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1,3-di-4-piperidylpropane derivatives as potential acetyl cholinesterase antagonists: Molecular docking, synthesis, and biological evaluation

Ahsaan Ahmed[1,2]*, Shamim Akhtar[3], Nousheen Mushtaq[1], Shazia Haider[1], Rabya Munawar[4], Hassaan Ahmed Siddique[1,2], Arifa Akram[5], Zafar Saeed Saify[6] and Muhammad Arif [3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan
[2]Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
[4]Department of Pharmaceutical Chemistry, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Federal Urdu University, Karachi, Pakistan
[6]ICCBS, HEJ Research Institute of Chemistry, University of Karachi, Karachi, Pakistan

Abstract: Acetylcholine esterase (AChE) is a key biological target responsible for the management of cholinergic transmission, and its inhibitors are used for the therapy of Alzheimer's disease. In the present study, a small library of molecules with 1,3-di-4-piperidylpropane nucleus were docked on AChE. The selected compounds were synthesized and evaluated for their enzyme inhibition. P25 and P17 expressed significantly higher AChE inhibition than standards with IC_{50} values of 0.591µM and 0.625µM, respectively. Binding mode of derivatives in the active site of AChE revealed dual binding of molecules in peripheral anionic site (PAS) and catalytic anionic site (CAS) of enzyme cavity.

Keywords: Acetylcholine esterase (AChE), Alzheimer's disease (AD), molecular docking, Piperidine, MOE (molecular orbital environment), Ellman's activity.

INTRODUCTION

Alzheimer's disease (AD) is the most prevalent clinical syndrome growing epidemic worldwide. Its widespread prevalence is creating an alarming signal for public health globally (Generoso *et al*. 2020). It is a neurodegenerative disorder characterized by several clinical manifestations such as impairment in cognitive function, disturbance in memory and speech along with visuospatial orientation (Haque and Levey, 2019). Impaired cholinergic neurons or deficiency of cholinergic enzymes associated with AD leads to the decrease level of acetylcholine (ACh) (H. Ferreira-Vieira *et al.*, 2016). This develops a strong relationship between cholinergic dysfunction and AD severity (Hampel *et al.*, 2018). These findings led to a resurgence of interest in Acetylcholine esterase (AChE) as a crucial target in the therapy of Alzheimer's disease (Kiametis *et al.*, 2017; Akıncıoglu and Gulcin 2020).

AChE is one of the key enzyme predominantly available in the healthy brain and majorly responsible in regulating the level of acetylcholine by metabolizing into acetic acid and choline respectively (Montanari *et al.*, 2016). A number of reported drugs targeting AChE, including donepezil, tacrine and galantamine, used as anti-Alzheimer agents (Sharma, 2019). Donepezil a piperidine containing molecule is one of most used acetylcholine esterase inhibitor (AChEI) in AD therapy, acting on a dual binding sites as a reversible inhibitor (S. Doody *et al*., 2012).

To design a new molecule, the process of rational drug discovery requires deep insight into the mechanism at the molecular level to propose selective and more efficient inhibitors. Assistance of computational methods based on molecular docking and physicochemical descriptors are available to compute the binding affinity of the ligands, interaction of drug-receptor complex, and kinetics parameter of the drug (Sarkar B *et al.*, 2020).

Three-dimensional (3D) structure of AChE (Protein code: 4EY7) complex with co-crystallized ligand (donepezil or E20) fetched from Protein data Bank. The structure of E20 enters deep into the active site of the enzymatic gorge covering the catalytic anionic site (CAS) and peripheral anionic site (PAS) residues, respectively (Fang *et al.*, 2014; Saglık *et al.*, 2020).

Current study is based on the findings that emerged the structure-based drug designing using 1,3-di-4-piperidylpropane as basic nucleus for derivatization as potential AChE inhibitors and explicate our understanding about ligand-target interaction for potential inhibition.

MATERIALS AND METHODS

Computational Study

By using ChemDraw Ultra 8.0, a library of inhouse compounds was prepared and standards were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and then all ligands were minimized by using force field energy (MMFF94x) for generating stable conformers for molecular docking. MOE (Molecular Operating

**Corresponding author:* e-mail: ahsaan.ahmed@jsmu.edu.pk

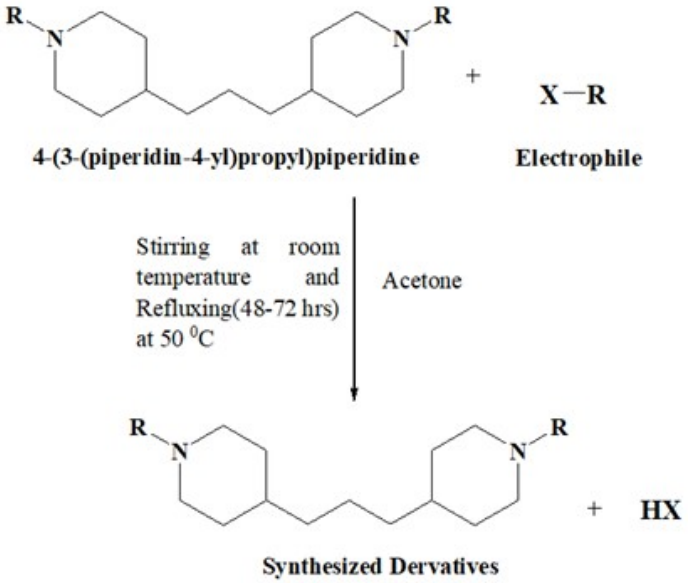
Environment version 2018.01) software was used to perform: a) refinement and minimization of protein (AChE; PDB ID= 4EY7) (Jang *et al.*, 2018); b) active site identification by alpha site finder; c) redocking of co-crystallized molecule (E20) into the active site of the target for the validation of docking protocol; and d) molecular docking of prepared library against the target protein. The pharmacokinetic profile of the compounds computed online by SWISS ADME (Daina, Michielin and Zoete, 2017).

Synthesis and characterization of the synthesized compounds

1,3-di-4-piperidylpropane(P) based phenacyl, benzyl and benzoyol derivatives were synthesized by using different reactants purchased from Sigma Aldrich and Tokyo chemical industry (TCI). Reactions were proceeded by utilizing 0.0025moles of parent (P) and 0.005moles of reactants in acetone (fig. 1). Melting point and thin layer chromatography confirmed the accomplishment of each reaction. Products were recrystallized using hot acetone for purification. Furthermore, characterization of the molecules was done by spectroscopic (Ultraviolet-UV-1601, Infra-red-FTIR-8900, Proton-Nuclear magnetic resonance-Bruker advance at 400 & 500MHz) and spectrometric (Fast atomic bombardment-Mass spectrometry +/-JOEL 600H-2) techniques.

2,2'-[propane-1,3-diyldi(piperidine-4,1-diyl)]bis[1-(4-methylphenyl)ethan-1-one] (P17)

Brown color (gummy product) with 60% yield, soluble in DMSO and methanol, $C_{31}H_{42}N_2O_2$ (Molecular Formula). UV λ_{max} (CH_3OH): 258.50 nm. IR (cm^{-1}): 2980 (C-H stretching, strong), 1670 (C=O ketone, strong peak), 1581 (C=C aromatic ring stretching, medium, sharp), 1472 (CH_2 bending peak). FAB positive (mass/charge) M+1: 475. ^{1}H-NMR (500 MHz, DMSO-d6) δ (ppm): 1.17-1.313 (10H,m,H-1,2,3(propyl),H-5,8,9,12(piperidine)), 1.4-1.77 (6H,m,H-4,5,8,9,12,13(piperidine)), 1.85 (4H,s,H-6,7,10, 11(piperidine)), 2.38-2.4(4H,t,J=10Hz,H-6,7,10,11



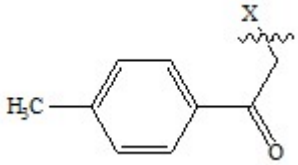
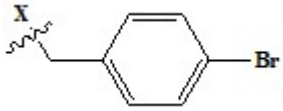
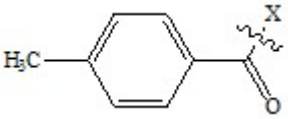
Compound	R	X
P17		Br
P25		Br
P26		Cl

Fig. 1: General scheme of synthesis based on Benzyl, Benzoyl and Phenacyl substituted derivatives

Scheme 1: Ellman's Activity

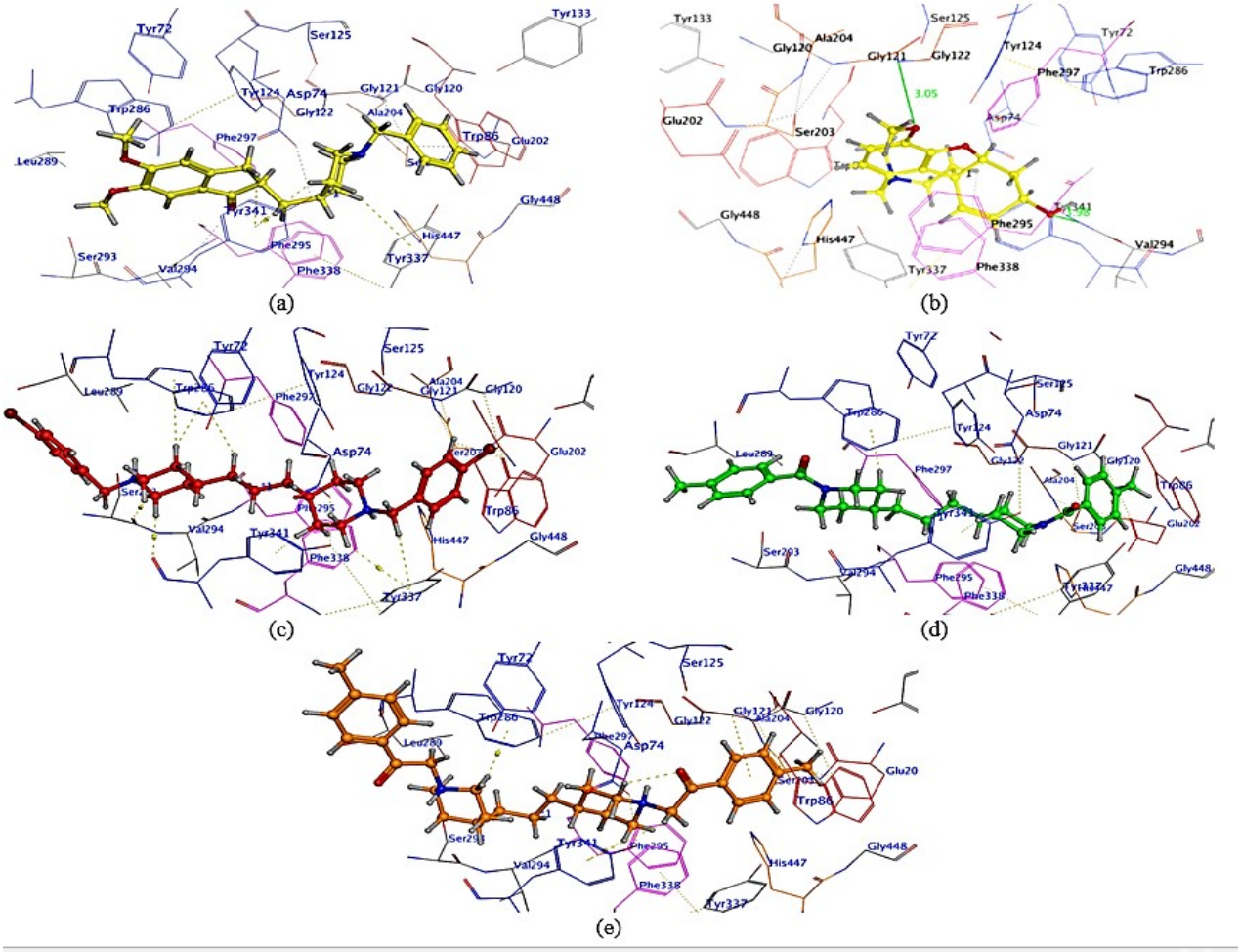


Fig. 2: 3D Pose of Donepezil (2a); Galantamine (2b); P25 (2c); P26 (2d) and P17 (2e) in the active binding site of AChE enzyme

(piperidine)), 2.8-3.2(6H,m,H-24,25(para-methyl)), 3.49-3.69(2H,m,H-14(methyl)), 4.13-5(2H,m,H-15(methyl)), 7.36-7.43(4H,m,H-17,18,21,22(Ar)), 7.87-8.18(4H,m,H-16,19,20,23(Ar)).

4,4'-(propane-1,3-diyl)bis{1-[(4-bromophenyl)methyl]piperidine} (P25)

Off-white color (Solid powder) with 60% yield, soluble in DMSO and methanol, $C_{27}H_{36}Br_2N_2$ (Molecular Formula), m.p 290 - 298 ^{0}C. UV λ_{max} (CH_3OH): 228.50 and 259.50 nm. IR (cm^{-1}): 2930 (C-H), 1580 (C=C aromatic ring), 1472 (CH_2 bending peak), 650 (C-Br). FAB positive (mass/charge): M+2 (548) and M+4 (550). 1H-NMR(500 MHz, DMSO-d6) δ (ppm): 1.128-1.402(8H,m,H-1,2,3(propyl), H-4,13(piperidine)), 1.73-1.91(8H,m,H-4,5,8,9,12(piperidine)), 2.83-2.95(4H,m,H-6,7,10,11 (piperidine)), 3.4-3.42 (4H,d,J=10Hz,H-6,7,10,11 (piperidine)), 4.3-4.8(4H,m,H-13,14(methyl)), 7.44-7.56 (4H,m,H-16,19,20,23(Ar)), 7.67-7.76(4H,m,H-17,18,21, 22(Ar)).

[propane-1,3-diyldi(piperidine-4,1-diyl)]bis[(4-methylphenyl)methanone] (P26)

Pale color (Solid powder) with 60% yield, soluble in DMSO and methanol, $C_{29}H_{38}N_2O_2$ (Molecular Formula), m.p 265 - 268 ^{0}C. UV λ_{max} (CH_3OH): 249 nm. IR (cm^{-1}): 3000 (C-H stretching, strong peak), 1650 (C=O of amide, sharp, strong peak), 1580 (C=C aromatic ring stretching, sharp peak), 1472 (CH_2 bending peak). FAB positive (mass/charge) M+1: 447. ^{1}H-NMR (500 MHz, DMSO-

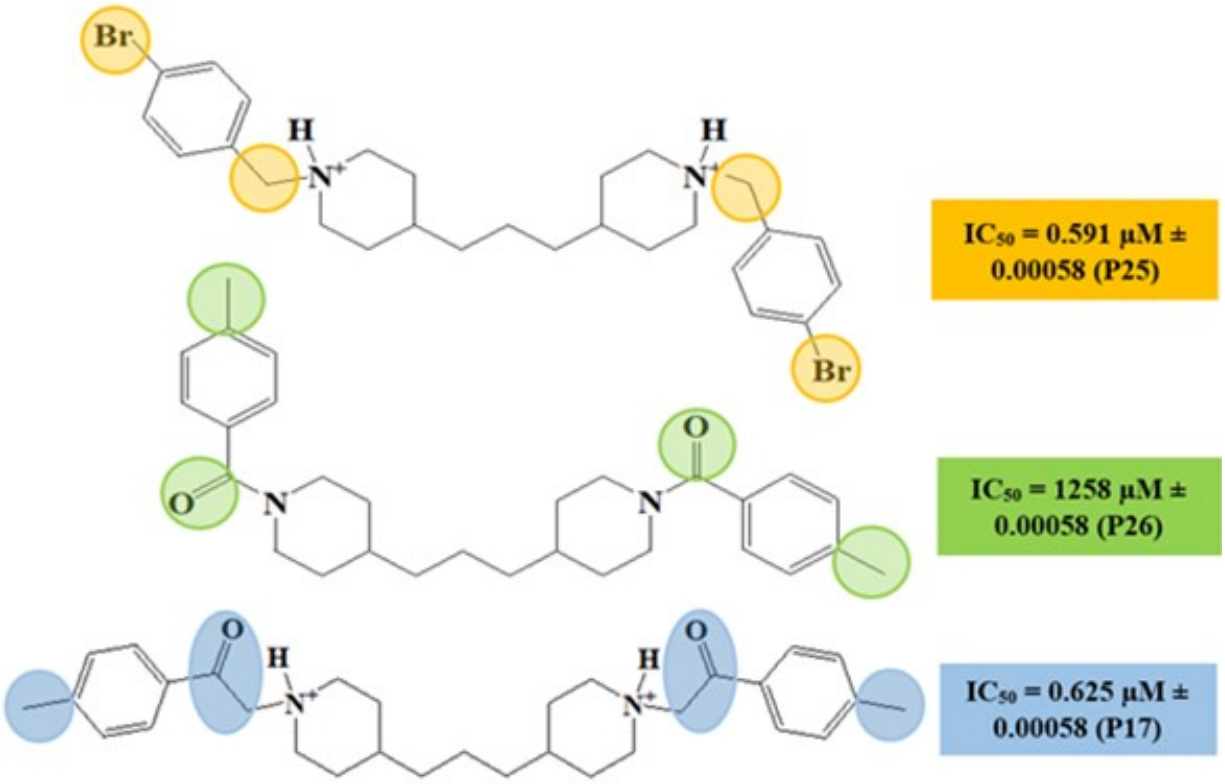


Fig. 3: Structural activity relationship of compound P25, P26 and P17

d6) δ (ppm): 1.17-1.313(10H,m,H-1,2,3(propyl), H-5,8,9,12(piperidine)), 1.4-1.77(6H,m,H-4,5,8,9,12,13 (piperidine)), 1.85 (4H,s,H-6,7,10,11(piperidine)), 2.38-2.4(4H,t,J=10Hz,H-6,7,10,11(piperidine)), 2.8-3.2(6H,m, H-24,25(para-methyl)), 7.36-7.43(4H,m,H-17,18,21,22 (Ar)), 7.87-8.18(4H,m,H-16,19,20,23(Ar)).

Acetyl choline esterase inhibitory activity (Ellman's method)

Inhibitory activity (*in-vitro*) of the prepared derivatives and standard drugs were done on an ultra-violet spectrophotometer by studying modified Ellman's method (Ellman *et al.*, 1961; Mohammadi-Khanaposhtani *et al.*, 2015). Different dilutions of micromolar (µM) concentration were made (0.5 to 1400) by dissolving parent (P), synthesized compounds (P17, P25 and P26) in DMSO + distilled water (1:1 ratio) and standard (Donepezil) in CH_3OH + distilled water. Absorbance of each dilution was detected in triplicate at 25°C (Scheme-1). Similar procedure was repeated for blank readings without adding the sample solution.

STATISTICAL ANALYSIS

Inhibitory concentration (IC_{50}) was calculated by applying simple linear regression between % inhibition and concentration (Graph Pad Prism8 software) and values are expressed as standard error of the mean (Mughal *et al.*, 2018).

RESULTS

Derivatives of 1,3-di-4-piperidylpropane were synthesized in search of potential AChE inhibitors as anti-Alzheimer's agents. Synthesis protocol and characterization are discussed in the experimental section and outlined in fig. 1. All the derivatives were synthesized based on findings of computational results (*in-silico*) and were evaluated for enzymatic inhibition (*in-vitro*) table 1. Outcomes of molecular docking and predicted pharma-cokinetic profile are illustrated in table 1 and fig. 2.

DISCUSSION

Molecular Docking

Molecular docking was performed against the target protein (PDB ID: 4EY7) by employing a library of proposed 1,3-di-4-piperidylpropane derivatives and known standards (Donepezil and Galantamine) to compare binding energy and interacting residues.

Donepezil showed significant docking energy (-8.0 Kcal/mol) as compared to other standard by making hydrophobic interactions with Tyr72, Trp86, Trp286, Phe295, Phe297, Tyr341, Tyr337 and Phe338. It is making π-π and hydrogen bonding in PAS and acyl pocket. Its piperidine is involved with Trp86, Tyr337, and Phe338 making π-cation interaction while benzyl ring is busy with Trp86 in choline binding site by π-π staking

Table 1: Summarized table based on Molecular docking, Pharmacokinetic profile & AChE (IC_{50})

Compound / Standards	Molecular Docking (PDB ID = 4EY7)				Pharmacokinetic Profile				AChE Activity
	Binding Energy (kcal/mol)	Hydrophobic Interacting Residues	π-π, CH-π & π-cation Residues	Hydrogen Bonding < 3 A°	BBB [c]	Drug likeness (violations) [d]	TPSA [e]	GI Absorption [f]	IC_{50} (µM) ± SEM [g]
Donepezil	-8.00	Trp86, Trp286, Phe295, Tyr337,Phe338,Tyr341	Trp86[ab],Trp286[a],Tyr337, Phe338[b],Tyr341[b]	Phe295 (N--OH)	Yes	Yes	38.77 Å^2	High (0.55)	2.7±0.001
Galantamine	-6.89	Trp286,Phe295,Phe297, Tyr337,Phe338,Tyr341	-	Gly122 (N--OCH_3)	Yes	Yes	41.93 Å	High (0.55)	60±0.28
P25	-8.81	Tyr72,Trp86,Tyr124,Trp286,Leu289,Phe295,Phe297,Tyr337,Phe338,Tyr341, His447	Trp86[ab],Trp286[b],Tyr337[b]	-	Yes	Yes (with one violation: MW)	8.88 Å^2	High (0.55)	0.591±0.00058
P26	-9	Trp86,Tyr124,Trp286,Leu289,Phe295, Phe297,Tyr337,Phe338,Tyr341,His447	Trp86[a],Trp286[a],Tyr337[b]	-	Yes	Yes	40.62 Å^2	High (0.55)	1285±0.00058
P17	-9.07	Tyr72,Trp86,Tyr124,Trp286,Val294,Phe295,Phe297, Tyr337,Phe338,Tyr341	Tyr72[a],Trp86[a],Trp286[b],Tyr341,Tyr337[b]	Tyr124 (OH--N)	Yes	Yes	43.02 Å^2	High (0.55)	0.625±0.00058

Note: [a]π-π stacking; [b]π-cation (between Piperidine 'N' and amino acid aromatic ring); [c] blood brain barrier; [d]Lipinski's rule of five (violation); [e]topological polar surface area; [f] Gastrointestinal absorption and bioavailability score; [g]Values are expressed as ± SEM (standard error of mean)

(fig. 2a). *Galantamine* (-6.89 kcal/mol) making hydrophobic interactions (Tyr286, Phe295, Phe297, Tyr337, Phe338 and Tyr341) in the PAS and acyl pocket while its methoxy group is making hydrogen bond with the nitrogen of Gly122 (fig. 2b).

All derivatives fit in to the active pocket site of AChE by interacting with key amino acid residues and presenting better docking energy than the standards. They occupied the PAS and CAS region with the help of hydrophobic, π-π staking and hydrogen bonding. P25 is a p-bromo substituted benzyl derivative showing docking energy (-8.81 kcal/mol). Ligand-target complex is stabilized with amino acids (Tyr72, Trp86, Tyr124, Trp286, Leu289, Phe295, Phe297, Tyr337, Phe338, Tyr341, His447) forming hydrophobic area in PAS and cationic site (CAS) of AChE. Piperidine ring in PAS facing Trp286 for π-cation interaction. Piperidine in CAS aligned in such a way to contact Trp86 and Tyr337 by making π-cation linkage, while an aromatic ring is also engaged by Trp86 for π-π staking (fig. 2c). P26 is a p-methyl benzoyl derivative exhibited energy of -9.0 kcal/mol. Both the aromatic rings in PAS and CAS make parallel interactions (π-π stacking) with Trp86 and Trp286 respectively, whereas piperidine facing the cavity is involved with Tyr337 via π-cation interaction. Hydrophobic interactions generated with Trp86, Tyr124, Trp286, Leu289, Phe295, Phe297, Phe338, Tyr341, His447 extended in the PAS and CAS of enzyme (fig. 2d). P17 with significant energy score (-9.0 kcal/mol) received by aromatic residues (Tyr72,Tyr124, Trp286, Tyr341), located on the top of the active site of the gorge creating space for the molecule to glide in the narrow acyl pocket where Phe295, Phe297, Phe337, Phe338 lining providing the hydrophobic interactions to fix the propyl chain and piperidine rings. PAS, there is an aromatic ring making π-π staking with Tyr72 along with π-cation interaction with Trp286. Piperidine ring facing the cavity communicating with Tyr124 through hydrogen bonding and adjusted well by Tyr337 and Tyr341 through π-cation connection. Aromatic ring in choline binding site facing Trp86 creating π-π staking (fig. 2e). All the derivatives possessed favorable pharmacokinetic characteristics (GI absorption, blood brain permeability) and good drug-likeness property (table 1).

Enzyme inhibition

P25 and P17 exhibited better ACE inhibition as compared to standards while in P26 inhibitory potential reduced to more than 1000 folds. Enzyme inhibition ability of molecules can be attributed to their structural features. The effect of different substitution on the aromatic ring and the connecting chain is having a great impact on biological activity (fig. 3).

P25 is a *para*-bromo benzyl derivative displaying best inhibitory potential with IC_{50} value 0.591µM (table 1 and fig. 3). In P17 substitution of a simple *para*-methyl group

in the phenacyl ring is reducing the stearic effect and lowering the inhibitory potential of the molecule (IC_{50}= 0.625µM) as compared to P25 but still better than the standards. Methyl group though adjusting the terminal ring to interact with Trp86 in CAS region providing opportunity to the piperidine amine for making π-cation (Tyr337 and Tyr341) interactions and hydrogen bonding (Tyr124) in the upper area of gorge. P26 having carbonyl linker with *para*-methyl substitution on the aromatic ring presented lowest inhibitory potency with IC_{50} of 1285µM.

SAR of P25 and P26 displaying the significance of linkers connecting aromatic rings with amines (fig. 3). Replacing the rigid carbonyl with lipophilic alkyl group as a linker making the molecule more potent.

CONCLUSION

It is concluded from the study that P25 and P17 are an excellent candidate for further advancement and could play an immense role as promising drug molecules for the treatment of AD.

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