Report

International Journal of Integrative Biology
A journal for biology beyond borders

IJIB
ISSN 0973-8363

Structure based designing of new inhibitors against acetylcholine esterase associated with Alzheimer's disease

Sriramamurthy Boppana [1], Nataraj Sekhar Pagadala [2], Kanchugarakoppal S Rangappa [1,*]

[1] *Department of Studies in Chemistry, University of Mysore, Mysore, India*
[2] *Department of genetics, Osmanai University, Hyderabad, India*

Submitted: 30 Sep. 2009; Revised: 6 Dec. 2009; Accepted: 10 Dec. 2009

Abstract
Structure-based generation of a novel series of AchE inhibitors were carried out with crystal structure of acetylcholine esterase complexed with choline (PDB: 2HA3) obtained from protein data bank. Extensive structure-activity relationship work was carried out with these molecules, compared with the know AChE inhibitors by performing the docking studies on crystal structure of acetylcholine esterase with choline (PDB: 2HA3) and a number of potent and selective AchE inhibitors were identified. These molecules were designed by substituting different chemical groups on imidazo (1,2-a) pyridine-8-carboxamide replacing 1 H-indene group in rutacarpine with different groups like 2-(2-oxo-2H-chromen-3-yl) imidazo (1,2-a) pyridine-8-carboxamide, N-methyl-2- (2-thienyl) imidazo (1,2-a) pyridine-8-carboxamide, imidazo (1,2-a) pyridine-8-carboxamide, 2-(2-furyl) imidazo (1,2-a) pyridine-8-carboxamide, 2-(4-nitrophenyl) imidazo (1,2-a) pyridine-8-carboxamide, 2-(4-methoxyphenyl) imidazo (1,2-a) pyridine-8-carboxamide, and 2-(3,4-dichlorophenyl) imidazo (1,2-a)pyridine-8-carboxamide. Several scoring functions were evaluated using OPENEYE software and the protein-ligand interaction score (total score) was correlated to pIC50. The results indicated that these molecules have very good binding affinity towards mouse AChE enzyme. Docking studies show that there is a general binding mode for nearly all compounds interacting with Tyr338 halfway down the gorge, and Tyr341 at the peripheral anionic site at the mouth of the gorge. The criteria for selecting compounds having greater chance of activity was developed by these protein-ligand interaction scores, predicted binding modes and key protein-ligand interactions. Accordingly synthetic studies are promisingly in progress.

Keywords: Acetylcholine esterase, structure based drug designing, Alzheimer's disease.

INTRODUCTION

Alzheimer's disease **(**AD) is the major disease of a group, which is characterized by loss of cognitive function leading to dementia. AD is estimated to account for between 50 and 60% of dementia cases in persons over 65 years of age (Francis *et al*., 1999) and is a progressive, neurodegenerative disease that primarily affects the elderly population. It is a major public health concern in developed countries because of the strains imposed on careers and financial resources by the increasing numbers of sufferers. The main symptoms associated with AD involve a decline in cognitive dysfunction, primarily memory loss (Desgranges *et al.*, 1998, Förstl *et al*., 1995) and in the later stages of the disease language deficits, depression, agitation, mood disturbances and psychosis are often seen (McGuffey *et al*., 1997). Although AD, as a defined medical condition, has only existed for about 100 years, age-related loss of memory and cognitive decline has been documented for thousands of years in human history. Postmortem studies have shown that AD is characterized by low amounts of the enzyme choline acetyl transferase (ChAT) and enzyme abnormalities that would produce levels of the neurotransmitter ACh 1 (Perry *et al*., 1978, Giacobini *et al*., 1990) and there also appears to be a depletion of nicotinic function. Attention deficit in AD is reversed with nicotine 3, which is reported to up regulate nicotinic receptors and to increase ACh release, enhancing cholinergic neurotransmission (Balfour *et al*., 1996, Whitehouse *et al*., 1995). ACh is certainly associated with cognitive function, since situations where it is blocked from acting on the cholinergic receptors by drugs such as hyoscine (scopolamine) 4,

[*] ***Corresponding author:***
Kanchugarakoppal S. Rangappa, Ph.D., FRSC., FNASc.
Department of Studies in Chemistry,
University of Mysore, Manasagangotri,
Mysore-570006, India
Email: rangappaks@gmail.com

which is a muscarinic antagonist; result in severe cognitive impairment in the patient. It is still unclear if the low levels of ACh in the CNS are cause or effect as far as AD is concerned, but the repletion of levels has been exploited therapeutically with some success in the last 15 years in the symptomatic relief of AD. The synthetic compound tacrine was the first drug introduced into the clinic and it increased the levels of ACh by inhibition of acetyl cholinesterase (AChE), the enzyme responsible for fast breakdown of ACh after its release from the nerve ending. This inhibition results in ACh having a longer half-life and therefore increasing in concentration at the synapse. Tacrine was the first of several AChE inhibitors, which have come into clinical use, but it is no longer used since the more recent introductions, generally named second-generation inhibitors, are safer and have longer-lasting effects. It should be stressed that AChE inhibitors only alleviate some of the cognitive symptoms of the disease for a time and ultimately do not arrest the cognitive decline of the patient. Another approach, which has been proposed, is the employment of cholinergic and, to some extent, nicotinic agonists, but this has not proved to be as useful therapeutically as the inhibition of cholinesterase. More recently prevention of glutamate-mediated neurotoxicity has been a therapeutic target in AD. The N-methyl-*D*-aspartate (NMDA) receptor antagonist, memantine, has been introduced in some countries for clinical use in AD patients. It should be noted that other types of activity, besides increase of neurotransmitter levels, are also being explored as leads for chemotherapeutic treatment of AD. In this work, the characterization of a new imidazo (1,2-a) pyridine-8-carboxamide derivatives, a family of orally active, potent, and selective AChE inhibitors is reported using structure based drug designing. The work includes computation of docking scores of N, 2-dimethylimidazo (1,2-a) pyridine-8-carboxamide derivatives, a correlation between the inhibitory concentration values (pIC_{50}) and the docking scores for a series of known inhibitors and a mechanistic analysis of the energy of interaction for the inhibitor with the enzyme residues.

MATERIALS AND METHODS

Docking studies of new inhibitors

Starting enzyme structure of AChE (PDB: 2HA3) was obtained from the Protein Data Bank (Bourne *et al*., 2006) and was treated as a template molecule to dock the known and unknown inhibitors. The ligands, N-methyl-2- (2-oxo-2H-chromen-3-yl) imidazo (1,2-a) pyridine-8-carboxamide analogs (Table 1), N-methyl-2-(2-thienyl) imidazo (1,2-a) pyridine-8-carboxamide analogs (Table 2), 8-(10b, 10c-dihydropyren-1-yl)-N-methyl-1, 7-diazabicyclo (4.3.0) nona-2, 4,6,8-tetraene-5-carboxamide analogs (Table 3), 2-(2-furyl)-N-methylimidazo (1,2-a) pyridine-8-carboxamide analogs (Table 4), N-methyl-2- (4-nitrophenyl) imidazo (1,2-a) pyridine-8-carboxamide analogs (Table 5), 2-(4-methoxyphenyl)-N-methylimidazo (1,2-a) pyridine-8-carboxamide analogs (Table 6), 2-(3,4-dichlorophenyl)-N-methylimidazo (1,2-a) pyridine-8-carboxamide analogs (Table 7) including all hydrogen atoms, were built and optimized with Chemsketch software suite (ACD/ChemSketch Freeware, version 10.00, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com). Extremely Fast Rigid Exhaustive Docking (FRED) version 2.1 was used for docking studies (Open Eye Scientific 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. The FRED process uses a series of shape-based filters and the default scoring function is based on Gaussian shape fitting (Schulz-Gasch ***et al.,*** 2003). The binding pocket was defined using the ligand-free protein structure and a box enclosing the binding site by 4A. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with OMEGA tool (Open Eye Scientific Software, Santa Fe, NM).A series of known inhibitors along with their inhibitory concentration values were also selected for the present study as shown in Table 8 (Yuhao *et al*., 2004; Chantal *et al*., 2004, Jamal *et al*., 2001, Marcus *et al*., 2004, Fumiyoshi *et al*., Patrick *et al*., 2004). Enzyme inhibitory activities in terms of docking scores were calculated in the present study.

Prediction of biological activity

The molecular properties and bioactivity for the inhibitors designed were predicted using Molinspiration server (www.molinspiration.com) that allows evaluation of few physicochemical properties to calculate log P (miLogP) by comparison with experimental log P provided by Syracuse Research Corporation. Molecular polar surface area (TPSA) was calculated using Erlt *et al*., 2000 approach to compute as a sum of fragment contributions. O and N centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, caco-2 permeability and blood-brain barrier penetration. Molecular volume is a method for calculation of molecule volume developed at Molinspiration is based on group contributions. These have been obtained by fitting sum of fragment contributions to "real" 3D volume for a training set of about twelve thousand, mostly drug-like molecules. 3D

molecular geometries for a training set were fully optimized by the semi empirical AM1 method. Rule of 5" Properties is set of simple molecular descriptors used by Lipinski in formulating his "Rule of 5" (Lipinski CA *et al*., 2001). First, the rule of 5 descriptors i.e., molecular mass, ClogP, the number of hydrogen bond acceptors, the number of hydrogen bond donors, were used to estimate oral absorption properties of the constituents. The molecules that met the rule of 5 were considered as good orally absorbable compounds. Secondly, the molecules that contained 10 or fewer rotatable bonds and topological molecular polar surface area (Ertl P *et al*., 2000) that were equal to or less than 140 $Å^2$ were regarded as good orally absorbable compounds (Veber D *et al*., 2002). The compounds that met both rules were considered as orally available compounds. Finally drug likeliness of these molecules was predicted using Molsoft server (http://www.molsoft.com/mprop/) based on a complex balance of various molecular properties and structure features, which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and presence of various pharmacophoric features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity and metabolic stability. Also, 6000 compounds from the WDI (World Drug Index) database were used to find a PLS regression model. The best model was found with Q2=0.99 and Rmse=1.56

RESULTS

Docking studies of new inhibitors

The new inhibitors listed in the Tables 1 to 7 were docked using OPENEYE software against AchE enzyme. These docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket. These new inhibitors were designed based on the knowledge of the ability of a protein to alter its conformation to accommodate a binding ligand and enabled us to directly compare the relative positions of the residues in the binding pocket. The active site is located 20Å from the protein surface at the bottom of a deep and narrow gorge. The only major conformational difference between the known complexes is the orientation of the phenyl ring of Phe330, a residue located in the middle of the gorge. However, the position of these inhibitors in the binding pocket is quite different, indicating that more than one clearly defined binding region exists. The known inhibitors of AChE complex were taken as positive controls for the performance of our docking studies with unknown molecules. The results of the docking studies helped us to identify the key features of the imidazo (1, 2-a) pyridine-8-carboxamide derivatives that are responsible for their high potency. The positively charged pyridine nitrogen makes a cation-p interaction with Phe330 and Trp84 and electrostatic interactions with Tyr121 and Ser122. Hydrophobic and Van-der-Waals interactions are also evident for the protein-inhibitor model. The gorge leading to the active site is lined with aromatic residues, which constitute 40% of the residues present in the binding pocket. Our inhibitors take advantage of these aromatic residues by making a variety of interactions. Van-der-Waals interactions of the pyridine ring occur with Phe331 and Tyr334. The benzyl ring of the inhibitor displays p-p stacking with the aromatic ring of Trp84. The imidazo (1, 2-a) pyridine-8-carboxymide part of the inhibitors is located at the entrance of the gorge and interacts with two aromatic residues (Trp279 and Tyr70). The favorable hydrophobic interactions analyzed using MOE software shows two main regions existing in the binding pocket. The aliphatic or aromatic fragments of the imidazo (1,2-a) pyridine-8-carboxymide inhibitors can occupy both regions. Inhibitors containing bulky groups attached to the imidazo (1, 2-a) pyridine-8-carboxymide show the highest potency among the series, pointing out the influence of the hydrophobic interaction in this area. The studies also show direct hydrogen bond between the polar groups of the inhibitors and the binding site except in case of M_{20}, M_{30} and M_{53}.

However, the polar atoms of these inhibitors are located in the proximity of polar amino acid residues. Thus, it is possible that water molecules bridge the distance between these inhibitors (M_{20}, M_{30} and M_{53}) and the protein. Water-bridged hydrogen bonds may occur between the piperidine nitrogen of the inhibitors and Tyr121 or Ser122. An additional water-bridged hydrogen bond may occur between the backbone atoms of Phe288 and the amino-group of the aminopyridazine structure. Comparable water-bridged hydrogen bonds have been observed in the X-ray structures of the AChE complexed with huperzine A, decamethonium or tacrine. The docking results of imidazo (1,2-a) pyridine-8-carboxamide analogs are given in Tables 1-7. From Tables 1-7, it is evident that the enzyme- N-(2-anthryl)-8-(2-oxochromen-3-yl)-1,7-diazabicyclo (4.3.0) nona-2,4,6,8-tetraene-5-carboxamide analog complexes have large favorable total docking score of -766.71.

Based on these findings, it appears that imidazo (1,2-a) pyridine-8-carboxamide analogs are the best inhibitors of the enzyme AchE. Also the results show that molecule 10 with NO_2 group in the fourth position on the benzene group is the best inhibitor of the series. It has the largest docking score of -766.71 (Fig. 1).The hydrogen bonds present in the enzyme- imidazo (1, 2-a) pyridine-8-carboxamide analogs complex along with their distances and angles for the top ten molecules are

listed in Table 8 and their docking poses are shown in Fig. 1. From Table 8 and Fig. 1 it was observed that these imidazo (1,2-a) pyridine-8-carboxamide analogs are binding with His287, Ser293, Val340, Phe295, Arg296, Tyr341, Asp283, Tyr124, Gln291, Trp286 and Tyr72 residues in the AChE enzyme play an important role in maintaining a functional conformation and are directly involved in donor substrate binding. Molecule10, which is shown to be the best inhibitor, binds with Ser293 and Val340, which are predicted to be the important determinant residue in the binding of inhibitors. In conclusion, we were able to design AChE inhibitors based on our molecular docking study, which seem to interact simultaneously with the cation-p sub-site of the catalytic site and the peripheral site of the enzyme. Preliminary to the design of new efficient inhibitors of the AChE enzyme with this computational approach it is useful to compare the docking scores of the known inhibitors. For this purpose, a series of known inhibitors reported in the literature have been chosen here (Table 8). Since many molecules have been reported in the literature from various sources, we have chosen a set of 30 molecules that represent the wide range of inhibition activity (from μm to nm) (Table 2). The structure rutacarpine 6 (Fig. 2) shows docking score of approximately –689.94 while the corresponding derivatives (Michael Decker 2005) show lesser values of docking score, presumably due to lesser non-bonding interactions (Table 9 and Fig. 2). A regression analysis of docking score and logIC50 for known inhibitors was carried out and the scatter plots were drawn as shown in Fig. 3. From Fig. 3 it was found that the correlation coefficient of docking score on X-axis and logIC50 values on Y-axis for known inhibitors is 2.3 (S=2.2, F=9.4 where s is the standard error of the estimate and F is the significance of the regression) with an R square value of 0.61.

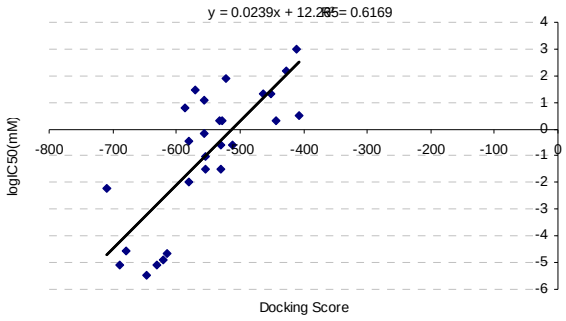

Figure 3: Correlation of experimental affinities (IC50) and their docking scores against wild type AchE calculated using OPENEYE software.

Docking studies show that quinoline ring of tacrine interacts with Trp84 with pi-stacking interaction. Studies also show that nitrogen atom of the quinoline ring of tacrine has hydrogen bond with the carbonyl group of the main chain of His440, and with the phenyl group of Phe330. (C.H.T.P. da Silva *et al*., 2006). To evaluate these docking studies site directed mutagenesis of some of these inhibitors were docked against the mutant's shows in Table 9 (Jeremy Kua *et al*., 2008). Apomarphine shows no inhibition activity against mutants W86F, W86A, E202A, E202D, E202Q and Y337F but shows reduced activity against the mutant Y337A. Aurisugacin-A shows equal activity against these mutants but shows reduced activity against Y337A by Aurisugacin-b. Atrophine shows equal activity against E202A, E202D and E202D mutants, increased activity against Y337A and reduced activity against W86F, W86A and Y337F. Cryptotranshhinone and disulphoton shows equipotent activity against E202A, E202D, and E202Q but shows increased activity against Y337F. It also shows equipotent activity W86F and W86A mutants. Oxetremorine shows equipotent activity against W86F, E202A, E202Q, and Y33F but shows increased activity against Y337A, E202D and reduced activity against W86A mutants. Rivastigmine shows equipotent activity against W86F, W86A, E202A, and E202Q but shows increased activity against E202D. It also shows reduced activity against Y337A and Y337F respectively. Rutacarpine shows equipotent activity E202A and E202Q but shows differential activity with other mutants respectively. Tarcine shows equipotent activity against W86A, E202A, E202Q, and Y337A but shows reduced activity against E202D and Y337F. Tarpene-4-ol shows equipotent activity against W86F, W86A, E202A, E202Q, and Y337F but shows increased activity against E202D and reduced activity against Y337A respectively as shown in Table 10.

Biological activity

The bioactivity of the molecules predicted using Molinspiration server showed that these inhibitors satisfy lipinskis rule of 5 with five rotatable bonds, LogP value between 1-4, molecular weight between 300 to 500 which confirms that these molecules act as best drug molecules against AchE (Table 11). The drug likeliness of the molecules calculated using molsoft server based on the comparison of the 5000 of marketed drugs (positives) and 10000 of carefully selected non-drug compounds (negatives) show that molecules 11-16, 19-20, 31-36, 38-40, 42-43, 46, 49, 53, 58, 68 and 69 given in Table 11 contain positive scores within the range of 0 to 0.7 conforming that these molecules act as drugs inhibiting AchE enzyme. The results also show that molecule 39 with a pIC50 value of -1.58μM is binding with Phe295, Ser293 and Tyr341 with a docking score of -578.92 and drug likeliness score of 0.69 predicts that the molecule if synthesized and tested against AChE mouse models show very good inhibition activity against AChE enzyme.

DISCUSSION

In the present study, to understand the binding affinity between AchE and different inhibitors, the inhibitor-protein (I-P) complex was generated using the OPENEYE software. The hydrogen bonds present in Inhibitor-protein complexes are listed in Table 4. Significant binding-site residues in the model are identified by the docking scores between the inhibitor and the amino acid residues in the active site of the enzyme.

From table 1 and 2 we can see that protein-inhibitor complex has large favorable docking score of –776.74 respectively. Through the interaction analysis, we know that His287, Ser293, Val340, Phe295, Arg296, Tyr341, Asp283, Tyr124, Gln291, Trp286 and Tyr72 important anchoring residues for the inhibitors and are the main contributors to the ligand interaction. Though the docking scores do not include the contribution from the water or the extended protein structure, this preliminary data along with the list of Hydrogen bonds (listed in Table 3) between the ligand and the active site residues in protein, supports the rationale for the apparent binding of inhibitors to AchE. In order to achieve the stated goals, a highly generic docking protocol was employed to extract the most common features of the wide variety of possible binding modes.

The Brookhaven Protein Data Bank (PDB) (http://www.rcsb.org.) contains several small molecule-AChE complexes. Some of them were already used for docking experiments, including the Torpedo californica AChE (TcAChE) complex with decamethonium (1ACL), edrophonium (2ACK), tacrine (1ACJ), and huperzine A (1VOT), respectively (Pilger *et al*., 2001; Sippl *et al*., 2001). Prior to the docking studies three assumptions were made. The first postulate refers to the orientation of Phe330. This side chain controls the access to the bottom of the gorge and was identified to adopt three major conformations, an open, a closed, and an intermediate access position, respectively (Pilger *et al*., 2001).

The closed position was identified for the native enzyme as well as for the complexes with huperzine A, edrophonium, and the transition state analog m-(N,N,N-trimethylammonio) trifluoro-acetophenone (1AMN) (Harel *et al*., 1996). All these compounds bind close to the bottom of the gorge. A different behavior can be found for tacrine. Here, Phe330 adopts an intermediate access conformation, which allows a 'sandwich-like' arrangement with tacrine and Trp84. For gorge-spanning ligands such as donepezil (1EVE) (Kryger *et al.,* 1998) and decamethonium Phe330 adopts an open access position pointing towards the gorge wall. Owing to their size and shape a gorge-spanning binding mode for the new inhibitors was assumed.

Structural modifications of rutacarpine inhibitor were carried out with different substituent's on imidazo (1,2-a) pyridine-8-carboxamide replacing 1 H-indene group in rutacarpine with different groups like 2-(2-oxo-2H-chromen-3-yl) imidazo (1,2-a) pyridine-8-carboxamide, N-methyl-2-(2-thienyl) imidazo (1,2-a) pyridine-8-carboxamide, imidazo (1,2-a) pyridine-8-carboxamide, 2-(2-furyl) imidazo(1,2-a)pyridine-8-carboxamide, 2-(4-nitrophenyl)imidazo(1,2-a)pyridine-8-carboxamide, 2-(4-methoxyphenyl)imidazo (1,2-a)pyridine-8-carboxamide, and 2-(3,4-dichlorophenyl)imidazo(1,2-a)pyridine-8-carboxamideto dissociate AchE inhibition. Docking studies of newly designed molecules predicted that N, 2-dimethylimidazo (1,2-a) pyridine-8-carboxamide analogs are the best inhibitors against the enzyme AchE. Docking studies show that M50 shows two hydrogen interactions with Ser293 and Val340 with main chain oxygen atoms, M_{10}, M_{11}, M_{31} and M_{51}, shows one hydrogen bonding interaction with Oγ atom of Ser293;M_{40}, M_{15}, M_{65} and M_{39} shows one hydrogen bonding with main chain oxygen of Tyr341M_1 and M_{38} shows one hydrogen bonding interactions with main chain oxygen atom of Ser293; M_8 shows two hydrogen bonding interaction with nitrogen atom of Arg296 and main chain oxygen atom of Tyr341; M_{35} shows two hydrogen bonding interactions with Oγ of Ser293 and nitrogen atom of Phe295; M_{70} shows one hydrogen bonding interactions with Nδ1 atom of His287; M_{12} shows one hydrogen bonding interaction with OH atom of Trp124 on the peripheral anionic site of the AChE and M_{47} shows three hydrogen bonding interaction with main chain oxygen of Trp286, Nδ1 of His287 and nitrogen atom of Phe295 respectively.

These studies show that His287, Ser293, Val340, Phe295, Arg296, Tyr341, Asp283, Tyr124, Gln291, Trp286 and Tyr72 are the important residues showing hydrogen-bonding interactions with these derivatives. Site directed muatgenic studies also shows that W86, E202 and Y337 are important determinant residues for binding of the substrate and inhibitors against the enzyme AChE. Investigation of the active site of AChE allows the identification of one peripheral anionic site (PAS) and at least five major binding sites: the oxyanion hole (OH), the esteratic site (ES), the anionic substrate binding site (AS), the active site-selective aromatic binding site (AACS), and the acyl binding site (ACS) (Lin *et al*., 1999; Eichler *et al.,* 1994).

These studies show that these inhibitors of AchE bind to the active site or to the peripheral anionic site (PAS), an allosteric site located at the active site center gorge entrance, or they span the two sites there by occupying much of the active center gorge. Mutagenesis and structural studies have revealed the functional role of the residues Tyr72, Asp74, Tyr124, Trp286, and Tyr341 at the PAS (Bourne *et al.,* 1995; Radic *et al.,*

1993; Shafferman *et al.,* 1992; Barak *et al*., 1994; Harel *et al*., 1995; Bourne *et al*., 2003, 2004). Due to this wide variety of possible binding sites, docking results were expected to be highly diverse.

Since no general statement on conserved water molecules is available, all water molecules were deleted. This adds a further degree of freedom in terms of variability in the docking results. For these reasons, the ligand-enzyme complexes (in the following referred to as docking configurations) obtained using OPENEYE were surprisingly homogenous. Inspection showed that one overall binding mode could be identified for nearly all docked compounds among these scoring docking configurations. This binding mode incorporates face-to-face interaction (π-π and cation-π, respectively) of the pyridine ring with Tyr341, which is found for nearly all compounds (Fig. 3).

Another common feature of these binding modes is the location of one of the benzyl rings outside the active site cavity. In order to interact with the protein surface this part of the molecule needs to be moved in a sterically unfavorable manner. Therefore, it is expected that interactions with this substructure will have little or no effect on the binding of the ligand to the enzyme, since the intramolecular penalty will overcompensate the gain in interaction energy.

Studies using Molsoft server predicted that N, 2-dimethylimidazo (1, 2-a) pyridine-8-carboxamide analogs are the best inhibitors against AchE. The inhibitory activity depends sensitively on the substitution pattern on the benzyl rings. This experimental finding can be explained by docking studies whose goal was to thoroughly explore possible binding modes of the imidazo (1, 2-a) pyridine-8-carboxamide analogs. One major observation is that almost all of the compounds docked display a general binding mode. These types of interaction are already known for other crystallized inhibitors of AChE (e.g. donepezil, galanthamine) and therefore the docking results are likely to be meaningful for the new compounds. Moreover, all compounds are potentially able to bind inside the active side gorge, although, the whole molecule is not able to interact with amino acid residues of the enzyme. This 'size problem' of the ligands may be one reason for their reduced activity as compared to other AChE inhibitors. McCammon and coworkers addressed this problem with extensive molecular dynamics studies (Shen *et al*., 2002). These authors were able to show that the primary entrance to the gorge of AChE is working as a bottleneck, which is not likely to hinder the binding of ACh significantly. Larger ligands, however, may escape by diffusion before fluctuations open the bottleneck wide enough to allow binding.

Correlation of the docking scores and predicted IC_{50} values showed R^2 value of 0.61. Since the R^2 values are 0.6, they indicate that these molecules act as best inhibitors against AchE. The results obtained so far will be used to develop novel second generation AChE inhibitor candidates structurally related to the known compounds*** n the present study, to understand the binding affinity between AchE and different inhibitors, the inhibitor-protein (I-P) complex was generated using the OPENEYE software. The hydrogen bonds present in Inhibitor-protein complexes are listed in Table 4. Significant binding-site residues in the model are identified by the docking scores between the inhibitor and the amino acid residues in the active site of the enzyme. From table 1 and 2 we can see that protein-inhibitor complex has large favorable docking score of –776.74 respectively. Through the interaction analysis, we know that His287, Ser293, Val340, Phe295, Arg296, Tyr341, Asp283, Tyr124, Gln291, Trp286 and Tyr72 important anchoring residues for the inhibitors and are the main contributors to the ligand interaction. Though the docking scores does not include the contribution from the water or the extended protein structure, this preliminary data along with the list of Hydrogen bonds (listed in Table 3) between the ligand and the active site residues in protein, supports the rationale for the apparent binding of inhibitors to AchE. In order to achieve the stated goals, a highly generic docking protocol was employed to extract the most common features of the wide variety of possible binding modes. The Brookhaven Protein Data Bank (PDB) (http://www.rcsb.org.) contains several small molecule-AChE complexes. Some of them were already used for docking experiments, including the Torpedo californica AChE (TcAChE) complex with decamethonium (1ACL), edrophonium (2ACK), tacrine (1ACJ), and huperzine A (1VOT), respectively (Pilger *et al*., 2001; Sippl *et al*., 2001). Prior to the docking studies three assumptions were made. The first postulate refers to the orientation of Phe330. This side chain controls the access to the bottom of the gorge and was identified to adopt three major conformations, an open, a closed, and an intermediate access position, respectively (Pilger *et al*., 2001). The closed position was identified for the native enzyme as well as for the complexes with huperzine A, edrophonium, and the transition state analog m-(N,N,N-trimethylammonio) trifluoro-acetophenone (1AMN) (Harel *et al*., 1996). All these compounds bind close to the bottom of the gorge. A different behavior can be found for tacrine. Here, Phe330 adopts an intermediate access conformation, which allows a 'sandwich-like' arrangement with tacrine and Trp84. For gorge-spanning ligands such as donepezil (1EVE) (Kryger *et al.,* 1998) and decamethonium Phe330 adopts an open access position pointing towards the gorge wall. Owing to their size and shape a gorge-spanning binding mode for the new inhibitors was assumed. Structural modifications of rutacarpine inhibitor were carried out with different

substituent's on imidazo (1,2-a) pyridine-8-carboxamide replacing 1 H-indene group in rutacarpine with different groups like 2-(2-oxo-2H-chromen-3-yl) imidazo (1,2-a) pyridine-8-carboxamide, N-methyl-2-(2-thienyl) imidazo (1,2-a) pyridine-8-carboxamide, imidazo (1,2-a) pyridine-8-carboxamide, 2-(2-furyl) imidazo(1,2-a)pyridine-8-carboxamide, 2-(4-nitrophenyl)imidazo(1,2-a)pyridine-8-carboxamide, 2-(4-methoxyphenyl)imidazo(1,2-a)pyridine-8-carboxamide, and 2-(3,4-dichlorophenyl)imidazo(1,2-a)pyridine-8-carboxamideto dissociate AchE inhibition. Docking studies of newly designed molecules predicted that N, 2-dimethylimidazo (1,2-a) pyridine-8-carboxamide analogs are the best inhibitors against the enzyme AchE. Docking studies show that M50 shows with two hydrogen interactions with Ser293 and Val340 with main chain oxygen atoms, M_{10}, M_{11}, M_{31} and M_{51}, shows one hydrogen bonding interaction with Oγ atom of Ser293; M_{40}, M_{15}, M_{65} and M_{39}, shows one hydrogen bonding with main chain oxygen of Tyr341; M_1 and M_{38} shows one hydrogen bonding interactions with main chain oxygen atom of Ser293; M_8 shows two hydrogen bonding interaction with nitrogen atom of Arg296 and main chain oxygen atom of Tyr341; M_{35} shows two hydrogen bonding interactions with Oγ of Ser293 and nitrogen atom of Phe295; M_{70} shows one hydrogen bonding interactions with Nδ1 atom of His287; M_{12} shows one hydrogen bonding interaction with OH atom of Trp124 on the peripheral anionic site of the AChE and M_{47} shows three hydrogen bonding interaction with main chain oxygen of Trp286, Nδ1 of His287 and nitrogen atom of Phe295 respectively. These studies show that His287, Ser293, Val340, Phe295, Arg296, Tyr341, Asp283, Tyr124, Gln291, Trp286 and Tyr72 are the important residues showing hydrogen-bonding interactions with these derivatives. Site directed muatgenic studies also shows that W86, E202 and Y337 are important determinant residues for binding of the substrate and inhibitors against the enzyme AChE. Investigation of the active site of AChE allows the identification of one peripheral anionic site (PAS) and at least five major binding sites: the oxyanion hole (OH), the esteratic site (ES), the anionic substrate binding site (AS), the active site-selective aromatic binding site (AACS), and the acyl binding site (ACS) (Lin *et al*., 1999; Eichler *et al.,* 1994). These studies show that these inhibitors of AchE bind to the active site or to the peripheral anionic site (PAS), an allosteric site located at the active site center gorge entrance, or they span the two sites there by occupy much of the active center gorge. Mutagenesis and structural studies have revealed the functional role of the residues Tyr72, Asp74, Tyr124, Trp286, and Tyr341 at the PAS (Bourne *et al.,* 1995; Radic *et al.,* 1993; Shafferman *et al.,* 1992; Barak *et al*., 1994; Harel *et al*., 1995; Bourne *et al*., 2003, 2004). Due to this wide variety of possible binding sites, docking results were expected to be highly diverse. Since no general statement on conserved water molecules is available, all water molecules were deleted. This adds a further degree of freedom in terms of variability in the docking results. For these reasons, the ligand-enzyme complexes (in the following referred to as docking configurations) obtained using OPENEYE were surprisingly homogenous. Inspection showed that one overall binding mode could be identified for nearly all docked compounds among these scoring docking configurations. This binding mode incorporates face-to-face interaction (π-π and cation-π, respectively) of the pyridine ring with Tyr341, which is found for nearly all compounds (Fig. 3). Another common feature of these binding modes is the location of one of the benzyl rings outside the active site cavity. In order to interact with the protein surface this part of the molecule needs to be moved in a sterically unfavorable manner. Therefore, it is expected that interactions with this substructure will have little or no effect on the binding of the ligand to the enzyme, since the intramolecular penalty will overcompensate the gain in interaction energy. Studies using Molsoft server predicted that N, 2-dimethylimidazo (1, 2-a) pyridine-8-carboxamide analogs are the best inhibitors against AchE. The inhibitory activity depends sensitively on the substitution pattern on the benzyl rings. This experimental finding can be explained by docking studies whose goal was to thoroughly explore possible binding modes of the imidazo (1, 2-a) pyridine-8-carboxamide analogs. One major observation is that almost all of the compounds docked display a general binding mode. These types of interaction are already known for other crystallized inhibitors of AChE (e.g. donepezil, galanthamine) and therefore the docking results are likely to be meaningful for the new compounds. Moreover, all compounds are potentially able to bind inside the active side gorge, although, not the whole molecule is able to interact with amino acid residues of the enzyme. This 'size problem' of the ligands may be one reason for their reduced activity as compared to other AChE inhibitors. McCammon and coworkers addressed this problem with extensive molecular dynamics studies (Shen *et al*., 2002). These authors were able to show that the primary entrance to the gorge of AChE is working as a bottleneck, which is not likely to hinder the binding of ACh significantly. Larger ligands, however, may escape by diffusion before fluctuations open the bottleneck wide enough to allow binding. Correlation of the docking scores and predicted IC_{50} values showed R^2 value of 0.61. Since the R^2 values are 0.6, they indicate that these molecules act as best inhibitors against AchE. The results obtained so far will be used to develop novel second generation AChE inhibitor candidates structurally related to the known compounds.

References

Balfour DJK (1996) Pharmacology of nicotine and its therapeutic use in smoking cessation and neurodegenerative disorders. *Pharmacol. Ther.*, **72**: 51–81.

Barak D, *et al*. (1994) Acetylcholinesterase peripheral anionic site degeneracy conferred by amino acid arrays sharing a common core. *J. Biol. Chem.*, **269**: 6296-6305.

Bourne Y, *et al*. (1995) Acetylcholinesterease inhibition by fasciculin: crystal structure of the complex. *Cell*, **83**: 503-512.

Bourne Y, *et al*. (2003) Structural insights into ligand interactions at the acetylcholinesterase peripheral anionic site. *EMBO J.*, **22**: 1-12.

Bourne Y, *et al*. (2004) Freeze-frame Inhibitor Captures Acetylcholinesterase in a Unique Conformation. *Proc. Natl. Acad. Sci. USA*, **101**: 1449-1454.

Bourne Y, *et al*. (2006) Substrate and product trafficking through the active center gorge of acetyl cholinesterase analyzed by crystallography and equilibrium binding. *J. Biol.Chem.*, **281**: 29256-29267

Chantal JGM, *et al*. (2004) Block of Neuronal Nicotinic Acetylcholine Receptors by Organophosphate Insecticides. *Toxicological sciences*, **82**: 545–554

da Silva CHTP, *et al*. (2006) Molecular modeling, docking and ADMET studies applied to the design of a novel hybrid for treatment of Alzheimer´s disease. *Journal of Molecular Graphics and Modelling*, **25**: 169-175.

Desgranges B, *et al*. (1998) The neural substrates of memory systems impairment in Alzheimer's disease. *Brain*, **121**: 611–631.

Eichler J, *et al*. (1994) Differential effects of "peripheral" site ligands on Torpedo and chicken acetylcholinesterase. *Mol. Pharmacol.*, **45**: 335-340.

Ertl P, *et al*. (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *J. Med. Chem.*, **43**: 3714–3717.

Förstl H, *et al*. (1995) Age-associated memory impairment and early Alzheimer' disease. *Drug Res.*, **45**: 394–397.

Francis, *et al*. (1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J. Neurol. Neurosurg. Psychiatry*, **66**: 137–147.

Fumiyoshi Kuno, *et al*. (1996) Arisugacins A and B, Novel and Selective Acetylcholinesterase Inhibitors from Penicillium sp. FO-4259. *The journal of antibiotics*, **8**: 742-747.

Giacobini E (1990) The cholinergic system in Alzheimer disease. *Prog Brain Res.*, **84**: 321–332.

Harel M (1996) The structure of a transition state analog complex reveals the molecular origins of the catalytic power and substrate specificity of acetyl cholinesterase, *J. Am. Chem. Soc.*, **118**: 2340-2346.

Harel M, *et al*. (1995) Crystal structure of an acetylcholinesterase-fasciculin complex: interaction of a three-fingered toxin from snake venom with its target, *Structure*, **3**: 1355-1366.

Jamal E, *et al*. (2001) Prediction of Organophosphorus Acetylcholinesterase Inhibitio Using Three-Dimensional Quantitative Structure-Activity Relationship (3D-QSAR) Methods, *Toxicological sciences*, **63**: 223–232.

Jeremy Kua, *et al*. (2003) studying the roles of W86, E202, and Y337 in binding of acetylcholine to acetyl cholinesterase using a combined molecular dynamics and multiple docking approaches. *Protein Sci.*, **12**: 2675-2684

Kryger G, *et al*. 1998) Three-dimensional structure of a complex of E2020 with acetylcholinesterase from Torpedo californica. *J. Physiol. Paris*, **92**: 191-194.

Decker M (2005) Novel inhibitors of acetyl- and butyrylcholinesterase derived from the alkaloids dehydroevodiamine and rutaecarpine, *Eur. J. Med. Chem.*, **40**(3): 305-13.

Lin G, *et al*. (1999) Molecular recognition by acetylcholinesterase at the peripheral anionic site: structure–activity relationships for inhibitions by aryl carbamates. *Bioorg. Med. Chem.*, 7: 2683-2689.

Lipinski CA, *et al*. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Adv. Drug Del. Rev.*, **46**: 3-26.

Marcus A, *et al*. (2004) Compound library development guided by protein structure similarity clustering and natural product structure. *PNAS*, **101**: 16721–16726.

McGuffey EC (1997) Alzheimer's disease: An overview for the pharmacist. *JAMA*, **37**: 347–352.

Patrick Friederich MD, *et al*. (2000) Stereospecific Interaction of Ketamine with Nicotinic Acetylcholine Receptors in Human Sympathetic Ganglion-like SH-SY5Y Cells, *Anesthesiology*, **93**: 818–824.

Perry E, *et al*. (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *BMJ*, **2**: 1457–1459.

Pilger C (2001) Accurate prediction of the bound conformation of galanthamine in the active site of torpedo californica acetylcholinesterase using molecular docking, *J. Mol. Graphics Modell.*, **19**: 288-296.

Radic Z, *et al*. (1993) Three distinct domains in the cholinesterase molecule confer selectivity for acetyl and butyrylcholinesterase inhibitors. *Biochemistry*, **32**: 12074-12084.

Schulz-Gasch T, *et al*. (2003). Binding site characteristics in structure based virtual screening: evaluation of current docking tools. *J. Mol. Model.*, **9**: 47-57.

Shafferman A (1992) Mutagenesis of human acetylcholinesterase. Identification of residues involved in catalytic activity and in polypeptide folding. *J. Biol. Chem.*, **267**: 17640-17648.

Shen T, *et al*. (2002) Molecular Dynamics of Acetylcholinesterase. *Acc. Chem. Res.*, **35**: 332-340.

Sippl W, *et al*. (2001) Structure-based 3D QSAR and design of novel acetylcholinesterase inhibitors. *J. Comput.-Aided Mol. Design*, **15**: 395-410.

Veber D, *et al*. (2002) Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.*, **45**: 2615–2623.

Whitehouse PJ (1995) Nicotinic receptors and neurodegenerative dementing diseases: Basic research and clinical implications. *Alzheimer Dis. Assoc. Disord.*, **9**: 93–95.

Yuhao Ren1 Peter J, *et al*. (2004) Novel Diterpenoid Acetylcholinesterase Inhibitorsfrom Salvia miltiorhiza. *Planta Med.* **70**: 201- 204.