465, 1350, 1150, 1600, 1450, 1050 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2), 2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, –CH_3), 7.75–7.8 (d, 2H, –ArH), 7.5–7.6 (d, 2H, –ArH). mp 173–175 °C (Yield = 80%). Anal. ($C_{13}H_{18}C_{l1}N_1O_2S_1$) C, H, N.

*2.2.1.6. Synthesis of 1-(2,5-dichloro benzene)-sulfonyl-*cis-*2,6-dimethyl piperidine (**3f**).* The general synthetic method described above affords **3f** as a colorless crystalline solid from piperidine **1** (1 g, 8.85 mmol) and 2,5-dichloro benzene sulfonyl chloride **2f** (2.607 g, 10.62 mmol). IR ν_{max} 1370, 1465, 1350, 1150, 1600, 1480, 1050 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2), 2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, –CH_3), 8.1–8.4 (t, 1H, –ArH), 7.2–7.5 (t, 1H, –ArH), 8.5–8.7 (d, 1H, –ArH). mp 178–181 °C (Yield = 82%). Anal. ($C_{13}H_{17}C_{l2}N_1O_2S_1$) C, H, N.

*2.2.1.7. Synthesis of 1-(2-nitro benzene)-sulfonyl-*cis-*2,6-dimethyl piperidine (**3g**).* The general synthetic method described above affords **3g** as a yellow crystalline solid from piperidine **1** (1 g, 8.85 mmol) and 2-nitro benzene sulfonyl chloride **2g** (2.352 g, 10.62 mmol). IR ν_{max} 1370, 1465, 1350, 1150, 1600, 1480, 1530, 1330 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2), 2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, –CH_3), 7.8–8.0 (d, 1H, –ArH), 8.1–8.4 (t, 1H, –ArH), 7.2–7.5 (d, 1H, –ArH), 8.5–8.7 (d, 1H, –ArH). mp 180–183 °C (Yield = 75%). Anal. ($C_{13}H_{18}N_2O_4S_1$) C, H, N.

*2.2.1.8. Synthesis of 1-(3-nitro benzene)-sulfonyl-*cis-*2,6-dimethyl piperidine (**3h**).* The general synthetic method described above affords **3h** as a yellow crystalline solid from piperidine **1** (1 g, 8.85 mmol) and 3-nitro benzene sulfonyl chloride **2h** (2.352 g, 10.62 mmol). IR ν_{max} 1370, 1465, 1350, 1150, 1600, 1480, 1525, 1335 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2), 2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, –CH_3), 7.5–8.3 (m, 4H, –ArH). mp 181–183 °C (Yield = 77%). Anal. ($C_{13}H_{18}N_2O_4S_1$) C, H, N.

*2.2.1.9. Synthesis of 1-(4-nitro benzene)-sulfonyl-*cis-*2,6-dimethyl piperidine (**3i**).* The general synthetic method described above affords **3i** as a yellow crystalline solid from piperidine **1** (1 g, 8.85 mmol) and 4-nitro benzene sulfonyl chloride **2i** (2.352 g, 10.62 mmol). IR ν_{max} 1370, 1465, 1350, 1150, 1600, 1480, 1530, 1330 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.3–1.8 (m, 6H, –CH_2),

Table 1
The total energies of chemguass score, chem score, PLP score and shapeguass score of the best-docked conformations.

Compd	Chemguass score	Chem score	PLP score	Screen score	Shapeguass score	Total score	IC_{50} (nM) (in vitro assay)
3a	−34.89	−15.45	−36.54	−77.95	−225.21	−390.04	85, 75, 90
3b	−47.56	−15.90	−44.17	−118.89	−324.96	−551.48	362, 392, 388
3c	−50.41	−13.86	−43.46	−107.73	−331.54	−547.00	362, 368, 365
3d	−56.66	−14.04	−43.04	−86.89	−385.61	−586.24	463, 458, 450
3e	−50.65	−13.22	−42.23	−107.00	−336.48	−549.58	325, 318, 312
3f	−52.32	−18.65	−47.58	−125.17	−348.53	−592.25	75, 90, 81
3g	NF	NF	NF	NF	NF	NF	195, 185, 180
3h	−51.36	−13.15	−43.56	−112.88	−379.42	−600.37	186, 192, 200
3i	−54.75	−9.57	−38.78	−101.13	−360.43	−564.66	1200, 1150, 1210

NF = no fit.

2.3–2.8 (m, 2H, –CH), 1.0–1.1 (d, 6H, $-CH_3$), 8.1–8.7 (m, 4H, –ArH). mp 182–184 °C (Yield = 79%). Anal. ($C_13H_18N_2O_4S_1$) C, H, N.

2.2.2. *In vitro acetylcholinesterase (AChE) assay*

The acetylcholinesterase assay method of Ellman et al. [8] was used to determine the in vitro acetylcholinesterase (AChE) activity. The activity was measured by the increase in absorbance at 412 nm due to the yellow color produced from the reaction of acetylthiocholine iodide with the dithiobisnitrobenzoate (DTNB) ion. AChE was obtained from the brain of wistar rats by homogenizing with a Teflon blender for 5 min in 0.1 M KH_2PO_4 buffer pH 8. A stock solution of the enzyme in 0.1 M KH_2PO_4 buffer (pH 8) was kept frozen. The protein concentration was estimated by the method described by Lowry et al. [9] using bovine serum albumin as standard. For each assay 300 μg of enzyme was used. Acetylthiocholine iodide was prepared freshly using 0.1 M KH_2PO_4 buffer (pH 7). A 0.01 M solution of DTNB was prepared in 0.1 M KH_2PO_4 buffer (pH 7). Crude human AChE was prepared by mixing 9 mL of fresh blood (collected from healthy volunteers by vein puncture) with 1 ml of 3.8% (w/v) trisodium citrate and centrifuging at 3000 rpm at 4 °C for 20 min. The supernatant was used as a source of AChE. Electric eel AChE was obtained from Sigma Laboratory and similar procedure was employed for the assay as that of rat brain AChE.

2.2.3. *Experimental conditions and kinetics*

Enzyme activity was measured using a Shimadzu Spectrophotometer. The assay medium contained phosphate buffer, pH 8.0 (2.6 mL), DTNB (0.1 mL), 5 μL of enzyme, 20 μL of 0.075 M substrate. The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals for 10 min at 37 °C. In dose dependent inhibition studies, the substrate was added to the assay medium containing enzyme, buffer and DTNB with inhibitor after 10 min of incubation time. Calculations were performed as described by Ellman et al. [8]. All the experiments were carried out in duplicate and the mean values are reported here. The relative activity is expressed as percentage ratio of enzyme activity in the absence of inhibitor.

2.2.4. *IC_{50} determination*

AChE inhibitor neostigmine (a reversible cholinesterase inhibitor) was used in the concentration range 25–500 nM to inhibit AChE in electric eel, human serum, and rat brain homogenate. Inhibition by piperidine **3a**–**i** derivatives was studied in the presence of different concentrations of compounds and the percentage inhibition of enzyme activity was calculated. The inhibition of AChE by piperidine derivatives was analyzed with values obtained in comparison to that of neostigmine.

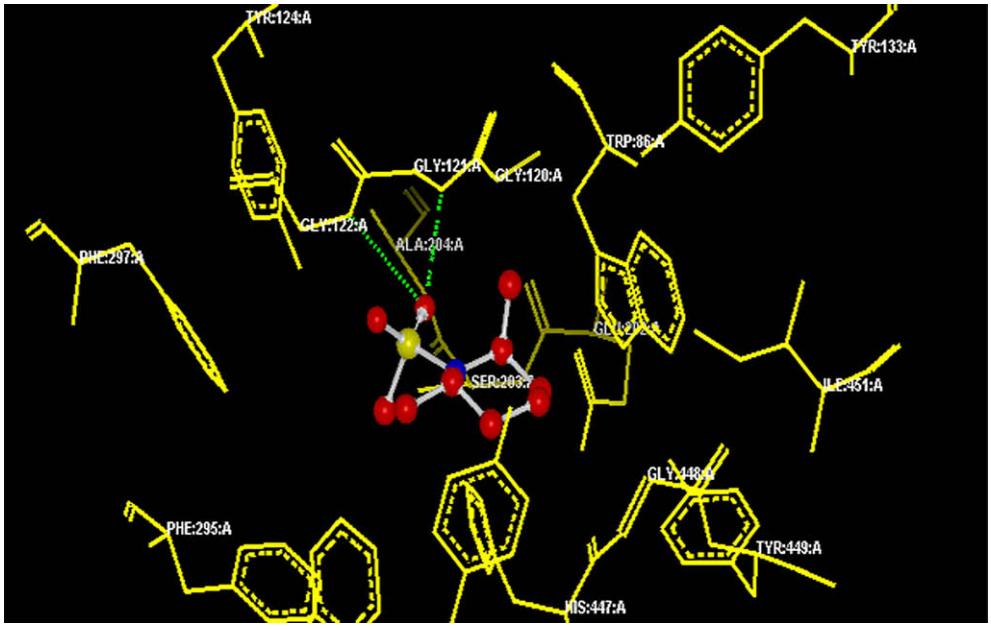


Fig. 1. Docking of *cis*-2,6-dimethyl-1-methyl sulfonyl piperidine (**3a**) on active site of mouse AChE.

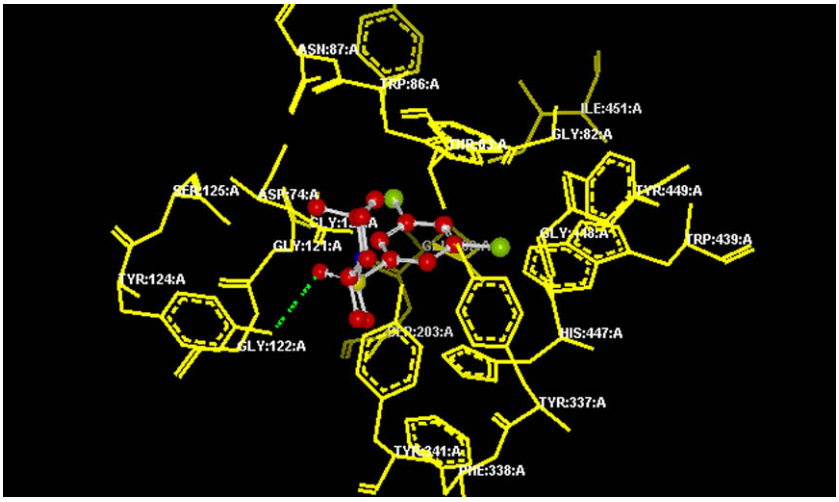


Fig. 2. Docking of 1-(2,5-chloro benzene)-sulfonyl-*cis*-2,6-dimethyl piperidine (**3f**) on active site of mouse AChE.

2.2.5. Antiamnesic effect

Antiamnesic behavioural studies were carried out for piperidine derivatives (**3a–i**) to check their ability to reverse scopolamine induced memory loss according to the method described by Sharma and Kulkarni [10,11].

2.2.6. Statistical analysis

The data was analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences and the correlations between rat brain, human serum and electric eel anticholinesterase activities were calculated by Pearson correlation using SPSS 16.0 computer software. The IC_{50} values were calculated by Boltzmann's dose–response analysis using Origin 6.1 computer software. The values were considered significant at $p \leq 0.01$.

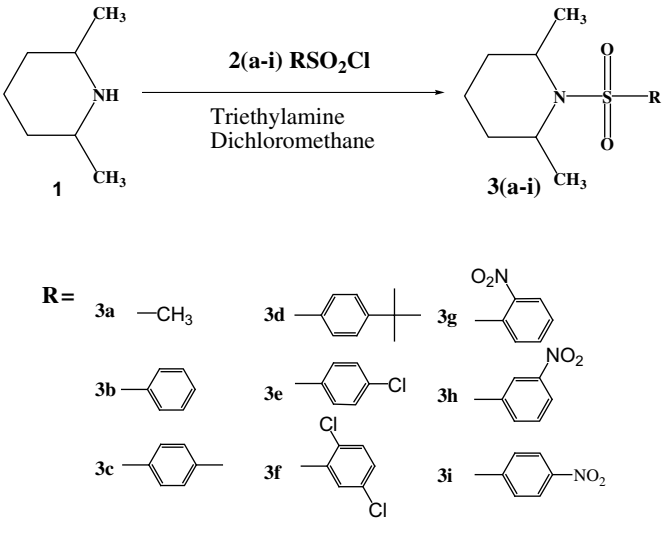
3. Results and discussion

3.1. Docking studies

Docking studies of piperidine sulfonamides (**3a–i**) on the active site of mouse AChE inhibited by tabun (PDB: 2C0P) revealed their mode of interaction, structural and positional requirements for potential AChE inhibition. These studies were further extended to in vitro AChE enzyme inhibition and in vivo pharmacological studies (rodent memory evaluation). To understand the interaction between mouse AChE and inhibitors, the AChE–inhibitor complex was generated using the OPENEYE software suite (Open Eye Scientific Software, Santa Fe, NM). Docking studies with these inhibitors show that **3a–i** are binding with a total score of −390.04, −551.48, −547.00, −586.24, −549.58, −592.25, −600.37 and −564.66 (more negative better the fit). These studies show that **3h** molecule is binding with high affinity with a chemguass score of −51.36, chem score of −13.15, PLP score of −43.56, screen score of −112.58 and, shapeguass score of −372.42 as shown in Table 1. It is evident from the figures that inhibitors are stabilized by hydrogen bonding interactions (Figs. 1 and 2). Table 1 shows the interaction energies' score including chemguass score [12], chem score [13], PLP score [14], screen score [15], shapeguass score, and consensus score for all the residues in the active site of enzyme–inhibitor complex. The hydrogen bonds present in enzyme–inhibitor complex along with their distances and angles are listed Table 2. This identification, compared with a definition based on the distance from the inhibitors can clearly show the relative significance for every residue. Hydrogen bonding interactions show that **3a** molecule is binding with nitrogen atom of Gly120 and Gly121, **3f** molecule is binding with OH group of Tyr124 as shown in Table 2 along with their distance and angles. Comparison of interaction energies of these inhibitors with other molecules shows that **3f** molecule has better binding affinity next to **3h** with a chemguass score of −52.32, chem score of −18.65, PLP score of −47.58, screen score of −125.17 and, shapeguass score of −348.53 as shown in Table 1 compared to other molecules. These results show that **3f** molecule is a very good inhibitor against the mouse AChE. These results in general also indicate that steric interactions and ligand acceptor–enzyme donor interactions, contribution from ligand

Table 2
Type of interaction between mouse AChE amino acids with **3a** and **f**. Significant binding key residues in the active site of the model were determined.

Molecule	Ligand atom	Atom protein	Distance (Å)	Angle (°)
3a	Lig O(10)	N GLY120	2.71	134.18
3a	Lig O(10)	N GLY121	3.01	101.73
3f	Lig O(15)	OH TYR124	3.27	106.09



Scheme 1.

Table 3
Comparative inhibitory activities of *cis*-2,6-dimethyl piperidine sulfonamides (**3a–i**) against AChE from different sources.

Compound	Rat brain homogenate AChE IC50 (nM)	Human serum AChE IC50 (nM)	Electric eel AChE IC50 (nM)
3a	85 ± 13.09	75 ± 10.14	90 ± 8.85
3b	362 ± 23.71	392 ± 25.30	388 ± 21.76
3c	362 ± 21.51	368 ± 23.78	365 ± 38.50
3d	463 ± 27.73	458 ± 25.51	450 ± 28.75
3e	325 ± 28.90	318 ± 25.87	312 ± 27.33
3f	75 ± 7.35	90 ± 7.35	81 ± 7.71
3g	195 ± 15.50	185 ± 11.10	180 ± 12.23
3h	186 ± 12.33	192 ± 10.55	200 ± 13.13
3i	1200 ± 55.73	1150 ± 53.71	1210 ± 49.56
Neostigmine	40 ± 5.32	41 ± 4.17	53 ± 6.73

Results are expressed as mean (±SEM).

donors' interaction with enzyme acceptors, aromatic–aromatic interactions, frozen rotatable bond penalty, lipophilic–lipophilic interactions, hydrogen bond interactions, penalty for ligand clashes with the enzyme, ligand hydrogen bond donors and acceptors, ligand non-polar atoms, interactions of ligand sulphur atoms, lipophlic–polar and polar–polar interactions, clash penalty, Piecewise Linear Potential, phenyl interactions with amides, methyl and aryl CH groups and Shape complementarity between ligand and active site of AChE are important for the protein interaction and complex interaction. Through the interaction analysis, it was found that Gly120, Gly121 and TYR124 were important anchoring residues for the inhibitor and are main contributors to the inhibitor interaction. Though the interaction energy does not include the contribution from the water or the extended enzyme structure, this preliminary data along with the list of hydrogen bond interactions between the enzyme and the active site residues clearly supports Gly120, Gly121 and TYR124 are more preferred residues in the inhibitors' binding. From the literature it is inferred that piperidine class of molecules form noncovalent bond with active site and also in vitro kinetic assay performed for these class compounds (**3a–i**) revealed that inhibition is of reversible type.

3.2. Synthetic chemistry

The reaction of *cis*-2,6-dimethyl piperidine **1** with different alkyl/aryl sulfonyl chlorides was carried out in the presence of triethylamine and dichloromethane as a solvent. The chemical structures of all the synthesized compounds (**3a–i**) are given in Scheme 1. The presence of N–H proton at 3.38 value in starting material *cis*-2,6-dimethyl piperidine **1** and the absence of this proton peak in proton NMR spectra confirm our products (**3a–i**) and also by the appearance of aromatic peaks at 7.3 to 8.7 δ value (except **3i**). Apart from this, IR data showed asymmetric stretching of S=O at 1320 cm^{-1}, bending absorption at 1370 cm^{-1} for terminal methyl group (**3a**, **c** and **d**), absorption at 1050 cm^{-1} for chloro aryl group (**3e** and **f**) and aromatic nitro group absorbs at 1525 and 1350 cm^{-1} (**3g–i**). We obtained all the products (**3a–i**) in good yield as mentioned in Scheme 1.

3.3. Experimental studies

Structure–activity relationship (SAR) of the synthesized piperidine derivatives (**3a–i**) can be drawn from in vitro findings (Scheme 1 and Table 3) that introduction of methyl group on sulfonyl-*cis*-2,6-dimethyl piperidine (**3a**) shows moderate AChE inhibition (IC_{50} = 85, 75 and 90 nm), whereas introducing electronegative chloro atom at positions 2 and 5 of phenyl ring (**3f**) on sulfonyl-*cis*-2,6-dimethyl piperidine demonstrated significant inhibitory activity (IC_{50} = 75, 90 and 81 nM). In a similar way electronegative nitro group at *meta* position (**3h**) of phenyl ring and nitro group at *ortho* position (**3g**) of the phenyl ring exerted prominent AChE inhibition (IC_{50} = 186, 192, 200 and 195, 185, 180 nM respectively). Although nitro group at *para* position (**3i**) of phenyl ring is found to be least active (IC_{50} = 1200, 1150 and 1210 nM), chloro atom at *para* position of phenyl ring (**3e**) weakens the inhibitory activity (IC_{50} = 325, 318 and 312 nM) as compared to *ortho*, *meta* substitution of chlorine atom (**3f**). Alkyl substitution at *para* position (methyl -**3c** and *tert-butyl*–**3d**) detrimental to AChE inhibitory activity (IC_{50} = 362, 368, 365, 463, 458 and 450 nM respectively). Docking studies also show that **3f** molecule was a better inhibitor compared to other molecules as shown in Table 1. A significant correlation ($r = 0.971$, $p \leq 0.01$) was observed between brain, human serum and electric eel anticholinesterase activities of all the compounds indicating effective inhibition of AChE from all the three biological sources.

Table 4
Study of antiamnesic effect *cis*-2,6-dimethyl piperidine sulfonamides (**3a–i**) against scopolamine induced memory loss.

Sl. No.	Experimental groups	Treatment (dose) mg/kg i.p.	Basal latency (s) of rat to reach shock free zone (SFZ)			Memory parameters	
			I	II	III	Latency (s)	Mistakes
1.	Control group*	Saline (0.9%)	16	03	0.7	01	07 ± 2.11
2.	Scopolamine treated group	0.4	35	09	08	05	38 ± 2.43
3.	**3a** + Scop.	0.1 + 0.4	20	04	0.7	02	10 ± 2.22
4	**3b** + Scop.	0.1 + 0.4	26	07	04	04	22 ± 3.25
5.	**3c** + Scop.	0.1 + 0.4	27	08	05	04	18 ± 2.14
6.	**3d** + Scop.	0.1 + 0.4	28	09	06	04	25 ± 1.85
7.	**3e** + Scop.	0.1 + 0.4	25	08	05	03	17 ± 2.81
8.	**3f** + Scop.	0.1 + 0.4	16	04	0.8	02	8 ± 2.43
9.	**3g** + Scop.	0.1 + 0.4	17	05	0.9	02	15 ± 2.84
10.	**3h** + Scop.	0.1 + 0.4	22	06	04	04	14 ± 2.74
11.	**3i** + Scop.	0.1 + 0.4	34	09	07	03	36 ± 2.73

Results are expressed as mean (±SEM), $n = 8$ (Scop. = Scopolamine).

In vitro AChE inhibition studies were done to support docking studies and extended these studies to in vivo pharmacological task involving the reversing amnesic effect of scopolamine induced memory loss, in passive avoidance step down task paradigm using rat as animal model, although slight structural differences are found in the active site of mouse and rat brain AChE, but showed comparable results. Compound **3a** with methyl substituent and **3f** with 2,5-dichloro substituent effectively reversed the average number of mistakes done by rats from 38 (scopolamine) to 10 and 8 respectively considerable extent (Table 4). Whereas *ortho* and *meta* nitro groups in **3g** and **h** respectively reversed the average number of mistakes (38 to 15 and 14 respectively) done by rats to moderate level. The in vivo and in vitro experimental data were correlated with the results obtained computationally.

4. Conclusion

As a part of our continued efforts in the design, synthesis and development of bioactive heterocyclic sulfonamides, herein, we have described the docking study and synthesis of alkyl and aryl *cis*-2,6-dimethyl piperidine sulfonamides along with their in vitro AChE inhibition and in vivo antidementia activity against scopolamine induced memory loss. *cis*-2,6-Dimethyl piperidine sulfonamides (**3a**–**i**) have been shown to be potentially useful, bioavailable and reversible inhibitors of AChE. These derivatives (**3a–h**, except **3g**) were superimposed on mouse AChE and was clearly shown that hydrogen bonding interactions of two derivatives (**3a** and **3f**) with active site of mouse AChE, whereas six derivatives (**3b–e, h** and **i**) just fit into active site of the enzyme and one derivative (**3g**) unable to occupy active site of the mouse AChE. Further methyl (**3a**), 2,5-dichloro phenyl (**3f**) substituents on sulfonyl-*cis*-2,6-dimethyl piperidine bind effectively and uniquely at the active site of AChE, and interact with amino acid residues Gly120, Gly121 and TYR124, that protonated piperidine binds to active site and the substituents containing alkyl or aryl groups bind to peripheral site of the enzyme. In correlation to docking results, methyl sulfonyl (**3a**) and 2,5-dichloro phenyl sulfonyl (**3f**) derivatives of *cis*-2,6-dimethyl piperidine exerted considerable AChE inhibition against different sources of AChE in vitro. Therefore it can be summarized that substitution of electronegative group at *ortho* or *meta* position of phenyl ring as R need to be explored for better AChE inhibitory activity.

Acknowledgments

The authors are grateful to the Department of Science and Technology (DST), New Delhi for financial support under the project SR/SO/HS-58/2003. The CHNS, IR and other data obtained from the instruments granted under DST-FIST and UGC-SAP (phase I) programmes are greatly acknowledged. Manish Malviya is grateful to the Indian Council of Medical Research (Ref. No. 45/51/2007/Pha/BMS) for providing the Senior Research Fellowship (IRIS Cell No. 2007-04110).

References

[1] Y.C. Martin, E.B. Danaher, C.S. May, D. Weininger, J.H. Van Drie, Strategies in drug design based on 3D-structures of ligands, in: J.L. Fauchere (Ed.), QSAR: Quantitative Structure–Activity Relationships in Drug Design, Alan R. Liss, New York, 1989, pp. 177–181.
[2] Y.C. Martin, Methods Enzymol. 203 (1991) 587–613.
[3] R.D. Cramer III, D.E. Patterson, J.D. Bunce, J. Am. Chem. Soc. 110 (1988) 5959–5967.
[4] D.B. Boyd, Successes of computer-assisted molecular design, in: D.B. Lipkowitz, Boyd (Eds.), Reviews in Computational Chemistry I, VCH Publishers, New York, 1990, pp. 355–372.
[5] C.E. Bugg, W.M. Carson, J.A. Montgomery, Sci. Am. 269 (1993) 92–98.
[6] A. Celeste Johns, Vahram Haroutunian, Drug Dev. Res. 5 (2004) 77–96.
[7] Mashkovhii, R.G. Glushkov, Pharm. Chem. J 35 (2001) 178–182.
[8] G.L. Ellman, K.D. Courtney, V. Andress Jr., F.M. Eartherstone, Biochem. Pharmacol. 7 (1961) 88–95.
[9] O.H. Lowry, O.H. Rosenbrough, A.L. Farr, R.J. Randall, J. Biol. Chem. (1951) 193–265.
[10] A.C. Sharma, S.K. Kulkarni, Methods Find. Exp. Clin. Pharmacol. 12 (1990) 175–180.
[11] A.C. Sharma, S.K. Kulkarni, Methods Find. Exp. Clin. Pharmacol. 13 (1991) 155–159.
[12] M.R. McGann, H.R. Almond, A. Nicholls, J.A. Grant, F.K. Brown, Biopolymers 68 (2003) 76–90.
[13] C.A. Baxter, C.W. Murray, D.E. Clark, D.R. Westhead, M.D. Eldridge, Proteins 33 (1998) 367–382.
[14] D.K. Gehlhaar, G.M. Verkhivker, P.A. Rejto, C.J. Sherman, D.B. Fogel, L.J. Fogel, S.T. Freer, Chem. Biol. 2 (1995) 317–324.
[15] M. Stahl, M. Rarey, J. Med. Chem. 44 (2001) 1035–1042.