

Structural insight into the inhibition of acetylcholinesterase by 2,3,4, 5-tetrahydro-1, 5-benzothiazepines

Sarfraz A. Nawaz, Sumaira Umbreen, Asaad Kahlid, Farzana L. Ansari, Atta-Ur-Rahman & M. Iqbal Choudhary

To cite this article: Sarfraz A. Nawaz, Sumaira Umbreen, Asaad Kahlid, Farzana L. Ansari, Atta-Ur-Rahman & M. Iqbal Choudhary (2008) Structural insight into the inhibition of acetylcholinesterase by 2,3,4, 5-tetrahydro-1, 5-benzothiazepines, Journal of Enzyme Inhibition and Medicinal Chemistry, 23:2, 206-212, DOI: [10.1080/14756360701533080](https://doi.org/10.1080/14756360701533080)

To link to this article: <https://doi.org/10.1080/14756360701533080>



Published online: 04 Oct 2008.



Submit your article to this journal [↗](#)



Article views: 703



View related articles [↗](#)



Citing articles: 2 View citing articles [↗](#)

Structural insight into the inhibition of acetylcholinesterase by 2,3,4,5-tetrahydro-1, 5-benzothiazepines

SARFRAZ A. NAWAZ¹, SUMAIRA UMBREEN², ASAAD KAHLID¹, FARZANA L. ANSARI², ATTA-UR-RAHMAN¹, & M. IQBAL CHOUDHARY¹

¹Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan, and ²Department of Chemistry, Quaid-i-Azam University, Islamabad-45320, Pakistan

(Received 5 January 2007; accepted 26 May 2007)

Dedicated to the memory of Dr. Mohammad Hussain Panjwani (1940–1992), a renowned philanthropist and a scholar

Abstract

Benzothiazepines **1–3** inhibited acetylcholinesterase (AChE; EC 3.1.1.7) enzyme in a concentration-dependent fashion with IC_{50} values of 1.0 ± 0.002 , 1.2 ± 0.005 and $1.3 \pm 0.001 \mu\text{M}$, respectively. By using linear-regression equations, Lineweaver-Burk, Dixon plots and their secondary replots were constructed which indicated that compounds **1–3** are non-competitive inhibitors of AChE with K_i values of 0.8 ± 0.04 , 1.1 ± 0.002 , and $1.5 \pm 0.001 \mu\text{M}$, respectively. Molecular docking studies revealed that all the compounds are completely buried inside the aromatic gorge of AChE, extending deep into the gorge of AChE. A comparison of the docking results of compounds **1–3** displayed that these compounds generally adopt the same binding mode in the active site of AChE. The superposition of the docked structures demonstrated that the non-flexible benzothiazepine always penetrate into the aromatic gorge through the six-membered ring A, which allowed the ligands to interact simultaneously with more than one subsites of the active center of AChE. The higher AChE inhibitory potential of compounds **1–3** was found to be the cumulative effect of hydrophobic contacts and π - π interactions between the ligands and AChE. The relatively high affinity of benzothiazepine **1** with AChE was found to be due to additional hydrogen bond in benzothiazepine **1**-AChE complex. The results indicated that substitution of halogen and methyl groups by hydrogen at aromatic ring of the benzothiazepine decreased the affinity of these molecules towards enzyme that may be due to the polar non-polar repulsions of these moieties with the amino acid residues in the active site of AChE. The observed binding modes of benzothiazepines **1–3** in the active site of AChE explain the affinities of benzothiazepines and provide a rational basis for the structure-based drug design of benzothiazepines with improved pharmacological properties.

Keywords: Benzothiazepines, acetylcholinesterase inhibition, molecular docking

Abbreviations: AChE, Acetylcholinesterase; PAS, Peripheral anionic site; AD, Alzheimer's disease

Introduction

The major function of AChE (EC 3.1.1.7) enzyme is the hydrolysis of the neurotransmitter acetylcholine [1]. According to the cholinergic hypothesis, the memory impairment in the patients with senile dementia of Alzheimer's disease (AD) results from a deficiency in cholinergic function in the brain [2]. Hence, the most promising therapeutic strategy for activating the central

cholinergic functions has been the use of cholinomimetic agents. The function of AChE inhibitors is to boost up the endogenous levels of acetylcholine in the brains of patients suffering from AD and thereby, to increase cholinergic neurotransmission.

The co-crystallization of AChE with transition-state analogs and different inhibitors has provided valuable information about the binding modes of substrate/inhibitor and possibility to apply the docking protocols

Correspondence: S. A. Nawaz, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical Sciences, University of Karachi, Karachi 75270, Pakistan. Tel: 92 21 4819010, 4824924. Fax: 92 21 4819018, 4819019. E-mail: hej@cyber.net.pk; E-mail: sarfraz_509@yahoo.com

for the protein-inhibitor complexes. The position of the known inhibitors in the binding pocket is often different; this suggests that more than one clearly defined binding sites exist. These binding sites are called esteratic and anionic subsites. Esteratic subsite contains catalytic triad (Ser200, His440 and Glu327)[3] which interact with the cationic substrates and oxyanion hole (Gly118, Gly119, and Ala201)[4]. The anionic subsite (Trp84, Phe330 and Glu199) is responsible for binding the quaternary trimethyl ammonium tail group of ACh by cation- π interaction [5]. The peripheral anionic site is situated >10 Å above the active site triad and near the opening of the aromatic gorge. The peripheral site of AChE includes Tyr70, Asp72, Tyr121, Glu278, Tyr334 and Trp279 residues. The aromatic residues lining the gorge at the outer rim of the gorge, has been postulated to be involved in the initial binding and guiding of the substrate towards the active site [6].

Presently, about 30% of life saving drugs perform their function through enzyme inhibition pathways. Therefore, the discovery of new enzyme inhibitors has been an exciting area of pharmaceutical research that has led to many interesting discoveries in drug development. We already have reported a number of new inhibitors of cholinesterase (AChE and BChE) enzymes [7–10]. The inhibition kinetics, pharmacological profiles, molecular docking, 3D-QSAR (CoMFA and CoMSIA) studies have also been conducted for a good number of compounds [11–15]. Continuing our on going search for new inhibitors of therapeutically significant enzymes through high-throughput screening assays, we recently synthesized benzothiazepines **1–3**, with valuable AChE inhibitory potential [16].

The main focus of the current study were to predict the possible binding interactions of benzothiazepines **1–3** in the active-site of AChE by employing kinetics and molecular docking techniques.

Materials and methods

Cholinesterases inhibition assay

Electric-eel (*Torpedo californica*) AChE (type VI-S), acetylthiocholine iodide, 5, 5'-dithiobis [2-nitrobenzoic acid] (DTNB), and galanthamine were purchased from Sigma (St. Louis, MO, USA). AChE inhibiting activities were measured by the spectrophotometric method developed by Ellman et al. [17]. The standard operational assay protocol was employed to determine the AChE inhibition activity of benzothiazepines **1–3** by modifying the spectrophotometric method [18]. Acetylthiocholine iodide was used as the substrate to assay AChE activity. The reaction mixture contained 150 μ L of sodium phosphate buffer (100 mM) (pH 8.0), 10 μ L of DTNB, 10 μ L of test-compound solution and 20 μ L of AChE which were mixed and incubated for 15 min (25°C). The reaction was then

initiated by the addition of 10 μ L acetylthiocholine. The hydrolysis of acetylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion resulting from the reaction of DTNB with thiocholine, catalyzed by acetylthiocholine at a wavelength of 412 nm (15 min). Test-compounds and the positive control (galanthamine) were dissolved in EtOH. As the extinction coefficient of the yellow anion is known, the rate of enzymatic reaction was finally determined by Ellman equation [17].

$$\text{Rate (mols/L/ min.)} = \frac{\text{Change in absorbance/ min.}}{13,600}$$

All the kinetic experiments were performed in 96-well microtitre-plates by using SpectraMax 340 (Molecular Devices, CA, USA).

Determination of kinetic parameters

The concentration of compounds that inhibited the hydrolysis of substrate (acetylthiocholine) by 50% (IC_{50}) was determined by monitoring the effect of various concentrations of the compound in the assays on the inhibition values. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA). Dissociation constant/inhibition constants (K_i) was determined by the interpretation of Dixon plots [19]. Lineweaver-Burk plot [20] and their secondary replots using initial velocities obtained over a substrate concentration range between 0.1–0.4 mM for acetylthiocholine iodide (ATCh). The dependency of $V_{\max}/K_m$ and $V_{\max}$ on inhibitor [I] can be described as:

$$V_{\max}/K_m = \left(\frac{(V_{\max}/K_m)_{K_i}}{K_i + [I]} \right)$$

Linear regression equations were used to determine the values of K_i , K_m and $V_{\max}$ in the Lineweaver-Burk plot and Dixon plots. The K_i value [dissociation constant/inhibition constant of AChE-inhibitor or free AChE and inhibitor] was determined graphically by Dixon plot and Lineweaver-Burk plots; firstly, $1/V_{\max_{app}}$ was calculated at each intersection points of lines of every inhibitor concentration on y-axis of the Lineweaver-Burk plot and then replotted against various concentrations of the inhibitor. Secondly, the slope of each line of the inhibitor concentration on Lineweaver-Burk plot was plotted against inhibitor concentrations followed by the replotting of slope versus various concentrations of inhibitor, K_i was the intercept on x-axis.

Statistical analysis

Graphs were plotted using GraFit program [21]. Values of the correlation coefficients, slopes, intercepts

and their standard errors were obtained by the linear regression analysis using the same program. The correlation for all the lines of all graphs was found to be >0.99 . Each point in the constructed graphs represents the mean of three experiments.

Molecular docking studies

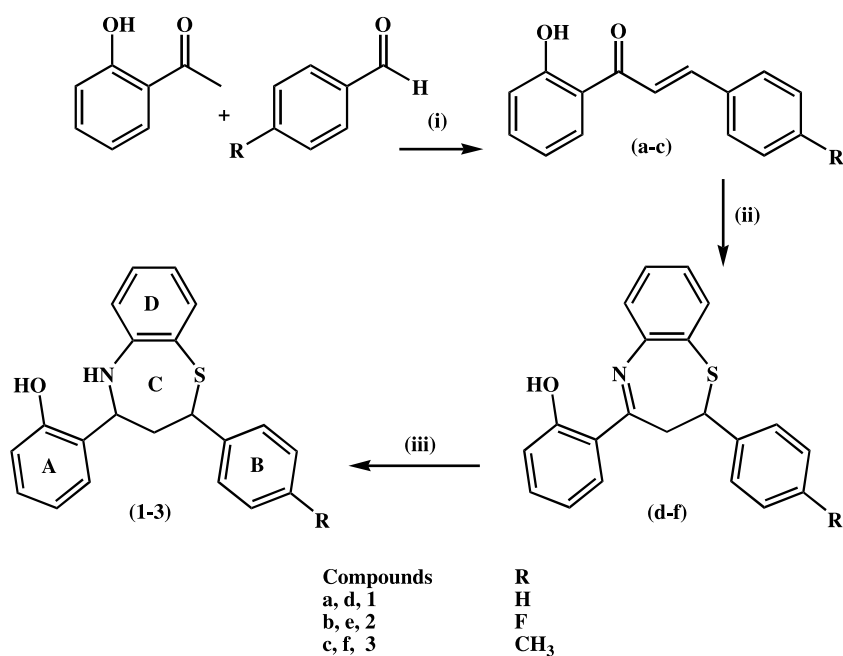
The three-dimensional structures of benzothiazepines **1–3** were constructed and optimized using the SYBYL program [22]. Energy minimization was performed using the tripos force field with a distance gradient algorithm with convergence criterion of $0.05 \text{ kCal}/(\text{mol}\text{\AA})$ and a maximum of 1000 interactions, respectively. The FlexX method was applied to dock compounds **1–3** with most of the default parameters, in the aromatic gorge of AChE complexed with decamethonium (PDB id; 1ACL) [23]. A radius of $6.5 \text{ \AA}$ was used to define the active site interactions points. The docking results were analyzed by VMD [24] and LIGPLOT softwares [25]. All the computational studies were performed by using compute server on a dual processor 1.5 GHz Intel based PC running the LINUX SUSE 8.2 (Kernel 2.4) operating system.

Results and discussion

The synthesis of variety of chalcones **2**, 3-dihydro-1, 5-benzothiazepines and their corresponding 2,3,4,5-tetrahydrobenzothiazepines has earlier been reported [16] and studies on their AChE and BChE inhibitory

potential were also carried out. However, it was found that only 2,3,4,5-tetrahydrobenzothiazepines showed a significant AChE inhibition; Therefore, it was considered worthwhile to have a deeper insight in the mechanism of interaction of these benzothiazepines with AChE by kinetics and molecular docking studies. AChE inhibitory potential of benzothiazepines **1–3** (see Scheme 1) was measured by using electric eel (*Torpedo californica*) (1ACL) AChE because oligomeric forms of electric eel AChE are similar to those of vertebrate's nerves and muscles AChE [7]. Furthermore, results of studies conducted on this enzyme can be correlated with the molecular modeling studies by the coordinates of electric eel AChE X-ray structure.

Benzothiazepines **1–3** inhibited AChE enzyme in a concentration-dependent manner with K_i values 0.8 ± 0.04 , 1.1 ± 0.01 , and $1.5 \pm 0.001 \mu\text{M}$, respectively. K_i values were calculated in three different ways; firstly, the slopes of each line in the Lineweaver-Burk plot were plotted against different concentrations of inhibitors, secondly the $1/V_{\text{maxapp}}$ was calculated by plotting different fixed concentrations of ATCh versus ΔV in the presence of different fixed concentrations of inhibitors in the assay of AChE. Then K_i was calculated by plotting different concentrations of inhibitor versus $1/V_{\text{maxapp}}$, K_i was the intercept on the x-axis. Thirdly, K_i was directly measured from Dixon plot as an intercept on x-axis. Determination of type of inhibition is important because it specifies the mechanism of enzyme action and the sites where inhibitor binds. Lineweaver-Burk,



(i) EtOH aq 4M NaOH, 3h, (ii) conc.HCl anh. MeOH, 2-aminobenzothiophenol, (iii) LiAlH₄ anh. THF, 20-120 min.

Scheme 1. General scheme for the synthesis of 2,3,4,5-tetrahydro-1,5-benzothiazepines (**1–3**).

Table I. Steady-state inhibition of AChE by benzothiazepines 1–3.

Benzothiazepines	IC_{50} (μM) $\pm$ SEM	K_i (μM) $\pm$ SEM	K_m (μM)	K_{mapp} (μM)	V_{max} ($\mu\text{mol L}^{-1}\text{min}^{-1}$)	V_{maxapp} (mean) ($\mu\text{mol L}^{-1}\text{min}^{-1}$)	V_{maxapp} / K_m ($\text{min}^{-1}\text{U}^{-1}$)	Type of Inhibition
1	1.0 ± 0.002	0.8 ± 0.04	0.12	0.12	5	2.0	16.6	NC
2	1.2 ± 0.005	1.1 ± 0.01	0.12	0.12	5	2.1	17.5	NC
3	1.3 ± 0.001	1.5 ± 0.001	0.12	0.19	5	2.2	18.3	NC
Gаланthамine*	0.5 ± 0.001	0.48 ± 0.002	0.12	0.12	5	2.5	20.8	MT

NC; Non-competitive, MT; Mixed-type, *Standard inhibitor of AChE; K_i (dissociation constant or inhibition constant) was determined from linear regression analysis by Dixon plot and secondary Lineweaver-Burk plot at various concentrations of benzothiazepine 1–3, K_m (Michaelis-Menten constant) is equal to the reciprocal of x-axis intersection, V_{max} (maximal velocity) is equal to the reciprocal of y-axis intersection of each line for each concentration of benzothiazepine 1–3 in the Lineweaver-Burk plot. The V_{maxapp} is equal to the reciprocal of y-axis intersection of each line for each concentration of benzothiazepine 1–3 in Dixon plot (Each point in Lineweaver-Burk, Dixon plot and in their secondary replots represents the mean of three experimental determinations).

Dixon plots and their secondary replots indicated that benzothiazepines 1–3 are non-competitive inhibitors of AChE as in the presence of these compounds there was a decrease in V_{max} without affecting the affinity (K_m values) of the AChE towards the substrate (ATCh). In other words, inhibitor and ATCh bind randomly and independently at the different sites of AChE, indicating that inhibition depends mainly on the concentration of inhibitor and dissociation constant (K_i). On the other hand, in the presence of galanthamine (standard inhibitor) a decrease in V_{max} was observed with an increase of K_m indicating a linear mixed-type of inhibition. Generally, this type of inhibition is the result of combination of partial competitive and a pure non-competitive type of inhibition. The K_i , K_m , K_{mapp} , V_{max} , V_{maxapp} , V_{maxapp} / K_m , and IC_{50} values and the type of inhibition has been listed in Table I. The graphical presentation of steady-state inhibition of benzothiazepine 1 for AChE has been presented in Figure 1.

In order to predict the interactions of benzothiazepines 1–3 in the active site of AChE, compounds 1–3 were subjected to molecular docking studies in the aromatic gorge of AChE (*Torpedo californica*). The docking position with the lowest energy was found most often during the docking procedure. This indicated that the phase space was sufficiently sampled out. The docking protocol was repeated many times for each compound and their respective minimum energies were consistently reproduced. The size and shape of compounds supported a gorge-spanning binding mode. Therefore, AChE co-crystallized with galanthamine was taken for the comparison in order to control the performance of our docking experiments [26].

The best ranking docking solutions showed that AChE can accommodate benzothiazepines 1–3 inside the aromatic gorge, interacting only with the anionic subsite near the top and middle of the aromatic gorge of AChE. A general binding mode for benzothiazepines 1–3 in the active site of AChE was identified by this study. In this binding mode, the docked compounds span the narrow, deeply buried active-site gorge, interacting with the anionic subsites halfway down the gorge, and near the mouth of the aromatic gorge. The principle interactions that keep the benzothiazepine-AChE complexes stable are summarized as follow.

Benzothiazepine 1-AChE Complex:

The main stabilizing factors that keep the benzothiazepine 1-AChE complex stable were found to be the hydrophobic contacts, π - π interactions and hydrogen bonding mainly with the amino acid residues of the peripheral anionic site (PAS) region of the AChE. The strong binding affinity ($K_i = 0.8 \pm 0.04 \mu\text{M}$) of compound 1 with AChE may be attributed to the presence of strong hydrogen bond (2.6 Å) which

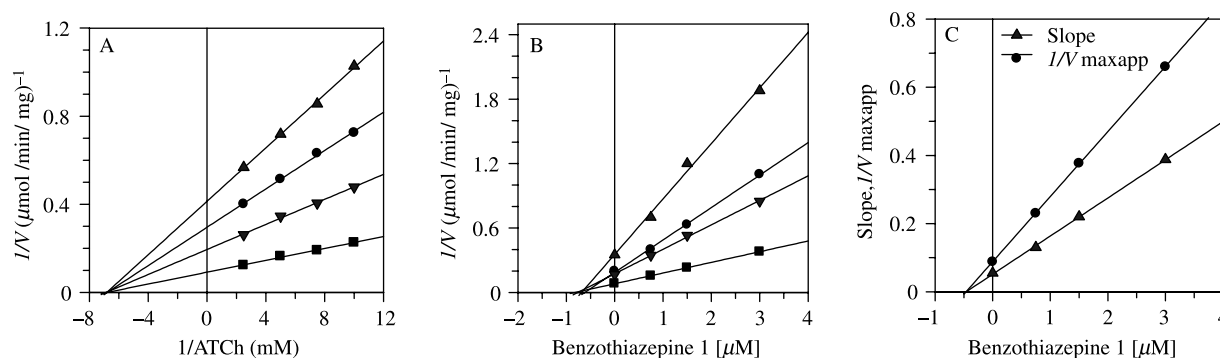


Figure 1. Inhibition of AChE by benzothiazepine 1 (A) is the Lineweaver-Burk plot of reciprocal of initial velocities and reciprocal of four fixed ATCh concentrations in the absence (■) and in the presence of 0.5 μM (▼), 1.0 μM (●), 2.0 μM (▲) of benzothiazepine 1. (B) is the Dixon plot at fixed ATCh concentrations, (■) 0.4 mM, (▼) 0.2 mM, (●) 0.133 mM and (▲) 0.1 mM. (C) is the secondary replots of the Lineweaver-Burk plot, $1/V_{\text{maxapp}}$ or slope and various concentrations of benzothiazepine 1.

is formed between hydroxyl group at the aromatic ring A with the hydroxyl group of Tyr121 of the peripheral anionic binding site residues. Similarly, PAS amino acid residue Tyr70 was found to have π - π interaction with ring A of the compound 1. Furthermore, PAS amino acid residue Tyr334 has π - π interaction and hydrophobic contact with rings A and B while Asp72 was found to make hydrophobic contacts with rings C and D. Ser122 has hydrophobic contacts with aromatic ring D. These results were found to be comparable with galanthamine which was used as a standard inhibitor of AChE. In galanthamine-AChE complex residues of quaternary ammonium binding locus (Trp84, Phe330 and Glu199) were found exclusively to be involved in the stabilization of galanthamine-AChE complex. Similarly, Trp84 is involved in hydrophobic contacts as well as in π - π interactions in galanthamine-AChE complex. Phe330 is involved in hydrophobic contacts while Glu199 forms a hydrogen bond with hydroxyl group of galanthamine. The determined galanthamine ($K_i = 0.48 \pm 0.002 \mu\text{M}$) inhibitory potential was found to be higher than benzothiazepine 1 ($K_i = 0.8 \pm 0.04 \mu\text{M}$), which may be due to the fact that galanthamine has interactions with all the four subsites (anionic and esteratic subsites) of the active site of the AChE, whereas benzothiazepine 1 has interactions mainly with anionic subsites. As benzothiazepine 1 does not have interaction with the amino acid residues of the catalytic triad (His440, Ser200 and Glu327). Therefore, compound 1 displayed a non-competitive type of AChE inhibition. The kinetic measurements were found to be in agreement with the docking results, indicating benzothiazepine 1 as a non-competitive inhibitor of AChE.

Benzothiazepine 2-AChE Complex:

Benzothiazepine-2-AChE complex was found to be stabilized mainly with the interactions of the amino acid residues of the quaternary ammonium-binding

locus (Trp84, Phe330 and Glu199) with the ligand (Figure 2). The low inhibitory potential of compound 2 ($K_i = 1.1 \pm 0.01 \mu\text{M}$) than compound 1 ($K_i = 0.8 \pm 0.04 \mu\text{M}$) may be due to the absence of hydrogen bonding in benzothiazepine-2-AChE complex as well as due to the repulsion between polar fluorine and non-polar Phe330 and Phe331 amino acid residues of AChE. The amino acid residue Trp84 was found to have π - π interactions with rings A and D of the ligand whereas Glu199 has hydrophobic contact with ring B of ligand. Similarly, Phe330 has π - π interactions with ring A. Peripheral anionic subsite amino acid residues Tyr121 and Asp72 have hydrophobic contacts with the rings C and D, respectively. Phe331 (acyl-binding locus) has π - π interactions with ring A. Gly118 and Ser122 have hydrophobic contacts with the rings B and D of compound 2, respectively. As all the interactions of the compound 2 are with anionic subsites, therefore, docking results are in agreement with the experimental kinetic data, indicating a non-competitive type of inhibition.

Benzothiazepine 3-AChE Complex:

Benzothiazepine 3-AChE complex was found to be stabilized mainly with the interactions of the amino acid residues of the quaternary ammonium-binding locus (Trp84, Phe330 and Glu199) with the ligand (Figure 2). Trp84 has π - π interactions with rings B, C and D. Glutamate 199 has hydrophobic contacts with ring B whereas Phe330 has hydrophobic contacts with methyl group at ring A as well as π - π interaction with ring A of the compound. Phe331 (acyl-binding locus amino acid residue) has π - π interaction with ring A. Gly118 has hydrophobic contacts with rings A and C. As all the four subsites are not involved in interactions with the ligand, therefore, docking results and kinetic experiments are in agreement with each other, indicating benzothiazepine 3 as a non-competitive inhibitor of AChE.

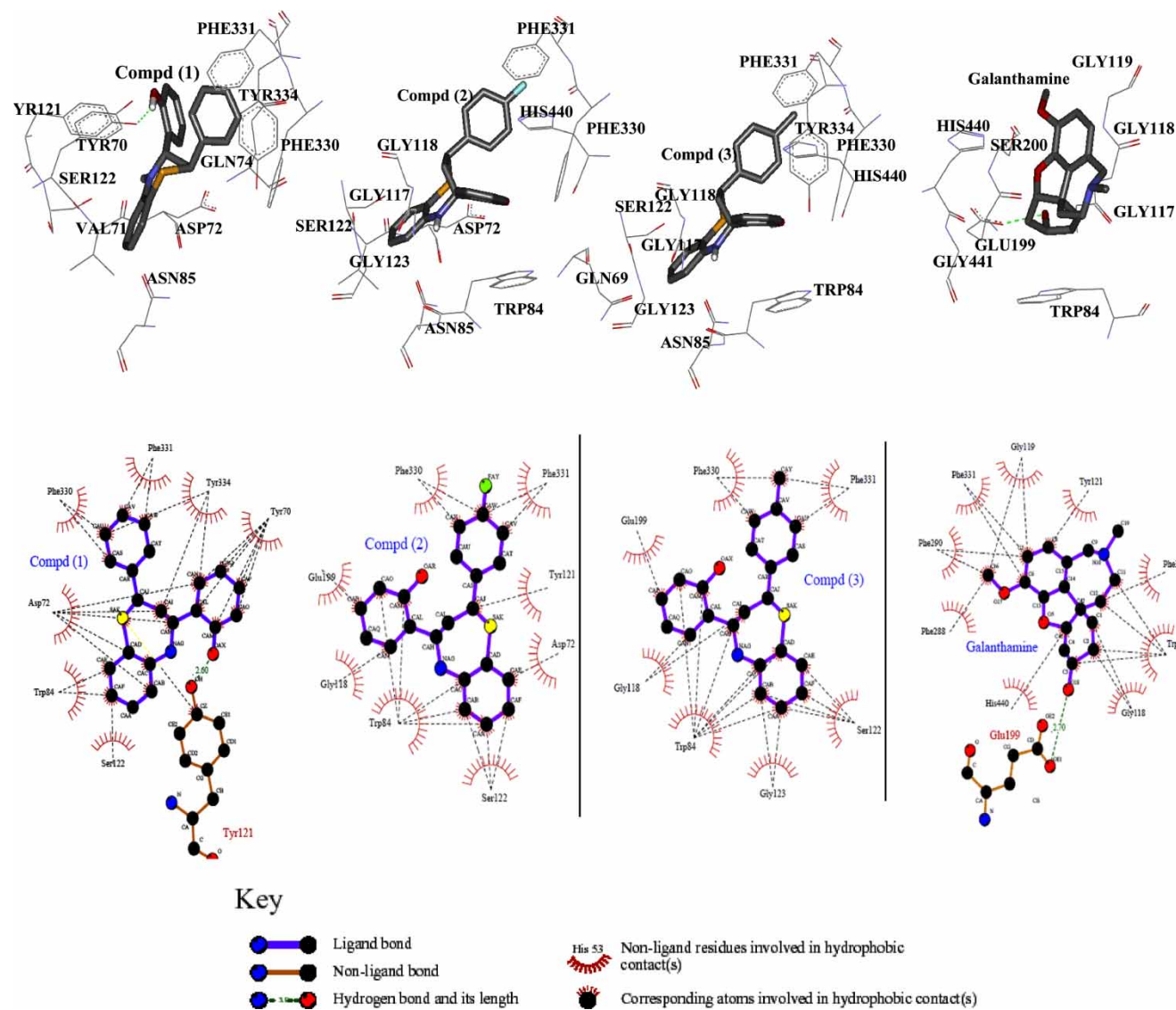


Figure 2. LIGPLOTS showing interactions of the benzothiazepines 1–3 and galanthamine in the active site of AChE.

4. Conclusion

Mechanism-based kinetic study revealed that benzothiazepines **1–3** are the non-competitive inhibitors of AChE. The molecular docking results were found to be well correlated with the steady-state inhibition measurements. The docking study revealed that all the ligands are completely buried inside the aromatic gorge extending down to the anionic subsite of the gorge. Compounds **1–3** have almost similar binding mode that was not unexpected because all the ligands are identical, differing only at the side chain substitutions at ring B. AChE-inhibitor complexes were found to be mainly stabilized through π - π and hydrophobic interactions, exclusively with the amino acid residues of anionic subsites of AChE. Benzothiazepine **1**-AChE complex was further stabilized by hydrogen bond. For specific analog, additional interactions were found to be responsible for their deviating activity. On the basis of our findings, we can conclude that strong interactions of benzothiazepines **1–3** with the anionic subsite of AChE indicated high inhibitory potential against AChE. Furthermore, observed binding modes of benzothiazepines **1–3** in the active site of AChE explains the affinities of these compounds with AChE and provides a rational basis for the structure-based drug design of benzothiazepines with improved pharmacological properties.

Acknowledgements

The authors express their gratitude to Dr. Mohammad Hussain Panjwani Memorial Trust for the establishment of Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi. They also extend their thanks to Prof. Dr. Khalid Iqbal (Department of Neurochemistry, New York State Institute for Basic Research in Developmental Disabilities) for his meaningful suggestions and fruitful discussions during his visits to enzyme inhibition laboratory of PCMD.

References

- [1] Quinn DM. *Chem Rev* 1987;87:955–979.
- [2] Perry EK. *Br Med Bull* 1986;42:63–69.
- [3] Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, Silman I. *Science* 1991;253:872–879.
- [4] Harel M, Quinn DM, Nair HK, Silman I, Sussman JL. *J Am Chem Soc* 1996;118:2340–2346.
- [5] Szegetes T, Mallender WD, Rosenberry TL. *Biochemistry* 1998;37:4206–4216.
- [6] Harel M, Schalk I, Ehret-Sabatier L, Bouet F, Goeldner M, Hirth C, Axelsen PH, Silman I, Sussman JL. *Proc Natl Acad Sci USA* 1993;90:9031–9035.
- [7] Atta-ur-Rahman, Parveen S, Khalid A, Farooq A, Choudhary MI. *Phytochemistry* 2001;5:963–968.
- [8] Atta-ur-Rahman, Zaheer-ul-Haq, Feroz F, Khalid A, Nawaz SA, Choudhary MI, Khan MR. *Helv Chim Acta* 2004;87:439–448.
- [9] Atta-ur-Rahman, Feroz F, Nawaz SA, Khan MR, Choudhary MI. *Steroids* 2004;69:735–741.
- [10] Choudhary MI, Devkota KP, Nawaz SA, Ranjit R, Atta-ur-Rahman. *Steroids* 2005;70:295–303.
- [11] Zaheer-ul-Haq, Wellenzohn B, Liedl KR, Rode BM. *J Med Chem* 2003;46:5087–5090.
- [12] Zaheer-ul-Haq, Wellenzohn B, Tonmunpuean S, Khalid A, Choudhary MI, Rode BM. *Bioorganic Med Chem Lett* 2003;13:4375–4380.
- [13] Choudhary MI, Nawaz SA, Zaheer-ul-Haq, Azim MK, Ghayur MN, Lodhi MA, Jalil S, Rode BM, Atta-ur-Rahman, Gilani AH, Ahmad VU. *Biochem Biophys Res Commun* 2005;332:1171–1179.
- [14] Choudhary MI, Nawaz SA, Zaheer-ul-Haq, Lodhi MA, Ghayur MN, Jalil S, Yousuf S, Malik A, Gilani AH, Atta-ur-Rahman. *Biochem Biophys Res Commun* 2005;334:276–287.
- [15] Nawaz SA, Zaheer-ul-Haq, Ghayur MN, Lodhi MA, Jalil S, Rode BM, Atta-ur-Rahman, Gilani AH, Choudhary MI. accepted in *Biochem Biophys Acta* 2006.
- [16] Ansari FL, Umbreen S, Hussain L, Makhmoor T, Nawaz SA, Lodhi MA, Khan SN, Shaheen F, Choudhary MI, Atta-ur-Rahman. *Chem Biodivers* 2005;2:487–496.
- [17] Ellman GL, Courtney KD, Andres V, Featherstone RM. *Biochem Pharmacol* 1961;7:88–95.
- [18] Choudhary MI, Devkota KP, Nawaz SA, Shaheen F, Atta-ur-Rahman. *Helv Chim Acta* 2004;87:1099–1108.
- [19] Dixon M. *Biochem J* 1953;5:170–171.
- [20] Segel IH. Non-competitive inhibition (Simple intersecting linear non-competitive inhibition). In *Enzyme kinetics: Behavior and analysis of rapid equilibrium and steady state enzyme systems*. New York: John Wiley and Sons; 1993. p 101–112.
- [21] Leatherbarrow RJ. *GraFit Version 4.09*; Erithacus Software Ltd 1999.
- [22] Stains K. SYBYL molecular modeling software. St. Louis MO: Tripos Associated Ltd.
- [23] Rarey M, Kramer B, Lengauer T, Klebe G. *J Mol Biol* 1996;261:127–134.
- [24] Humphrey W, Dalke A, Schulten KJ. *Molec Graphics* 1996;14:33–38.
- [25] Wallace AC, Laskowski RA, Thornton JM. *Protein Eng* 1995;8:127–134.
- [26] Greenblatt HM, Guillou C, Guenard D, Argaman A, Botti S, Badet B, Thal C, Silman I, Sussman JL. *J Am Chem Soc* 2004;126:15405–15411.