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Synthesis and Molecular Docking Study of Some New 4-{[4-(2-Furoyl)-1-piperazinyl]methyl}-N-(substituted-phenyl)benzamides as Possible Therapeutic Entrants for Alzheimer's Disease

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Abstract

In the present work, a new series of different 4-{[4-(2-furoyl)-1-piperazinyl]methyl}-*N*-(substituted-phenyl) benzamides (5a-h) have been synthesized as possible therapeutic agents for the treatment of Alzheimer's disease. The structural confirmation of all the synthesized compounds was carried out by their IR, ^{1}H-NMR and EI-MS spectral data. Enzyme inhibition activity was performed against butyrylcholinestrase enzyme, which revealed that, 4-{[4-(2-furoyl)-1-piperazinyl]methyl}-*N*-(4-ethylphenyl)benzamide (5b) showed excellent IC_{50} value 0.82 ± 0.001 μM relative to Eserine, a reference standard having IC_{50} value of 0.85 ± 0.0001 μM. The enhanced potential of this molecule may be attributed to the 4-ethylphenyl group. As the cholinesterase enzyme inhibitors are good targets for Alzheimer's disease, therefore, the inhibition study of these synthesized molecules was carried out to discover their possible therapeutic effect as target for aforesaid disease.

Keywords: 4-Chloromethyl benzoylchloride; Substituted anilines; Butyrylcholinesterase; Hemolytic activity

Introduction

Piperazine nucleus is one of the most important heterocycles exhibiting remarkable pharmacological activities. Piperazine is consists a six-membered ring containing two nitrogen atoms opposite to one another. Slight change in substitution pattern in piperazine nucleus causes distinguishable difference in their pharmacological activities, such as anti-psychotic, anti-convulsant, anti-arrhythmic, anti-microbial, anti-malarial, cytotoxic and anti-oxidant activities [1-3].

Benzamides have been reported as relaxant for smooth muscle and activators of potassium channel. Some synthetic benzamides are antihelmintic agent [4], while some others are anti-inflammatory and analgesic [5]. Therapeutically active compounds are listed under heterocyclic benzamides, show activity in central nervous system. These heterocyclic compounds also act as anti-psychotics, anti-emetics and gastric motility stimulants [6-8].

Butyrylcholinesterase (BChE, EC 3.1.1.8) belongs to a family of serine hydrolases. The active sites of BChE contain different amino acid residues which promote their specifications for substrates and inhibitors for these enzymes. The enzyme system is highly involved in the termination of acetylcholine at cholinergic synapses [9]. These cholinesterase inhibitors promote acetylcholine for neuronal and neuromuscular transmission reversibly or irreversibly. It has been found that BChE (E.C 3.1.1.8) inhibition is an effective tool to cure Alzheimer's disease and dementias. The amount of BChE is significantly high in Alzheimer's plaques when compared in plaques present among normal age-related brains without dementia. BChE is produced in the liver and enriches blood circulation. It is also present in adipose tissue, and can also be found in the intestine, smooth muscle cells, white matter of the brain, and in many other tissues. For the treatment of Alzheimer's and related disease, it is of great importance to search new cholinesterase inhibitors as possible drug candidates [10].

In continuation of our previous efforts for the search of cholinesterase inhibitors [11-13], here we report the synthesis of some new 4-{[4-(2-furoyl)-1-piperazinyl]methyl}-*N*-(substituted-phenyl) benzamides as valuable therapeutic agents aiming to play a pivitol role in the treatment of Alzheimer's disease.

Materials and Methods

Chemicals were purchased from Sigma Aldrich and Alfa Aesar (Germany) and solvents of analytical grade from local suppliers. By using open capillary tube method, melting points were taken on Griffin and George apparatus and were uncorrected. By using thin layer chromatography using various percentages of ethyl acetate and *n*-hexane as mobile phase, initial purity of compounds was detected at 254 nm. IR peaks were recorded on a Jasco-320-A spectrometer by using KBr pellet method. ^{1}H-NMR signals were recorded at 500 MHz in $CDCl_3$ using Bruker spectrometers. EIMS signals were recorded by utilizing a JMS-HX-110 spectrometer.

Synthesis of 4-(chloromethyl)-*N*-(substituted-phenyl) benzamides (3a-h)

4-(Chloromethyl)benzoyl chloride (12.8 mmol; 1) was added in 100

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mL distilled water and pH was adjusted between 9.0 to 10.0 by addition of aqueous Na_2CO_3 (10 %) which was followed by dropwise addition of substituted anilines (12.8 mmol; 2a-h) in the reaction mixture under stirring for 3-4 hours at room temperature. The reaction mixture was stirred and monitored with TLC till completion of reaction. Then conc. HCl (4 mL) was added slowly till pH 2.0 and the reaction mixture was allowed at RT for 15 minutes, precipitates obtained were filtered and washed with distilled water and air dried to afford 4-(chloromethyl)-*N*-(substituted-phenyl)benzamides (3a-h).

Synthesis of 4-{[4-(2-furoyl)-1-piperazinyl]methyl}-*N*-(substituted-phenyl)benzamides (5a-h)

2-Furyl(1-piperazinyl)methanone (2-furoyl-1-piperazine; 0.00024 mol; 4) solubilized in acetonitrile (20-30 mL) was taken in 100 mL round bottom flask, followed by the addition of solid K_2CO_3 (0.0135 mol). The reaction mixture was refluxed for half an hour and then electrophiles, 4-(chloromethyl)-*N*-(substituted-phenyl)benzamides (0. 00024 mol; 3a-h) were added and reaction mixture was further refluxed for 4-5 hours. Thin layer chromatography was carried out to check the reaction completion. Distilled water was added in the reaction mixture to acquire the precipitates, which were filtered, washed and dried to get 4-{[4-(2-furoyl)-1-piperazinyl]methyl}-*N*-(substituted-phenyl) benzamides (5a-h).

Compound characterization

4-{[4-(2-Furoyl)-1-piperazinyl]methyl}-*N*-(2-methoxyphenyl) benzamide (5a): White amorphous solid; Yield: 85 %; m.p.: 145-147 °C; Mol. F.: $C_{24}H_{25}N_3O_4$; Mol. Mass: 419 g/mol; IR (KBr, cm^{-1}) ν_{max}: 3401 (N-H), 3089 (Ar C-H), 2882 (R C-H), 1655 (C=O), 1584 (Ar C=C), 1190 (C-O-C), 1104 (C-N-C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 8.26 (d, *J* = 7.6 Hz, 1H, H-6'''), 7.89 (d, *J* = 8.0 Hz, 2H, H-2'' and H-6''), 7.52 (d, *J* = 0.9 Hz, 1H, H-5), 7.27 (d, *J* = 8.0 Hz, 2H, H-3'' and H-5''), 7.08 (d, *J* = 2.7 Hz, 1H, H-3), 7.04 (t, *J* = 7.2 Hz, 1H, H-5'''), 6.92 (t, *J* = 7.6 Hz, 1H, H-4'''), 6.82 (d, *J* = 8.0 Hz, 1H, H-3'''), 6.48 (dd, *J* = 3.5, 1.7 Hz, 1H, H-4), 3.88 (br.s, 4H, CH_2-3' and CH_2-5'), 3.81 (s, 3H, OCH_3-1''''), 3.67 (s, 2H, CH_2-8''), 2.59 (br.s, 4H, CH_2-2' and CH_2-6'); EI-MS (*m/z*): 419 $[M]^+$, 324 $[C_{19}H_{22}N_3O_2]^+$, 295 $[C_{18}H_{19}N_2O_2]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 241 $[C_{15}H_{16}NO_2]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-(4-Ethylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl} benzamide (5b):** Off- white amorphous solid; Yield: 88 %; m.p: 151-153 °C; Mol. F.: $C_{25}H_{27}N_3O_3$; Mol. Mass: 417 g/mol; IR (KBr, cm^{-1}) ν_{max}: 3405 (N-H), 3095 (Ar C-H), 2883 (R C-H), 1651 (C=O), 1580 (Ar C=C), 1197 (C-O-C), 1119 (C-N-C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.86 (d, *J* = 8.2 Hz, 2H, H-2'' and H-6''), 7.58 (d, *J* = 8.3 Hz, 2H, H-2''' and H-6'''), 7.49 (d, *J* = 0.9 Hz, 1H, H-5), 7.47 (d, *J* = 8.0 Hz, 2H, H-3''' and H-5'''), 7.22 (d, *J* = 8.4 Hz, 2H, H-3'' and H-5''), 7.00 (d, *J* = 2.7 Hz, 1H, H-3), 6.49 (dd, *J* = 1.6, 3.4 Hz, 1H, H-4), 3.84 (br.s, 4H, CH_2-3' and CH_2-5'), 3.62 (s, 2H, CH_2-8''), 2.67 (q, *J* = 7.6 Hz, 2H, CH_2-1''''), 2.54 (br.s, 4H, CH_2-2' and CH_2-6'), 1.22 (t, *J* = 4.8 Hz, 3H, CH_3-2''''); EI-MS (*m/z*): 417 $[M]^+$, 322 $[C_{20}H_{24}N_3O]^+$, 293 $[C_{19}H_{21}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 239 $[C_{16}H_{17}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-(4-Ethoxyphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl} benzamide (5c):** Tea pink amorphous solid; Yield: 92 %; M.P: 153-155 °C; Mol. F.: $C_{25}H_{27}N_3O_4$; Mol. Mass: 433 g/mol; IR (KBr, cm^{-1}) ν_{max}: 3412 (N-H), 3084 (Ar C-H), 2885 (R C-H), 1659 (C=O), 1587 (Ar C=C), 1195 (C-O-C), 1117 (C-N-C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.88 (d, *J* = 8.3 Hz, 2H, H-2'' and H-6''), 7.59 (d, *J* = 8.3 Hz, 2H, H-2''' and H-6'''), 7.50 (d, *J* = 1.0 Hz, 1H, H-5), 7.46 (d, *J* = 8.0 Hz, 2H, H-3''' and H-5'''), 7.20 (d, *J* = 8.8 Hz, 2H, H-3'' and H-5''), 7.09 (d, *J* = 2.6 Hz, 1H, H-3), 6.44 (dd, *J* = 3.3, 1.4 Hz, 1H, H-4), 3.90 (q, *J* = 7.6 Hz, 2H, H-1''''), 3.87 (br.s, 4H, CH_2-3' and CH_2-5'), 3.58 (s, 2H, CH_2-8''), 2.57 (br.s, 4H, CH_2-2' and CH_2-6'), 1.38 (t, *J* = 7.5 Hz, 3H, CH_3-2''''); EI-MS (*m/z*): 433 $[M]^+$, 338 $[C_{20}H_{24}N_3O_2]^+$, 309 $[C_{19}H_{21}N_2O_2]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 255 $[C_{16}H_{17}NO_2]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-(2,3-Dimethylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl} benzamide (5d):** Light pink amorphous solid; Yield: 86 %; m.p: 132-134 °C; Mol. F.: $C_{25}H_{27}N_3O_3$; Mol. Mass: 417 g/mol; IR (KBr, cm^{-1}) ν_{max}: 3416 (N-H), 3075 (Ar C-H), 2878 (R C-H), 1652 (C=O), 1580 (Ar C=C), 1205 (C-O-C), 1107 (C-N-C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.86 (d, *J* = 8.0 Hz, 2H, H-2'' and H-6''), 7.54 (d, *J* = 7.5 Hz, 1H, H-6'''), 7.49 (d, *J* = 0.8 Hz, 1H, H-5), 7.23 (d, *J* = 8.2 Hz, 2H, H-3'' and H-5''), 7.11 (t, *J* = 8.0 Hz, 1H, H-5'''), 7.06 (d, *J* = 7.5 Hz, 1H, H-4'''), 7.00 (d, *J* = 2.8 Hz, 1H, H-3), 6.47 (dd, *J* = 3.2, 1.7 Hz, 1H, H-4), 3.81 (br.s, 4H, CH_2-3' and CH_2-5'), 3.57 (s, 2H, CH_2-8''), 2.51 (br.s, 4H, CH_2-2' and CH_2-6'), 2.34 (s, 3H, CH_3-1''''), 2.14 (s, 3H, CH_3-2''''); EI-MS (*m/z*): 417 $[M]^+$, 322 $[C_{20}H_{24}N_3O]^+$, 293 $[C_{19}H_{21}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 239 $[C_{16}H_{17}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-(2,4-Dimethylphenyl)4-{[4-(2-furoyl)-1-piperazinyl]methyl} benzamide (5e):** Off- white amorphous solid; Yield: 92 %; m.p: 141-143 °C; Mol. F.: $C_{25}H_{27}N_3O_3$; Mol. Mass: 417 g/mol; IR (KBr, cm^{-1}) ν_{max}: 3405 (N-H), 3078 (Ar C-H), 2882 (R C-H), 1649 (C=O), 1578 (Ar C=C), 1201 (C-O-C), 1115 (C-N-C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.88 (d, *J* = 7.6 Hz, 2H, H-2'' and H-6''), 7.78-7.51 (m, 3H, H-3''', H-5''' and H-6'''), 7.49 (d, *J* = 1.7 Hz, 1H, H-5), 7.10 (d, *J* = 8.1 Hz, 2H, H-3'' and H-5''), 7.01 (d, *J* = 3.2 Hz, 1H, H-3), 6.49 (dd, *J* = 3.4, 1.6 Hz, 1H, H-4), 3.85 (br.s, 4H, CH_2-3' and CH_2-5'), 3.65 (br.s, 2H, CH_2-8''), 2.55 (br.s, 4H, CH_2-2' and CH_2-6'), 2.34 (s, 3H, CH_3-2''''), 2.32 (s, 3H, CH_3-1''''); EI-MS (*m/z*): 417 $[M]^+$, 322 $[C_{20}H_{24}N_3O]^+$, 293 $[C_{19}H_{21}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 239 $[C_{16}H_{17}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-(2,6-Dimethylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl} benzamide (5f):** White crystalline solid; Yield: 90 %; m.p.: 140-142 C; Mol. F.: $C_{25}H_{27}N_3O_3$; Mol. Mass.: 417 g/mol; IR (KBr, cm^{-1}) ν_{max}: 3411 (N-H), 3078 (Ar C-H), 2882 (R C-H), 1650 (C=O), 1579 (Ar C=C), 1208 (C-O-C), 1114 (C-N-C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.89 (d, *J* = 8.2 Hz, 2H, H-2'' and H-6''), 7.49 (d, *J* = 0.9 Hz, 1H, H-5), 7.19 (d, *J* = 8.4 Hz, 2H, H-3'' and H-5''), 7.13-7.08 (m, 3H, H-3''' to H-5'''), 7.03 (d, *J* = 2.7 Hz, 1H, H-3), 6.46 (dd, *J* = 3.4, 1.6 Hz, 1H, H-4), 3.89 (br.s, 4H, CH_2-3' and CH_2-5'), 3.62 (s, 2H, CH_2-8''), 2.57 (br.s, 4H, CH_2-2' and CH_2-6'), 2.28 (s, 6H, CH_3-1'''' and CH_3-2''''); EI-MS (*m/z*): 417 $[M]^+$, 322 $[C_{20}H_{24}N_3O]^+$, 293 $[C_{19}H_{21}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 239 $[C_{16}H_{17}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-(3,5-Dimethylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl} benzamide (5g):** Light brown liquid; Yield: 86 %; Mol. F.: $C_{25}H_{27}N_3O_3$; Mol. Mass: 417 g/mol; IR (KBr, cm^{-1}) ν_{max}: 3409 (N-H), 3071 (Ar C-H), 2886 (R C-H), 1646 (C=O), 1575 (Ar C=C), 1209 (C-O-C), 1118 (C-N-C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.86 (d, *J* = 8.2 Hz, 2H, H-2'' and H-6''), 7.53 (d, *J* = 0.9 Hz, 1H, H-5), 7.27 (d, *J* = 8.4 Hz, 2H, H-3'' and H-5''), 7.16 (s, 2H, H-2''' and H-6'''), 7.09 (d, *J* = 2.6 Hz, 1H, H-3), 6.91 (s, 1H, H-4'''), 6.46 (dd, *J* = 3.4, 1.8 Hz, 1H, H-4), 3.81 (br.s, 4H, CH_2-3' and CH_2-5'), 3.63 (s, 2H, CH_2-8''), 2.59 (br.s, 4H, CH_2-2' and CH_2-6'), 2.28 (s, 6H, CH_3-1'''' and CH_3-2''''); EI-MS (*m/z*): 417 $[M]^+$, 322 $[C_{20}H_{24}N_3O]^+$, 293 $[C_{19}H_{21}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 239 $[C_{16}H_{17}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-(2-Ethyl-6-methylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl] methyl}benzamide (5h):** Light brown liquid; Yield: 90 %; Mol. F.: $C_{26}H_{29}N_3O_3$; Mol. Mass: 431 g/mol; IR (KBr, cm^{-1}) ν_{max}: 3409 (N-H), 3081 (Ar C-H), 2881 (R C-H), 1658 (C=O), 1586 (Ar C=C), 1197 (C-O-C), 1110 (C-N-C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.86 (d,

J = 8.2 Hz, 2H, H-2'' and H-6''), 7.57 (d, *J* = 0.9 Hz, 1H, H-5), 7.20 (d, *J* = 8.4 Hz, 2H, H-3'' and H-5''), 7.07 (d, *J* = 2.7 Hz, 1H, H-3), 6.98-6.90 (m, 3H, H-3''' to H-5'''), 6.46 (dd, *J* = 3.4, 1.6 Hz, 1H, H-4), 3.80 (br.s, 4H, CH_2-3' and CH_2-5'), 3.68 (s, 2H, CH_2-8''), 2.57 (br.s, 4H, CH_2-2' and CH_2-6'), 2.48 (q, *J* = 7.5 Hz, 2H, H-1''''), 1.96 (s, 3H, CH_3-3''''), 1.07 (t, *J* = 7.5, 3H, CH_3-2''''); EI-MS (*m/z*): 431 $[M]^+$, 336 $[C_{21}H_{26}N_3O]^+$, 307 $[C_{20}H_{23}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 253 $[C_{17}H_{19}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

Butyrylcholinesterase assays

The BChE inhibition study was performed according to the established method [14]. The percent inhibition was calculated by the following equation,

$$\text{Inhibition}(\%) = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

IC_{50} values (concentration at which there is 50% in enzyme catalyzed reaction) compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Statistical analysis

The results are written as mean ± SEM after performance in three-folds and statistical analysis by Microsoft Excel 2010. Minimum inhibitory concentration (MIC) was calculated by using different dilutions (ranging 5-30 μg/well) and EZFit Perrella Scientific Inc. Amherst USA software.

Hemolytic activity

Hemolytic activity was done by the reported method [15,16]. Bovine blood was obtained from the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. After centrifugation, separation and washing, the % RBCs lysis was computed by noting the absorbance.

Molecular docking methodology

To predict the bioactive conformations, various compounds (ligands) were docked into the binding pockets of the selected proteins (enzymes) by using the default parameters of MOE-Dock program.

Ligands preparation: The three dimensional (3D) structures of synthesized compounds were modeled by using the Build program of MOE 2009-10. Then the energies of the compounds were minimized by using the default parameter of MOE energy minimization algorithm (gradients: 0.05, force field: MMFF94X). Database was created in which all the compounds (3D structures) were saved in the mdb file format for the next step of docking.

Receptor protein preparation: The 3D structures of receptor protein molecules of α-glucosidase (PDB ID code: 3NO4; resolution: 2.02 Å), acetyl cholinesterase (PDB ID code: 1ZJO; Resolution: 1.64 Å) and butyrylcholinesterase (PDB ID code: 1POP; Resolution: 2.30 Å) were downloaded from Protein Data Bank. All water molecules were removed from the receptor proteins and 3D protonation was carried out by using Protonate 3D Option. Protein molecules were energy minimized by using the default parameters of MOE 2009-10 energy minimization algorithm (gradient: 0.05, Force Field: MMFF94X). By using default parameters of MOE-Dock Program, all the ligands were docked into binding sites of the above proteins. Re-docking procedure was also used to increase the validity of docking protocol [17].

Results and Discussion

Chemistry

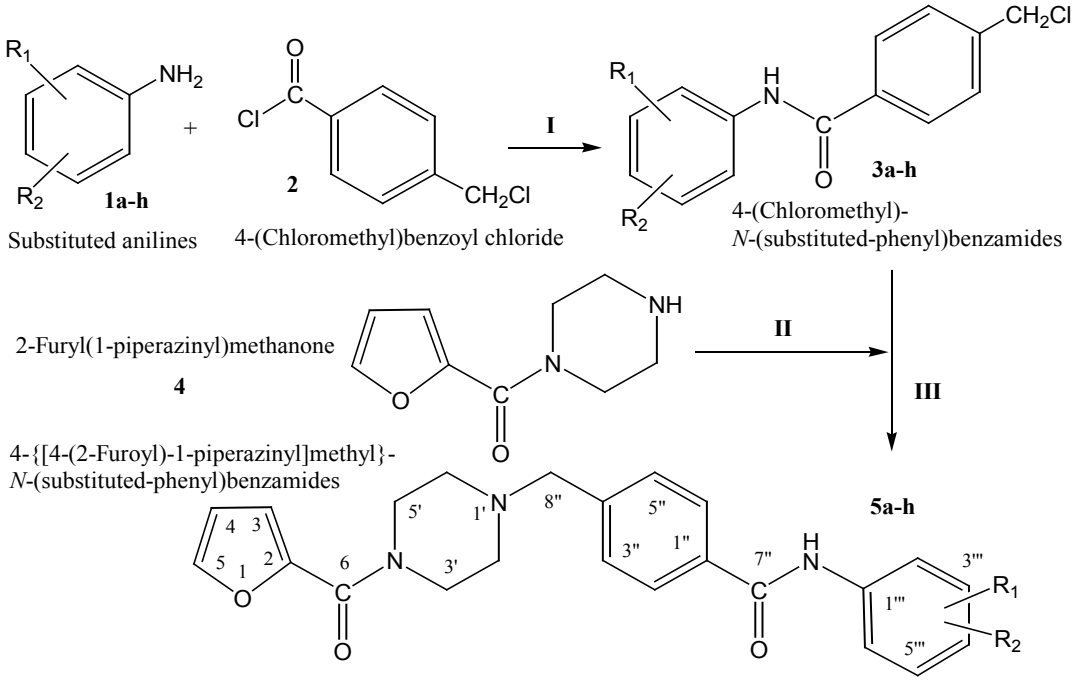
The aim of the present research work was to synthesize new molecules and to evaluate their biological activities against butyrylcholinesterase and their hemolytic activity was also checked. In the present research work, different 4-{[4-(2-furoyl)-1-piperazinyl]methyl}-*N*-(substituted-phenyl)benzamides (5a-h) were synthesized in a multiple steps as it is depicted in Scheme 1and Table 1.

The molecule 5b was synthesized as off white amorphous solid having melting point of 151-153°C and molecular formul of $C_{25}H_{27}N_3O_3$, which was confirmed by EI-MS by appearance of $[M]^+$ peak at *m/z* 417 and by by counting the protons in ^{1}H-NMR spectrum. The suggested mass fragmentation pattern and spectrum of this molecule is given in Figures 1 and 2. The distinct peak at *m/z* 239 was related to 4-ethylphenylmethylbenzamide and the peak at *m/z* 95 to furoyl part of the molecule. In IR spectrum, characteristic peaks appeared at 3405 (N-H), 3095 (Ar C-H), 2883 (R C-H), 1651 (C=O), 1580 (Ar C=C), 1197 (C-O-C), 1119 (C-N-C) which confirmed the presence of benzamide and 2-furoyl-1-piperazine ring. In ^{1}H-NMR spectrum signals of methylbenzamide moiety appeared at δ 7.86 (d, *J* = 8.2 Hz, 2H, H-2'' and H-6''), 7.22 (d, *J* = 8.4 Hz, 2H, H-3'' and H-5''), 3.62 (s, 2H, CH_2-8''). The signals of disubstituted aromatic proton appeared at 7.58 (d, *J* = 8.3 Hz, 2H, H-2''' and H-6''') and 7.47 (d, *J* = 8.0 Hz, 2H, H-3''' and H-5''') were assigned to 1,4-disubstituted aromatic ring. Furan ring showed three peaks in aromatic region at δ 7.49 (d, *J* = 0.9 Hz, 1H, H-5), 7.00 (d, *J* = 2.7 Hz, 1H, H-3), 6.49 (dd, *J* = 1.6, 3.4 Hz, 1H, H-4). In the aliphatic region ^{1}H-NMR spectrum for piperazine ring of eight protons appeared at δ 3.84 (br.s, 4H, CH_2-3' and CH_2-5') and 2.54 (br.s, 4H, CH_2-2' and CH_2-6'), while for ethyl group of disubstituted aromatic ring signal appeared at δ 2.67 (q, *J* = 7.6 Hz, 2H, CH_2-1'''') and 1.22 (t, *J* = 4.8 Hz, 3H, CH_3-2''''). The ^{1}H-NMR spectrum of this molecule is given in Figure 3. On the basis of these spectral evidences, the structure was assigned as *N*-(4-Ethylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl} benzamide. All the synthesized molecules (5a-h), were characterized by IR, ^{1}H-NMR and EI-MS spectral analysis same as mentioned above.

Pharmacology

Enzyme inhibition study (*in vitro*): The synthesized compounds exhibited valuable inhibitory potential against butyrylcholinesterase as it was evident from their too low IC_{50} values shown in Table 2. The most active inhibitors among all the molecules were *N*-(4-Ethylphenyl)4-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (5b) and *N*-(2-Ethyl-6-methylphenyl)4-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (5h) having IC_{50} values of 0.82 ± 0.001 μM and 0.91 ± 0.003 μM respectively, relative to Eserine, a reference standard with IC_{50} value of 0.85 ± 0.0001 μM. The enhanced potential of these molecule might be attributed to the presence of 4-ethylphenyl and 2-Ethyl-6-methylphenyl groups in these molecules. The comparison of molecules, 5b (0.82 ± 0.001) and 5c (115.63 ± 0.01), demonstrated that molecule (5b) bearing *para* ethyl substituted phenyl ring linked to acetamoyl group remained more efficient as compared to that (5c) bearing *para* ethoxyphenyl ring. Among all the molecules (5d-h) bearig disubstituted phenyl ring, the *meta* substitution decremented the biological activity of compounds like 5d (141.23 ± 0.03) and 5g (76.81 ± 0.02). The molecules bearing *ortho* substituted phenyl rings remained better inhibitors. Furthermore, the *ortho* substitution by ethyl group enhanced the inhibition ability, compound 5h. In future molecule 5b can further be investigated to introduce a potent drug candidate, as it has hown better IC_{50} value even from the reference standard (Eserine).

Hemolytic activity: The cytotoxicity of the synthesized molecules was also investigated through hemolytic activity analysis. The highest hemolytic activity (Table 2) was shown by 5c (80.55 %) but lower than the positive control (Triton-X-100). The lowest activity was shown by 5b (4.48 %) but higher than the negative controls (PBS). The least



Scheme 1: Outline for the synthesis of 4-{[4-(2-furoyl)-1-piperazinyl]methyl}-*N*-(substituted-phenyl)benzamides (**5a-h**). Reagents and Conditions: (I) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 3-4 hrs. (II) Acetonitrile/K_2CO_3/refluxing for 0.5 hrs. (III) Refluxing for 4-5 hrs.

Compd.	5a	5Tb	5c	5d	5e	5f	5g	5h
$-R_1$	2-OCH_3	-H	-H	2-CH_3	2-CH_3	2-CH_3	3-CH_3	2-C_2H_5
$-R_2$	-H	4-C_2H_5	4-OEt	3-CH_3	4-CH_3	6-CH_3	5-CH_3	6-CH_3

Table 1: Different substituents (-R1 and -R2) in Scheme 1.

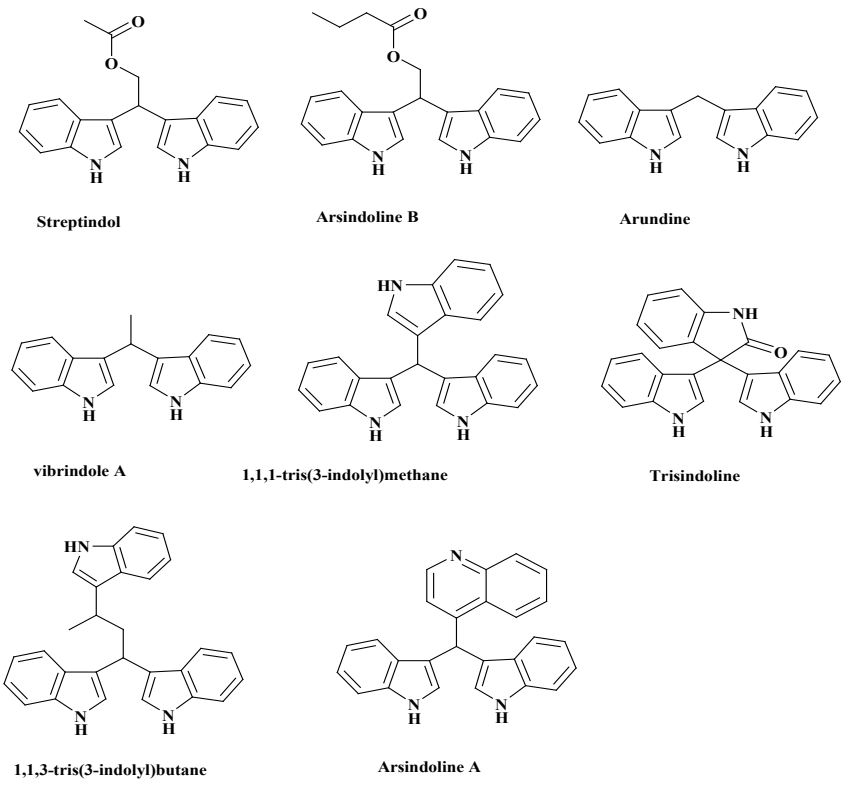


Figure 1: Suggested mass fragmentation pattern of *N*-(4-ethylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (5b).

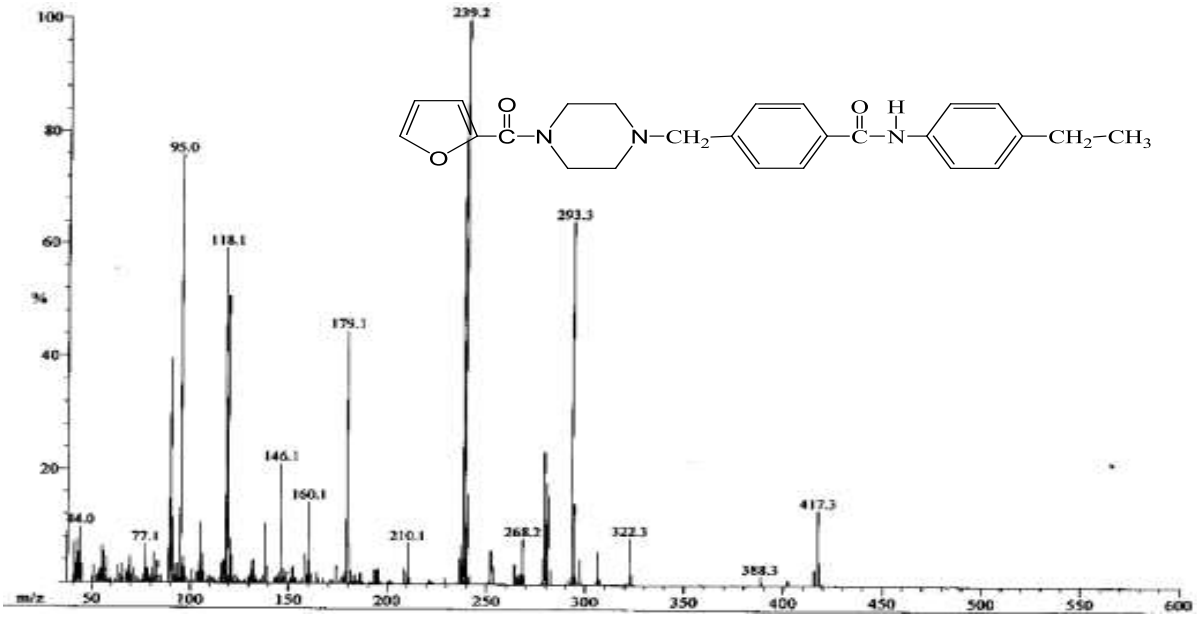


Figure 2: EI-MS spectrum of N-(4-ethylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (5b).

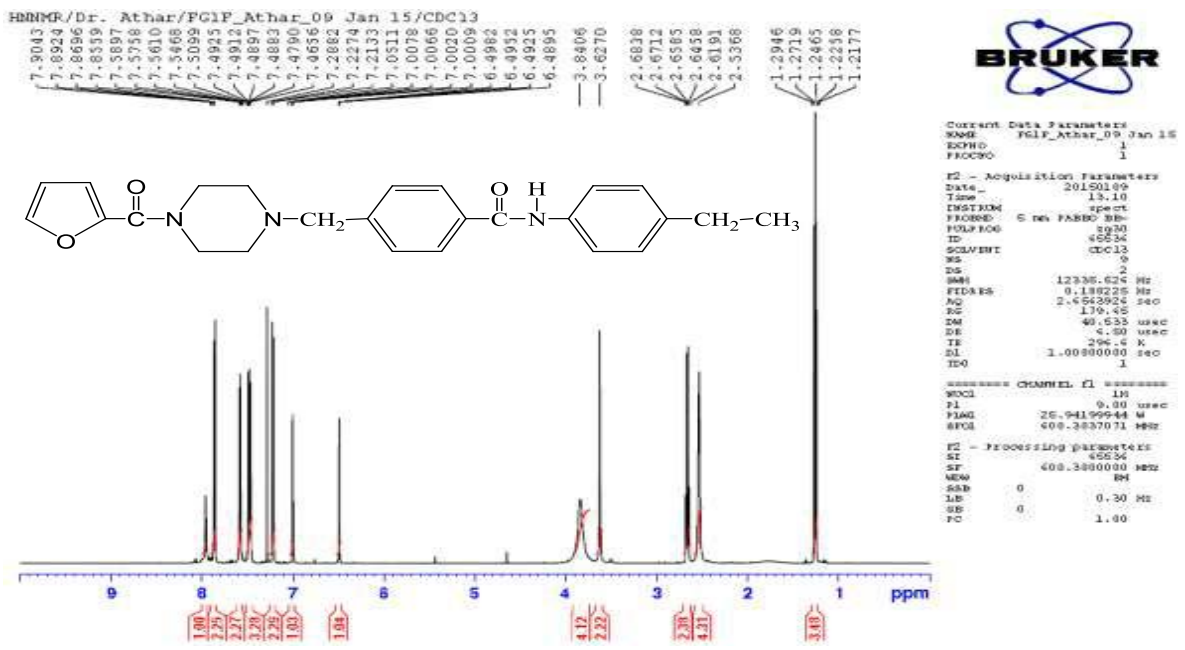


Figure 3: 1H-NMR spectrum of N-(4-ethylphenyl)-4-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (5b).

cytotoxicity and highest enzyme inhibition of 5b made it the most active compound among all the synthesized compounds. The range overall demonstrated that most of the molecules are not much cytotoxic and can be considered as possible therapeutic agents.

Computational docking: All compounds were docked into the active pocket of butyrylcholinesterase. Compound 5a make two arene-arene interactions. First interaction was observed between Trp82 and phenyl ring of the inhibitor with a bond length of 3.41 Å. Second was found between Tyr332 and benzyl ring of ligand giving a bond length of 4.42 Å as indicated in Figure 4 (2D and 3D). Compound 5b was deeply bound in the binding pocket of enzyme. It makes two important interactions with Ser287 and Trp82 amino acid residues. Ser287 interacted strongly through side chain acceptor with the NH group of ligand giving a bond length of 2.06 Å, while second arene-arene interaction was made between Trp82 and furoyl ring with a bond distance of 3.77 Å and 3.94 Å as indicated in Figure 5 (2D and 3D). Compound 5c showed two clear interactions with Tyr332 and Trp82

Tyr332 was bound with phenyl ring of the ligand through arene-arene interaction giving a bond distance of 3.52 Å. Trp82 was also involved in the same π-π interaction with furoyl ring giving a bond length of 3.68 Å as shown in Figure 6 (2D and 3D). Compound 5d has made a clear single side chain acceptor (basic) interaction through its NH group with Ser287. The bond distance of 2.27 Å was achieved as indicated in Figure 7 (2D and 3D). Compound 5e were docked into the active site of butyrylcholinesterase. It was found with two arene-arene interactions. First between Tyr332 and phenyl ring with a distance of 3.69 Å and second between Trp82 and furoyl ring of the ligand in a bond lengths of 3.46 Å and 4.14 Å as indicated in Figure 8 (2D and 3D). Compound 5f made two π-π interactions with Trp82 and Tyr332 active site residues. Trp82 interacted with phenyl ring having a bond distance of 3.46 Å, but Trp82 gave interaction with benzyl ring having a length of 3.90 Å as shown in Figure 9. Pro285, Ser72 and His438 etc were also present very close to the ligand. Compound 5g was observed that carbonyl oxygen make a single strong side chain donor interaction with Thr120 giving a bond length of 3.24 Å. Trp82, Tyr332, Met437, Asp70 and Ser287 etc were also present in the closest region of the interaction as shown in Figure 10 (2D and 3D). Compound 5h also made two interactions. Tyr332 bonded with phenyl ring of the ligand through a π-π interaction. The bond length calculated was 3.68 Å. Trp82 made arene-arene interaction with furoyl ring of the compound giving a bond distance of 3.85 Å as shown in Figure 11 (2D and 3D).

Compounds	BchE		Hemolytic activity
	Inhibition % age	IC_{50} (μM)	% age
5a	95.24 ± 0.08	8.32 ± 0.005	69.34
5b	97.27 ± 0.03	0.82 ± 0.001	4.48
5c	94.52 ± 0.06	115.63 ± 0.01	80.55
5d	75.37 ± 0.09	141.23 ± 0.03	7.19
5e	95.54 ± 0.07	3.41 ± 0.002	15.17
5f	96.17 ± 0.05	4.51 ± 0.006	16.06
5g	82.14 ± 0.08	76.81 ± 0.02	13.58
5h	93.15 ± 0.08	0.91 ± 0.003	41.51
Control	**82.82 ± 1.09[a]**	**0.85 ± 0.0001[a]**	
PBS			**0.09**
Triton			**100**

Note: All compounds were dissolved in methanol and experiments were performed in triplicate (mean ± SEM, n = 3), a = Eserine, BChE = Butyrylcholinesterase.

Table 2: Enzyme inhibitory and hemolytic activity of synthesized 4-{[4-(2-furoyl)-1-piperazinyl]methyl}-*N*-(substituted-phenyl)benzamides (**5a-h**).

Conclusion

The structures of the synthesized molecules were confirmed through spectral data in a very lucid manner. All the synthesized molecules were investigated for their activity against butyrylcholinesterase enzyme along with cytotoxic behavior. Overall, these molecules can be considered as possible therapeutic entrants for the Alzheimer's disease, and particularly, molecule 5b, being a potent inhibitor of butyrylcholinesterase enzyme along with the least cytotoxicity, is much suited candidate for aforementioned disease. Among the synthesized molecules bearing monosubstituted phenyl ring, *para* ethyl remained more efficient inhibitors. Likewise among disubstituted ones, again molecule bearing *ortho* ethyl phenyl ring was the most efficient. The molecules bearing *meta* substituted groups remained least efficient in

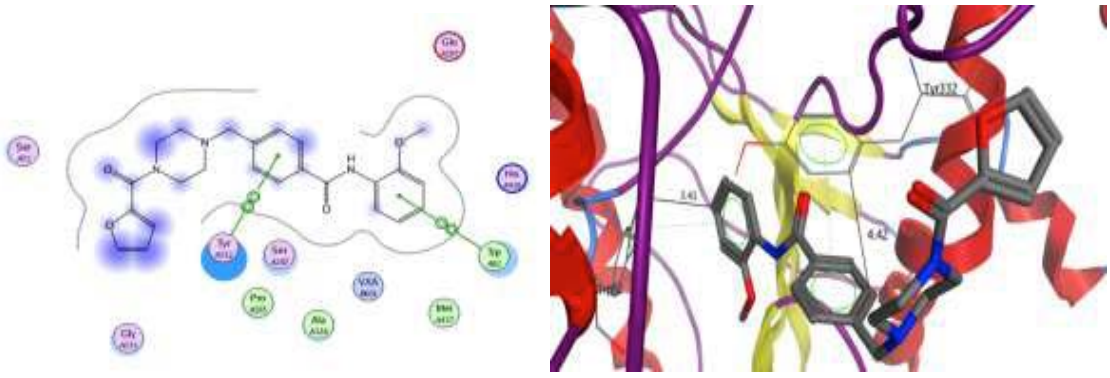


Figure 4: (2D and 3D): The interaction analysis of 5a.

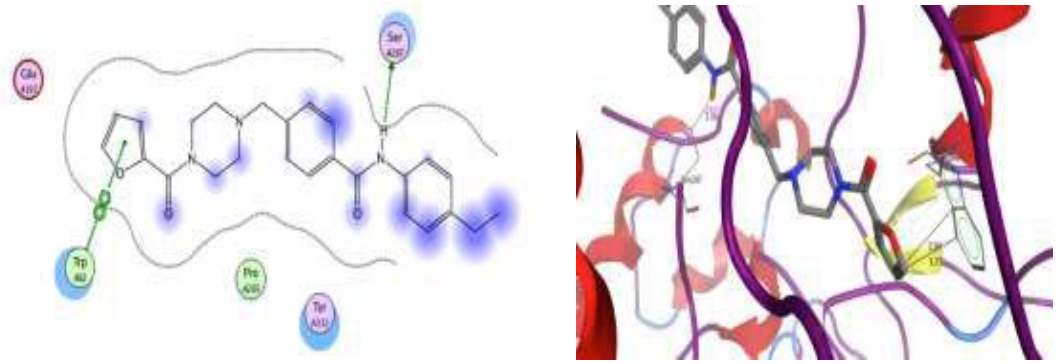


Figure 5: (2D and 3D): The interaction analysis of 5b.

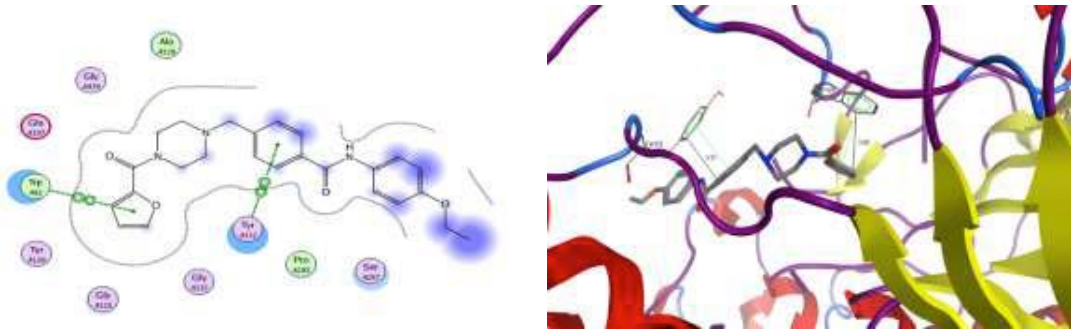


Figure 6: (2D and 3D): The interaction analysis of 5c.

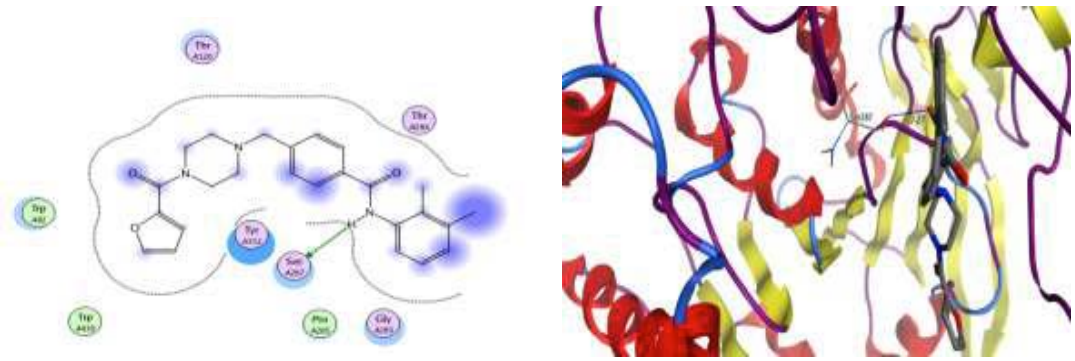


Figure 7: (2D and 3D): The interaction analysis of 5d.

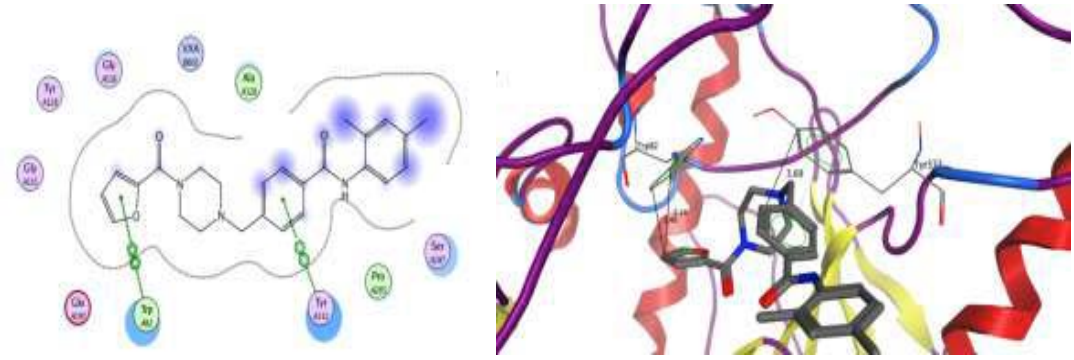


Figure 8: (2D and 3D): The interaction analysis of 5e.

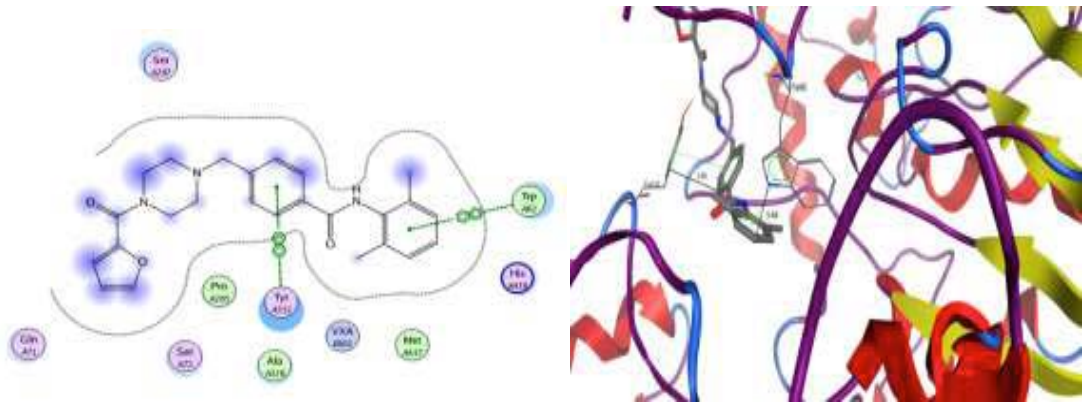


Figure 9: (2D and 3D): The interaction analysis of 5f.

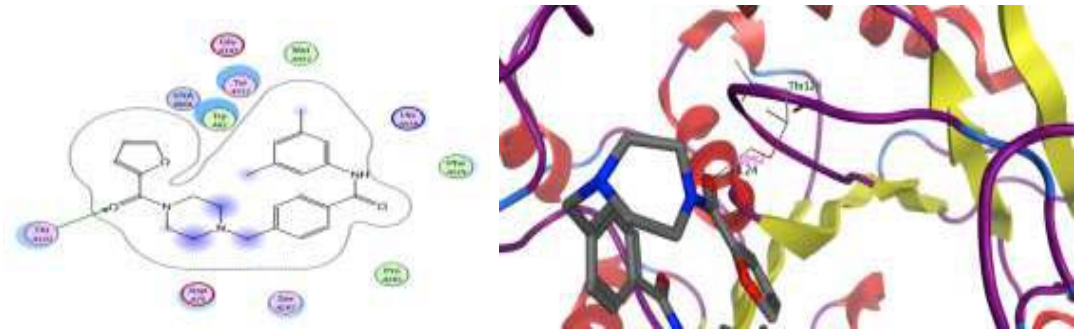


Figure 10: (2D and 3D): The interaction analysis of 5g.

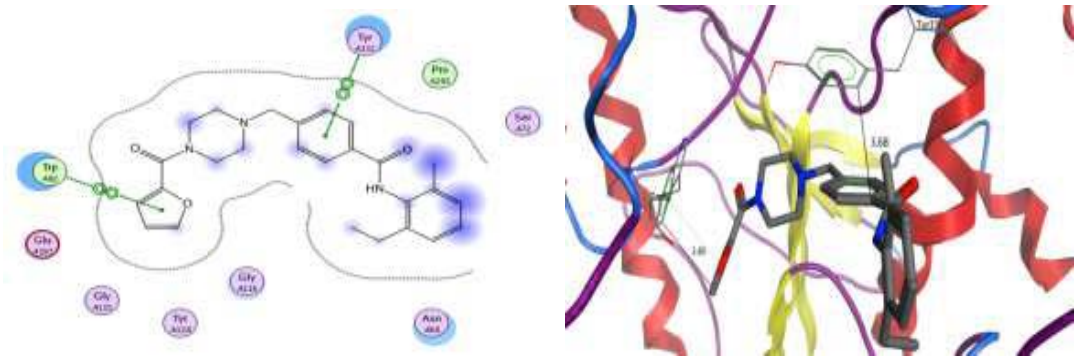


Figure 11: (2D and 3D): The interaction analysis of 5h.

inhibiting the enzyme, although their cytotoxicity was low as compared to others. Hence, the synthesized molecules are foreseen for the drug designing program in future.

Acknowledgements

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