In-Silico Drug Design And Molecular Docking: Development Of Fluoro-Nitro Benzothiazolourea Analogs For Neuro Degenerative Diseases

Chandrashekhar a S[1], M Gnanarubapriya[2], Dhana shree N[3], Rekha S[4]*, Panag ante Sagar[5]

Abstract

Alzheimer is a chronic neurodegenerative syndrome. It is irreversible disease predominantly degenerates the brain cells and predominantly affects the person aptitude to function autonomously. Even though after active investigation, no appropriate treatment but can edge their determined outcome in early stages. In explore of supplementary effective drugs for the cure of Alzheimer. In the present work we synthesized fluoro-nitro benzothiazolourea derivatives by three schemes and were characterized by various spectroscopic techniques. The parameters of Drug likeness of the synthesized analogs were analyzed *in-silico*, followed by molecular modeling to establish the binding mode of AChE with protein **4EY7**. All the derivatives exhibited excellent docking scores ranging from -9.7 to -11.2, the scores were compared with the reference standard donepezil with -11.6 dock scores. Apart from docking scores, all the derivatives exhibit excellent hydrogen bonding with amino acids. *In-vitro* studies were performed for further investigation of lead candidate against acetyl cholinesterase enzyme under positive control of donepezil. Seven derivatives **55(III), 55(III)b, 55(I)d, 55(II)a, 55(II)d, 55(II)f** and **55(II)g** showed more promising activity against acetyl cholinesterase enzyme and IC_{50} values of **55(III)** and **55(I)d** is 94.58±1.74, **55(II)d** is 92.39±1.72 and **55(II)g** is 98.55±1.17.

Keywords: Alzheimer, Fluoro-nitrobenzothiazolourea, Molecular modelling, Drug likeness, Donepezil, Acetyl cholinesterase.

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INTRODUCTION

Alzheimer's disease (AD) is the most widespread degenerative disease of brain and is explained collectively with the mutilation of cognitive function. Patients will be unable to regulate original memories. The improvement of novel drugs in this vicinity continues at an enormous pace[1]. But till time - the exact root and method of this disease is completely unfamiliar but as per common consent there are assured factors like increased chlorination of neuron protein in brain, decreased absorption of acetylcholine (Ach), accumulation of beta amyloid peptide and oxidative pressure play crucial roles to prompt the disease further [2].

Fluoro-nitrobenzothiazolourea derivatives are a fascinating group with a broad range of activities. The literature has reported activity for this class of compounds were aldose-reductase activity [3], Raf-1 inhibitor activity [4], anticonvulsant [5], anti-microbial [6], anti-Alzheimer's activity [7, 8], anticancer activity [9], topoisomerase II inhibitor activities [10] and potential PET cancer imaging agents [11].

In the present research we aimed to locate the appropriateness and probability of novel methods for the synthesis of fluoro-

***Corresponding Author: -** Rekha S
Address: [1]Department of Pharmaceutics, Dr. Ravi Patil College of Pharmacy, Belgaum, Karnataka, India.
[2,3,5]Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Dayananda Sagar University, Bangalore, Karnataka, India.
*[4]Assistant Professor Dept. of Pharmaceutical chemistry College of Pharmaceutical sciences Dayananda Sagar University Bengaluru -560 078 Karnataka, India. E-mail: rekha.maheshh@gmail.com, Mobile no: (+91) 9980402125
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nitrobenzothiazolourea derivatives. Based on literature survey we made an attempt to incorporate urea into fluoro-nitrobenzoth iazole ring system with the hope that it enhances the clinical activity.

Molecular docking is a fast and efficient computational method to predict the preferred orientation of the bioactive compounds to a specific protein, when bound to each other they form a stable complex [12].

MATERIALS AND METHODS

Drugs & Chemicals: Procurement of 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), was from Sigma-Aldrich. The compounds used were highest analytical grade. **Protein data bank (PDB)**

PDB is a database for the three-dimensional structural data for large biological molecules, which includes proteins and nucleic acids. Most of the structures are determined by X-ray diffractions and NMR studies. Each structure published PDB receives a four-character alphanumeric identifier called PDB ID Eg: 4EY7 [13].

Molecular docking

Docking studies were carried out to analyse the different types of biomolecular interactions and ligand receptor binding affinities. Molecular docking is achieved by means of Autodock vina. The 3D crystallographic protein structure is discovered from protein data bank (PDB ID- 4EY7). Autodock Vina is an open-source program with a complete molecular viewer and graphical support for doing molecular docking. PyMOL produce a high-quality 3D image of protein as well as its visualization. PyRx is for docking analysis [14]. The docking study was performed on PPARG Nuclear protein (PDB ID: **4EY7**). The computational work was performed on a **HP 15s-eq0132au Laptop running on AMD Ryzen 7 3700U processor**.

Protein preparation

The protein selected from protein data bank that is 4EY7 which is crystal structure of recombinant human acetylcholinesterase in complex with Donepenzil, which is Homo -2-mer with resolution of 2.35A. This protein is acetylcholinesterase enzyme which has been evidently seen responsible for depletion of acetylcholine in alzheimer's disease extensively. Hydrogen atom should be added to protein structure.

In the present study rigid docking is performed. The first a binding site on 4EY7 is predicted using the software called CASTp 3.0 (Computational Atlas of Surface Topology of protein), it is based on recent theoretical and algorithm results of computational geometry. Using Auto dock vina 1.5.7 remove chain A , C ,D and keeping Chain B in that the co-crystalized ligand along with drug is also deleted, the water molecule which can interrupt the docking process is also removed. Add kollman charges, here the protein selected has (3.264) and saved in PDBQT format. This is followed by additional step by adding polar contacts to find out the types of amino acid interactions during ligand-receptor binding [15].

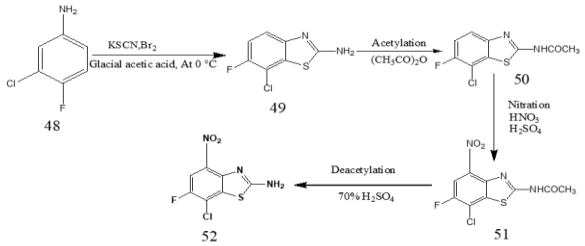
Ligand Preparation

The 2D chemical structures of ligands are drawn using ACD Lab Chemsketch ver 12.0 and generated smiles notation. The 3D structures of the ligands were downloaded from RCSB PDB (https://www.rcsb.org) and uploaded in BIOVIA Discovery Studio Visualizer-2020. Ligand minimization was done and using small molecule wizard in 'SMALL MOLECULE' wizard in BIOVIA Discovery Studio Visualizer-2020 and was saved as a cluster sdf file [16].

Donepezil

Donepezil is an effective, selective, noncompetitive, and quick acting reversible inhibitor of acetylcholinesterase (AChEI) qualified for the healing of Alzheimer disease (AD); and is the primary and solitary AChEI accredited in the management of rigorous AD [17].

SCHEME 1



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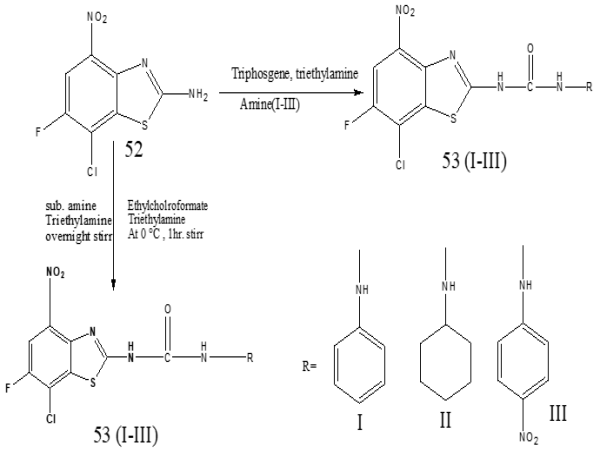
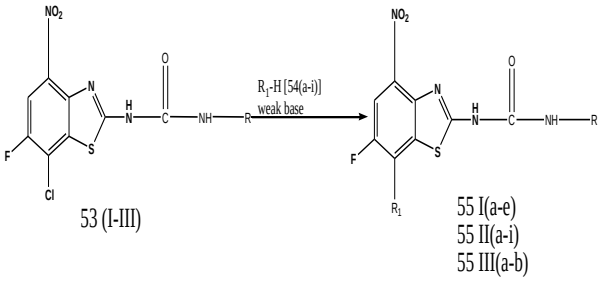
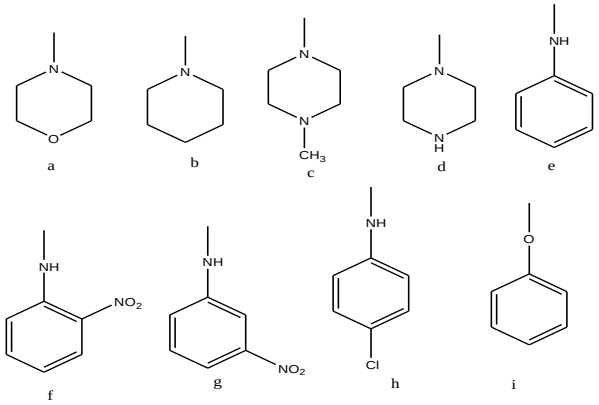


SCHEME 3

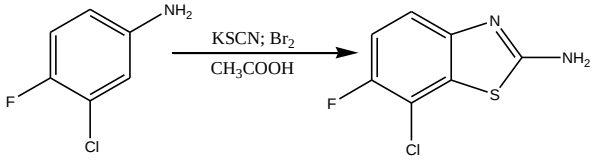


for II R1



EXPERIMENTAL PROCEDURE.

SYNTHESIS OF 2-AMINO- 7-CHLORO-6-FLUORO BENZOTHIAZOLE

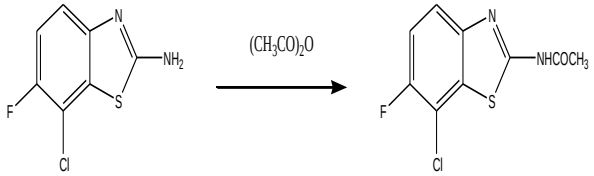


48 49

To 40 ml pre-cooled (5 ˚C) glacial acetic acid, add 40 g (2.4mol) of potassium thiocyanate and 7.25 g (0.05mol) of 3-fluoro-4-chloroaniline. The mixture of 6 ml of bromine in 24 ml of glacial acetic acid was added drop wise at 0 ˚C temperature for 10hrs. After overnight orange precipitate will settle at the bottom, to this add 30 ml water and solution was heated at on a steam bath at 85 ˚C and filter. To the orange residue add 10 ml of glacial acetic acid heat again to 85 ˚C and filter. Combine both the filtrates and neutralized with concentrated ammonia solution to pH 6. Dark yellow precipitate obtained is recrystallized using toluene[18].

Chemical Name	7-Chloro-6-fluorobenzo[d]thiazole-2-amine.
Molecular formula	$C_7H_4ClFN_2S$
Molecular weight	202.02
Melting point	180 ˚C
% yield	85
***R_f* values**	0.75
Mobile phase	n-Hexane: Ethyl acetate2:1
Solubility	DMSO, Chloroform, Acetone
IR spectral studies (KBr) cm⁻¹:	**(**Ar C=C) 1527, (C-H) 2924, (C-F) 1261, (NH_2) 3441, 3363, (C-Cl) 736.

SYNTHESIS OF 2-ACETAMIDO- 7-CHLORO-6-FLUORO BENZOTHIAZOLE (50):-

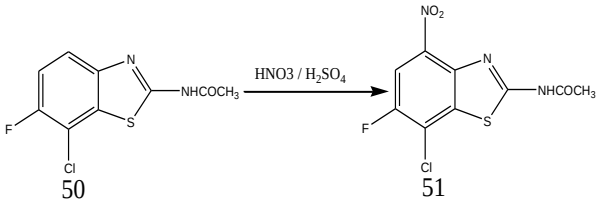


49 50

Took 0.01ml of 2-amino-6-fluoro-7-chloro-benzothiazole into a 100 ml RBF, add 10 ml of acetic anhydride and refluxed it for 1hr. Cool it and pour it in ice water and boil to remove the excess of acetic anhydride. Filtered product is recrystallized from ethanol[19].

Chemical Name	N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)acetamide
Molecular formula	$C_9H_6ClFN_2OS$
Molecular weight	244.678
Melting point	240 ˚C
% yield	82.5.
***R_f* values**	0.69
Mobile phase	n- Hexane: Ethyl acetate2:1, Cyclohexane: Chloroform [2:1].
Solubility:	DMSO, Chloroform, Acetone.
IR spectral studies (KBr) cm⁻¹	**(**Ar C=C) 1530, (C-H) 2952, (C-F) 1278, (NH_2) 3120, (CH_3) 1442, (C-Cl) 751, (C=O) 1654.

SYNTHESIS OF 2-ACETAMIDO-7-CHLORO-6-FLUORO-4- NITROBENZOTHIAZOLE 51):-

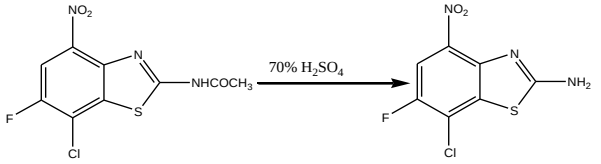


50 51

The above acetamide (100 mg) is added to ice cooled conc. H_2SO_4 (0.3 ml) and to this is conc. HNO_3 (0.1 ml) at 0 ˚C and stirred for 2 hr. Add additional conc. HNO_3 (0.1 ml) and mixture stirred at 30 ˚C overnight. The reaction mixture is added large amount of water and neutralized with aqueous $NaHCO_3$, Filter, add excess of water to wash the residue. The acetone and water used for recrystallization[20].

Chemical Name	N-(7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl) acetamide
Molecular formula	$C_9H_5ClFN_3O_3S$
Molecular weight	289.675
Melting point	270-280 ˚C
% yield	79.5.
***R_f* values**	0.563.
Mobile phase	n-hexane: Ethyl acetate.2:1.
Solubility	DMSO, Chloroform, Acetone.
IR spectral studies (KBr) cm-1:	(NO_2) 1502, 1325, (Ar str C=C) 1535, (C-H) 2924, (C-F) 1253, (C-Cl) 736, (NH) 3163, (C=O) 1656, (CH_3)1350

DEACETYLATION-2-ACETAMIDO-7-CHLORO-6-FLUORO-4 NITROBENZOTHIAZOLE (52):-

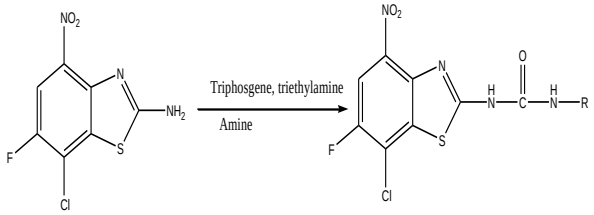


51 52

Reflux a mixture of 15 g (0.083) of N-(7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)acetamide with 75 ml of 70% w/w sulphuric acid for 20-30 min. Pour the clear hot solution in to 500 ml of ice water and adding more of 10% percent sodium hydroxide solution (or) of ammonia solution yellow crystalline amines precipitate outs. The acetone and water used for recrystallization [21].

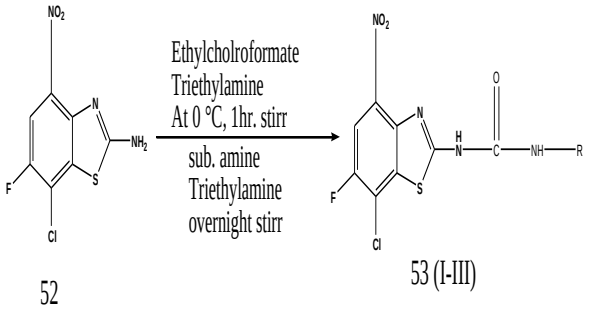
Chemical Name	7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-amine
Molecular formula	$C_7H_3ClFN_3O_2S$
Molecular weight	247.63
Melting point	230 ˚C
***R_f* values**	0.97
% yield	82.5.
Mobile phase	n - Hexane: Ethyl acetate.2:1.
Solubility:	DMSO, Chloroform, Acetone.
IR spectral studies (KBr) cm-1:	(NO_2) 1523, (Ar str C=C) 1553, (C-H) 2928, (C-F) 1327, (C-Cl) 781.81, (NH_2) 3298, 3120.

SYNTHESIS OF UREA DERIVATIVES [53 (I-III)]:-



52 53 (I-III)

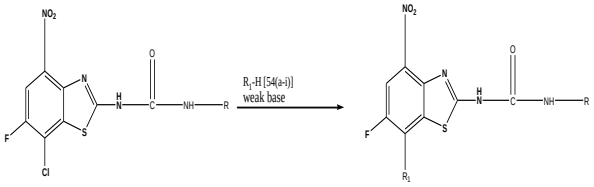
I. To a solution of 2-amino-6-fluoro-7-chloro-4-nitrobenzothiazole (0.8 mM) in dry dicholoromethane was added triethyl amine (2.4 mM) and triphosgene. After stirring for 15 min at 25 ˚C various amines (aniline, cyclohexylamine) were added and monitored by TLC. Add water and extract with ethyl acetate. By using anhydrous $MgSO_4$ dry organic layer and concentrate [22].



52 53 (I-III)

II. To a solution of 2-amino-6-fluoro-7-chloro-4-nitrobenzothiazole in tetrahydro furan at 0 ^{0}C was added triethyl amine and ethyl choloro formats, stir for 1-hour, various amines (aniline, cyclohexylamine) were added. Continue stirring for overnight simultaneously the temperature slowly rose to room temperature. Add water and extract with ethyl acetate. By using anhydrous $MgSO_4$ dry organic layer and concentrate [23].

REMOVAL OF CHLORINE BY DIFFERENT GROUPS:-

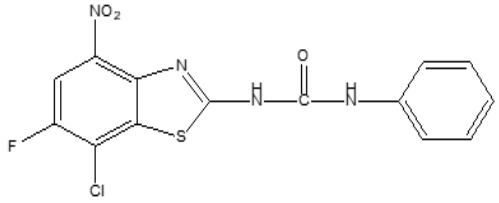
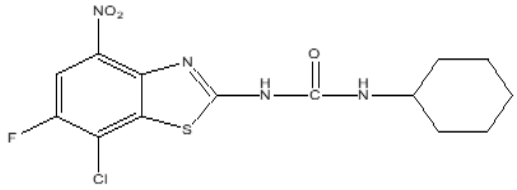
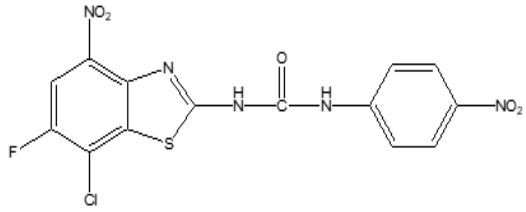
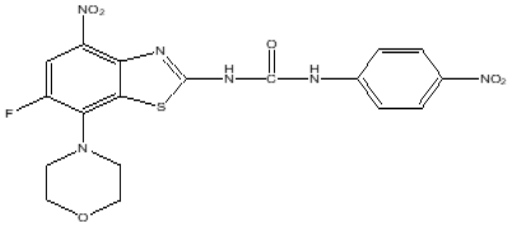
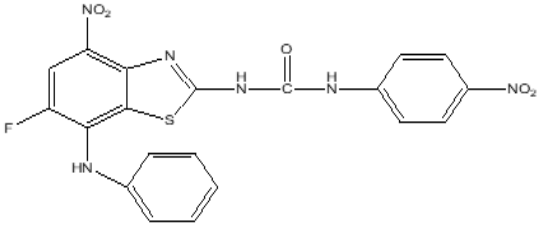


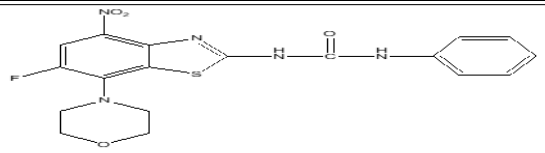
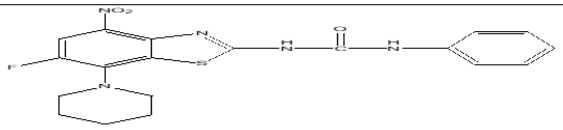
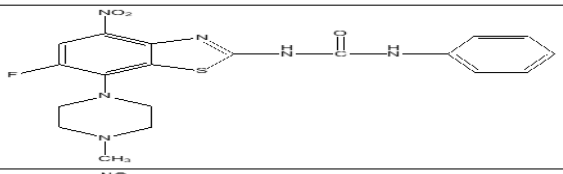
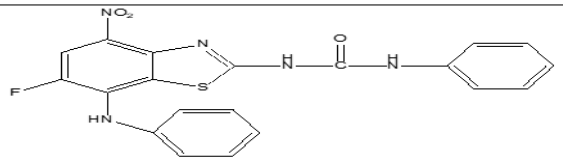
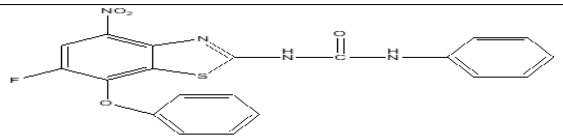
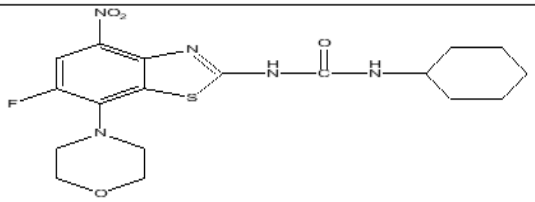
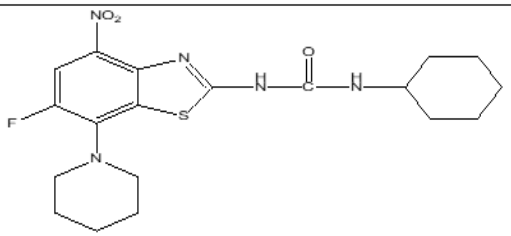
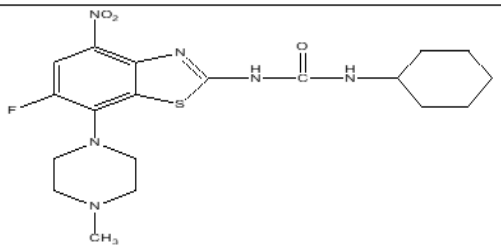
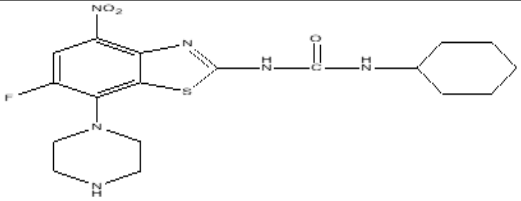
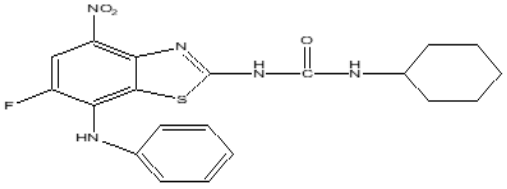
53 (I-III)

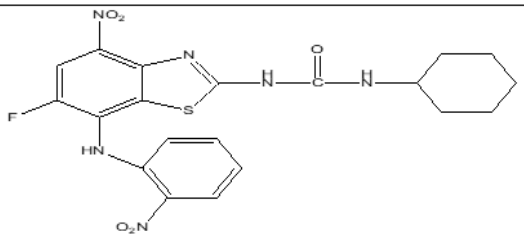
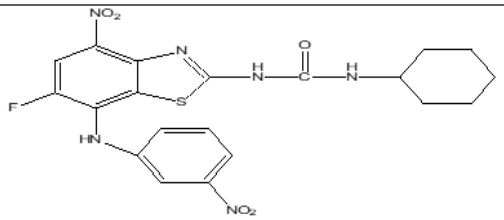
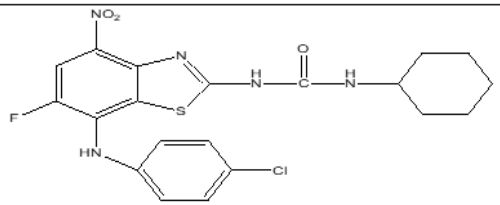
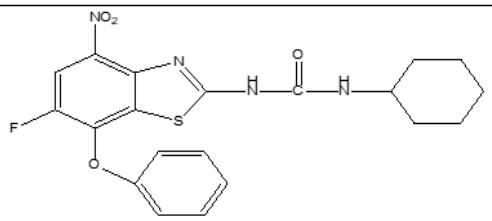
55 I(a-e)
55 II(a-i)
55 III(a-b)

Reflux the urea derivative with different amines (aniline, morpholine, and piperidine etc.) or phenol with equivalent amount of 10 ml dioxane and 2-3 drops of triethyl amine for 12-14 hours and monitored by TLC, then add ice cold water and filter [24].

List of synthesized compounds

Sl. no	Compound code	Chemical Name	Structure
01	55(I)	1-(7-choloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-phenylurea	
02	55 (II)	1-(7-choloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-cyclohexylurea	
03	55 (III)	1-(7-choloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(4-nitrophenyl)urea	
04	55(III)a	1-(6-fluoro-7morpholin-4-yl-4-nitro- benzothiazol-2-yl)-3-(4-nitrophenyl)urea	
05	55(III)b	1-(6-fluoro-4-nitro-7-(phenylamino)benzo[d]thiazol-2-yl)-3-(4-nitrophenyl)urea	

06	55(I)a	1-(6-fluoro-7-morpholino-4-nitrobenzo[d]thiazol-2-yl)-3-phenylurea	
07	55(I)b	1-(6-fluoro-4-nitro-7-(piperidin-1-yl)benzo[d]thiazol-2-yl)-3-phenylurea	
08	55(I)c	1-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-nitrobenzo[d]thiazol-2-yl)-3-phenylurea	
09	55(I)d	1-(6-fluoro-4-nitro-7-(phenylamino)benzo[d]thiazol-2-yl)-3-phenylurea	
10	55(I)e	1-(6-fluoro-4-nitro-7-phenoxybenzo[d]thiazol-2-yl)-3-phenylurea	
11	55(II)a	1-cyclohexyl-3-(6-fluoro-7-morpholino-4-nitrobenzo[d]thiazol-2-yl)urea	
12	55(II)b	1-cyclohexyl-3-(6-fluoro-4-nitro-7-(piperidin-1-yl)benzo[d]thiazol-2-yl)urea	
13	55(II)c	1-cyclohexyl-3-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-nitrobenzo[d]thiazol-2-yl)urea	
14	55(II)d	1-cyclohexyl-3-(6-fluoro-4-nitro-7-piperazin-1-yl-benzothiazol-2-yl)-urea	
15	55(II)e	1-cyclohexyl-3-(6-fluoro-4-nitro-7-(phenylamino)benzo[d]thiazol-2-yl)urea	

16	55(II)f	1-cyclohexyl-3-(6-fluoro-4-nitro-7-(2-nitrophenylamino)benzo[d]thiazol-2-yl)urea	
17	55(II)g	1-cyclohexyl-3-(6-fluoro-4-nitro-7-(3-nitrophenylamino)benzo[d]thiazol-2-yl)urea	
18	55(II)h	1-(7-(4-chlorophenylamino)-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-cyclohexylurea	
19	55(II)i	1-cyclohexyl-3-(6-fluoro-4-nitro-7-phenoxybenzo[d]thiazol-2-yl)urea	

IN VITRO SCREENING FOR ACETYL CHOLINESTERASE INHIBITORY ACTIVITY:

Acetylcholinesterase activity was calculated by means of a modified 96-well microplate assay followed by Ellman *et.al* [25]. The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2- nitrobenzoate which can be detected at 412 nm.

(Enzyme)

Acetylthiocholine ----------→ thiocholine + acetate

Thiocholine + DTNB ----------→ yellow color

Reagents

Buffer: Phosphate, 0.1 M, pH 8.0.

Substrate: Acetylthiocholine iodide, 0.075 M (21.67 mg/ml). This solution was used effectively for 10-15 days if kept refrigerated.

Ellman's reagent: Dithiobisnitrobenzoic acid (DTNB) 0.01 M (39.6 mg) was dissolved in 10 ml pH 7.0 phosphate buffer (0.1 M) and 15 mg of sodium bicarbonate were added.

Procedure

After the behavioral study, the rats were decapitated and brains were excised and kept on a cool petridish, which is kept on ice. Brains were washed with isotonic saline to take out blood. The tissue was homogenized, approximately 20 mg of tissue per ml of phosphate buffer (pH 8.0, 0.1 M) in a homogenizer.

In a cuvette take 0.4 ml of homogenated brain solution; add 2.6 ml of phosphate buffer (pH 8.0, 0.1 M) and 20 µL of 10 mM of DTNB (5, 5'-dithio-bis[2- nitrobenzoic acid]) were added.Positive control is donepezil. The absorbance was measured at 412 nm every 10 sec for 3 mins (SHIMADZU UV-1800, UV-VIS Spectrophotometer. Blank with buffer instead of enzyme solution was used [26, 27].

Calculation

$$\textbf{\% inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Molecular Docking Studies

Docking studies predicts the preferred orientation the binding mode of compounds

55(III), 55(III)b, 55(I)d, 55(II)a, 55(II)d, 55(II)f and **55(II)g** to AChE enzyme. Studies were carried out by using the X-ray crystal structure of *Homo sapiens* AChE (*h*AChE PDB ID: **4EY7**) [13] obtained from Protein Data Bank server (www.pdb.org).

The docking poses of the compounds **55(III), 55(III)b, 55(I)d, 55(II)a, 55(II)d, 55(II)f** and **55(II)g** are shown in (**Figures 2-3**). By the review of literature it is recognized that donepezil has high affinity to the pharmacophore due to its dual binding sites. The moiety of 6-fluoro-4-nitro-7-(2-nitrophenylamino) binds the enzyme active region i.e., peripheral anionic site (PAS) by interacting with the Tyr124 and Trp286 amino acid. Remaining groups like benzothiazole group including urea, binds to the catalytic anionic site (CAS) by interacting with Tyr341.

By analyzing the molecular docking outcomes of **55(III), 55(III)b, 55(I)d, 55(II)a, 55(II)d, 55(II)f** and **55(II)g** proves that active binding site of these compounds are similar to that of donepezil.

The fluoro/chloro-benzthiazole ring constitutes lipophilic part, while basic polar centre is 1-cyclohexyl-3-(6-fluoro-4-nitro-7-(2-nitrophenylamino)benzo[d]thiazol-2-yl)urea.

The PAS region of AChE interacts with lipophilic groups by interacting with Tyr124 and Trp286, whereas the hydrophilic and basic groups bind to the CAS region of the enzyme by interacting with the Tyr341 amino acid residue.

RESULTS AND DISCUSSION

The new compounds prepared during the present investigation have been authentically established by spectroscopic methods.

The compound **49** was prepared by bromination of 4-fluoro-3-chloroaniline with potassium thiocyanate **(scheme 1).** IR spectrum and showed its characteristic peak at 1527 cm^{-1} due to Ar C=C, 2924 cm^{-1} due to C-H, 1261 cm^{-1} due to C-F, 3441, 3363 cm^{-1} due to NH_2 str, 736 cm^{-1} due to C-Cl.

Upon acetylation of compound **49** give compound **50 (scheme 1).** The formation of compound **50** has been confirmed by peaks at 1442 cm^{-1} due to CH_3 str, 1654 cm^{-1} due to C=O str (carbonyl) in IR spectrum. Nitration of compound **50** gives Compound **51 (scheme 1)** with peaks at 1502 $cm^{-1}$and 1325 cm^{-1}due to nitro group**.** Further the conformation of compound **51** done by ^{1}H NMR**.** The presence of signals at δ 13.210 (s, 1H, NH at I), 8.4075-8.3839(d 1H, J= 9.44 Hz, (fluorine coupling at ortho position) CH at II), 2.2793 (s, 3H, CH_3, at III). This is on further deacetylation with 70% H_2SO_4 gave compound **52** with doublet peak at 3429cm^{-1} and 3302 cm^{-1} due to NH_2 str **.** The signals at δ 7.36- 7.34 (d, 1H, CH at I), 1.65 (s, 2H, NH_2 at II) clearly shown the formation of compound **52 (Table 5).**

Compound **52** is treated with ethyl choloroformate or triphosgene and different amine in the presence of triethyl amine to get urea derivatives **[53 (I -III)].** The formation of **53(I)** has been indicated by peaks at 1672 cm^{-1} due to C=O (amide) and 3294 cm^{-1} due to NH str **(Table 4, Scheme 2).**

A series of derivatives was synthesized **(scheme 3)** by replacement chlorine moiety by different amines (morpholine, piperazine, aniline) and phenol from urea derivatives **[53 (I -III)].** The formation of **55(II) d** was indicated by its IR spectrum**.** The appearance peak at 3309 cm^{-1} due to N-H str, 1663 cm^{-1} due to C=O (carbonyl), 1523 cm^{-1} due to nitro group and 1442 cm^{-1} due to C=N str. Further confirmed by ^{1}H NMR spectrum at δ 6.25 (s, 2H, NHCO at II), δ 5.12 (s, 1H, NH at III), δ 2.01-3.12 (m, 18H, CH_2 at IV and V) and δ 7.36- 7.54(s, 1H, CH at I)**.** Our aim was to actually isolate **53 (III),** which was purified by column chromatography as the major product and confirmed by ^{1}H NMR. In chemexper data + sign indicate good drug and – sign indicate bad drug and moleinspiration shows viceversa.

In vitro AChE enzyme inhibitory was carried out by using DTNB Ellmans reagent. The Compounds were randomly selected for *in vitro* AChE inhibitory activity. The most excellent result in terms of AChE inhibitory activity, were shown by seven derivatives **55(III), 55(III)b, 55(I)d, 55(II)a, 55(II)d, 55(II)f** and **55(II)g (Table7).** The IC_{50} values of **55(III)** and **55(I)d** is 94.58±1.74, **55(II)d** is 92.39±1.72 and **55(II)g** is 98.55±1.17 were almost close to standard donepezil (**Table 6)**.

Benzothiazolourea derivatives against AChE demonstrated very good activity when *m*- nitro aniline, *p*-chloro aniline or chlorine moiety present on 7th position of benzothiazole. However, when *o*- nitro aniline is at the same position, lesser activity was obtained. This could be possibly being attributed to steric hinderance.

According to the docking poses, **55(III),**

55(III)b, 55(I)d, 55(II)a, 55(II)d, 55(II)f and **55(II)g** compounds have five common almost near to standard reference donepezil i.e. **-11.6** (**Table 6)**. The 6-fluoro-4-nitro -benzthiazole ring interacts with the benzyl of the Trp286 by π–π interaction. The urea contains a nitrogen atom which forms a hydrogen bond with the hydroxyl group of Tyr124. The 7-(2-nitrophenylamino) groups will undergo π–π interaction with benzyl group of Tyr341 vowing for polar interaction. The C4 nitro group on the benzyl ring forms hydrogen bonds with the carbonyl of Gly121 by acting as a hydrogen donor and the hydroxyl group of Tyr341 by acting as a hydrogen acceptor. The docking poses of 55(I)d, 55(II)g and 55(III) are interactions and there docking scores were recorded. **(Fig 1-3).**

CONCLUSION

AChE enzyme plays a key role in recall and in mental action. In order to ameliorate AChE inhibitory activity, a wide range of electron withdrawing group (NO_2 and Cl) at *m* and *p* position on the phenyl ring in case of substituted fluoro-nitrobenzothiazolourea derivatives need to be investigated as pharmacophore development for these classes of molecules. The supplementary lead optimization should be carried out for the better AChE inhibitory activity.

Table No. 1 Predicted data of synthesized compounds

Sl. no.	Compound code	Calculated % of element (C, H, N)	Clog p	Drug likeness	Drug score
01	55 (I)	45.85, 2.20, 15.28	4.27	-2.76	0.25
02	55 (II)	45.10, 3.79, 15.03	4.28	-4.44	0.23
03	55 (III)	40.84, 1.71, 17.01	4.30	-2.67	0.24
04	55 (I)a	51.79, 3.86, 16.78	3.04	-1.05	0.34
05	55 (I)b	54.93, 4.37, 16.86	4.25	-1.87	0.24
06	55 (I)c	53.01, 4.45, 19.52	3.16	4.29	0.57
07	55 (I)d	56.73, 3.33, 16.54	5.53	-2.87	0.17
08	55 (I)e	56.60, 3.09, 13.20	5.07	-2.56	0.18
09	55 (II)a	51.05, 5.24, 16.54	3.05	-3.34	0.27
10	55 (II)b	54.17,5.74, 16.62	4.26	-1.24	0.26
11	55 (II)c	52.28, 5.77, 19.25	3.17	1.57	0.51
12	55 (II)d	46.75, 3.27, 18.17	2.88	-2.02	0.31
13	55 (II)e	55.93, 4.69, 16.31	5.54	-5.76	0.16
14	55 (II)f	50.63, 4.04, 17.71	5.07	-4.31	0.17
15	55 (II)g	50.63, 4.04, 17.71	5.27	-4.08	0.15
16	55 (II)h	51.78, 4.13, 15.10	5.27	-4.34	0.05
17	55 (II)i	55.80, 4.45, 13.02	6.15	-3.48	0.13
18	55 (III)a	51.17, 5.49, 19.89	2.78	-1.76	0.06
19	55 (III)b	51.28, 2.80, 17.94	5.52	-4.65	0.04

Table No. 2 Predicted Mole inspiration data of synthesized compounds

Sl. no	Compound code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	miLogP	TPSA(total polar surface area)
01	55 (I)	-0.18	-0.04	-0.11	-0.98	4.332	99.841
02	55 (II)	-0.07	0.01	-0.19	-1.00	4.540	99.841
03	55 (III)	-0.19	-0.06	-0.13	-0.89	4.291	145.665
04	55 (I)a	-0.17	-0.05	-0.04	-0.71	3.603	112.313
05	55 (I)b	-0.09	-0.02	-0.11	-0.63	4.665	103.079
06	55 (I)c	-0.01	0.04	0.02	-0.67	3.648	106.317
07	55 (I)d	-0.17	-0.05	0.02	-0.90	5.646	111.868
08	55 (I)e	-0.18	-0.03	-0.20	-0.53	5.408	109.075
09	55 (II)a	-0.08	-0.01	-0.10	-0.72	3.811	112.313
10	55 (II)b	-0.02	0.02	-0.15	-0.67	4.873	103.079
11	55 (II)c	-0.01	0.02	-0.27	-0.87	5.114	103.079
12	55 (II)d	0.04	0.07	-0.07	0.68	3.261	115.106
13	55 (II)e	-0.08	-0.02	-0.03	-0.92	5.854	111.868
14	55 (II)f	-0.15	-0.16	-0.05	-0.90	5.765	157.692
15	55 (II)g	-0.11	-0.10	-0.06	-0.97	5.789	157.692
16	55 (II)h	-0.08	-0.01	-0.03	-0.95	6.532	111.868
17	55 (II)i	-0.09	0.00	-0.25	-0.55	5.616	109.075
18	55 (III)a	-0.17	-0.09	-0.06	-0.66	3.562	158.137
19	55 (III)b	-0.17	-0.12	-0.00	-0.84	5.605	157.692

Table No. 3 Physical properties of synthesized compounds

Sl. No	Compound code	Molecular formula	Mol. weight	Melting Point (°C)	% yield	R_f value	Mobile phase
1.	55 (I)	$C_{14}H_8ClFN_4O_3S$	366.75	110	54%	0.34	n-H:EA 2:1
2.	55 (II)	$C_{14}H_{14}ClFN_4O_3S$	372.8	130	58%	0.42	n-H:EA 2:1
3.	55 (III)	$C_{14}H_7ClFN_5O_5S$	411.75	140	58%	0.44	n-H:EA 2:1
4.	55 (I)a	$C_{18}H_{16}FN_5O_4S$	417.41	160	50%	0.26	n-H:EA 2:1
5.	55 (I)b	$C_{19}H_{18}FN_5O_3S$	415.44	150	55%	0.60	n-H:EA 2:1
6.	55 (I)c	$C_{19}H_{19}FN_6O_3S$	430.46	155	48%	0.58	n-H:EA 2:1
7.	55 (I)d	$C_{20}H_{14}FN_5O_3S$	423.42	175	55%	0.54	n-H:EA 2:1
8.	55 (I)e	$C_{20}H_{13}FN_4O_4S$	424.41	152	53%	0.50	n-H:EA 2:1
9.	55 (II)a	$C_{18}H_{22}FN_5O_4S$	423.46	150	48%	0.23	n-H:EA 2:1
10.	55 (II)b	$C_{19}H_{24}FN_5O_3S$	421.49	170	49%	0.65	n-H:EA 2:1
11.	55 (II)c	$C_{19}H_{25}FN_6O_3S$	436.5	160	40%	0.60	n-H:EA 2:1
12.	55 (II)d	$C_{18}H_{15}FN_6O_6S$	462.61	145	45%	0.75	n-H:EA 1:1
13.	55 (II)e	$C_{20}H_{20}FN_5O_3S$	429.47	150	48%	0.73	n-H:EA 1:1
14.	55 (II)f	$C_{20}H_{19}FN_6O_5S$	474.47	220	43%	0.80	n-H:EA 1:1
15.	55 (II)g	$C_{20}H_{19}FN_6O_5S$	474.47	210	52%	0.73	n-H:EA 1:1
16.	55 (II)h	$C_{20}H_{19}ClFN_5O_3S$	63.91	195	49%	0.82	n-H:EA 1:1
17.	55 (II)i	$C_{20}H_{19}FN_4O_4S$	430.45	160	44%	0.78	n-H:EA 1:1
18.	55 (III)a	$C_{18}H_{23}FN_6O_3S$	422.48	168	50%	0.30	n-H:EA 2:1
19	55 (III)b	$C_{20}H_{13}FN_6O_5S$	468.42	174	49%	0.44	n-H:EA 2:1

Table No.4 Infra-Red spectral study of the synthesized compounds

Compound code	Molecular nature and Spectral peaks (cm^{-1})
55(I)	Ar-NO_2 1552, Ar C=C1598, C-H str (aromatic) 2918, C-F (aromatic) 1234, NH str 3294, C=O 1651, C-Cl 752
55 (II)	Ar-NO_2 1535, Ar C=C 1560, C-H str (aromatic) 2928, C-F (aromatic) 1271, NH str 3336, C=O 1653, C-Cl 752
55 (III)	Ar-NO_2 1504, Ar C=C 1573, C-H str (aromatic) 2924, C-F (aromatic) 1273, NH str 3423, C=O 1672, C-Cl 711
55 (III)a	Ar-NO_2 1543, Ar C=C 1602, C-H str (aromatic) 2926, C-F (aromatic) 1224, NH str 3379, C=O 1730
55 (III)b	Ar-NO_2 1543, Ar C=C 1600, C-H str (aromatic) 2951, C=N str 1442 C-F (aromatic) 1186, NH str 3219, C=O 1653
55(I)a	Ar-NO_2 1535, Ar C=C 1633, C-H str (aromatic) 2960, C=N str 1444, C-F (aromatic) 1112, NH str 3317, C=O 1683
55(I)b	Ar-NO_2 1543, Ar C=C 1562, C-H str (aromatic) 2939, C-F (aromatic) 1257, NH str 3248, C=O 1656
55(I)c	Ar-NO_2 1539, Ar C=C 1606, C-H str (aromatic) 2924, C-F (aromatic) 1261, NH str 3298 , C=O 1672
55(I)d	Ar-NO_2 1535, Ar C=C 1600, C-H str (aromatic) 2960, C-F (aromatic) 1259, NH str 3298, C=O 1653
55(I)e	Ar-NO_2 1543, Ar C=C 1595, C-H str (aromatic) 2928, C-F (aromatic) 1265, NH str 3367, C=O 1670, C-O 1301.
55 (II)a	Ar-NO_2 1301, Ar C=C 1558, C-H str (aromatic) 2926, C-F (aromatic) 1166, NH str 3269, C=O 1653, C-O 1236.
55 (II)b	Ar-NO_2 1543, Ar C=C 1562, C-H str (aromatic) 2933, C=N str 1442, C-F (aromatic) 1261, NH str 3196, C=O 1653.
55 (II)c	Ar-NO_2 1543, Ar C=C 1562, C-H str (aromatic) 2933, C=N str 1448, C-F (aromatic) 1234, NH str 3325, C=O 1685.
55 (II)d	Ar-NO_2 1523, Ar C=C 1618, C-H str (aromatic) 2958, C=N str 1442, C-F (aromatic) 1263, NH str 3309, C=O 1663.
55 (II)e	Ar-NO_2 1543, Ar C=C 1600, C-H str (aromatic) 2931, C=N str 1446, C-F (aromatic) 1114, NH str 3298, C=O 1653.
55 (II)f	Ar-NO_2 1512, Ar C=C 1624, C-H str (aromatic) 2931, C=N str 1442, C-F (aromatic) 1224, NH str 3346, C=O 1718.
55 (II)g	Ar-NO_2 1543, Ar C=C 1562, C-H str (aromatic) 2928, C=N str 1438, C-F (aromatic) 1235, NH str 3113, C=O 1649.
55 (II)h	Ar-NO_2 1543, Ar C=C 1580, C-H str (aromatic) 2929, C=N str 1444, C-F (aromatic) 1114, NH str 3309, C=O 1653.
55 (II)i	Ar-NO_2 1543, Ar C=C 1606, C-H str (aromatic) 2962, C=N str 1442, C-F (aromatic) 1219, NH str 3136, C=O 1655, C-O 1263.

Table No. 5 ^1H NMR Spectral data of synthesized compounds

Compound code	Chemical shift value δ and Proton nature
53	(400MHz; DMSO-d_6/TMS, ppm): 13.210(s, 1H, NH at I) 8.4075-8.3839(d 1H, J= 9.44 Hz, (o-F coupling) CH at II) 2.2793 (s, 3H, CH_3, at III.)
54	(200MHz; $CDCl_3$/TMS, ppm): 7.36-7.34(d, 1H, CH at I) 1.65(s, 2H, NH_2 at II)
55 (III)a	(200MHz; $CDCl_3$/TMS, ppm): 7.957-7.923(d, 2H, Ar-H) 6.649-6.604(d, 2H, Ar-H) 4.255(s, 2H, -NH)
55 (II)d	(200MHz; $CDCl_3$/TMS, ppm): 7.36(s, 1H, CH at I) 2.01-3.12 (m, 18H, CH_2, at IV and V) 6.25(s, 2H, -NH at II) 5.12 (s, 1H, -NH at III)

Table No. 6 Docking results and acetylcholinesterase inhibitory activity of substituted fluoro-nitro benzothiazolourea derivatives

Sl.no	Compound Code	Docking score	Amino acid residue	IC_{50}
1	55 (I)d	-11.0	Trp-86, His-447, Ala-204, Gly-122, Tyr-124, Trp-286	94.58 ± 1.74
2	55 (II)a	-10.5	Tyr-341, Trp–286, Tyr--124, Gly-122, Ala-204, Gly-121, Ser-203, His-441, Trp-86	87.26 ± 1.17
3	55 (II)d	-10.9	Tyr-124, Trp-286, His-447, Glu-202	92.39 ±1.72
4	55 (II)f	--10.7	Tyr-341, Trp-286, Tyr-72, Tyr-124, Tyr-337, phe-338, Phe-233	87.26 ±1.17
5	55 (II)g	-11.2	Ser-293, Tyr-337, Tyr-341, Tyr-124,Asp-74,Trp-286,Leu-289	98.55±1.17
6	55 (III)	-11.0	Phe-297, Arg-296, Phe-295, Tyr-341, Tyr-124, Ser-125, Gly-121,Gly-120,ser-293,Glu-448,His-447	94.58 ± 1.74
7	55(III)b	-10.6	Tyr-341,His-447,Tyr-337,Ser-293,Trp286	88.48 ± 1.72
8	Donepezil	-11.6	Trp-86,His-447,Tyr-337,Phe-338, Tyr-341,Tyr-124,Trp-286,Leu-289, Tyr72	99.42 ±1.85

Figure No. 1: The docking pose of compound 55 (II) g with AChE.

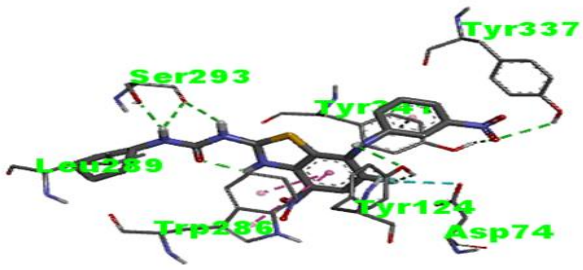


Figure No. 2: The docking pose of compound 55 (I) d with AChE.

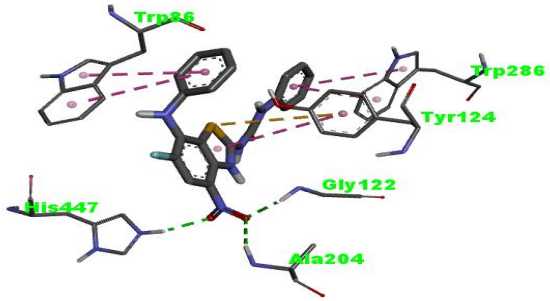
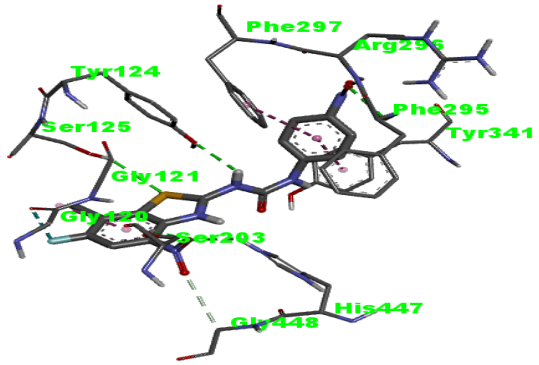


Figure No. 3: The docking pose of compound 55 (III) with AChE.



REFERENCES

Derya O, Begüm NS, Ulviye AC, Serkan L, Betül KÇ, Yusuf Ö *et.al*. Synthesis and AChE Inhibitory Activity of Novel Thiazolylhydrazone Derivatives. Molecules 2019; 24(2392): 1-15.doi:10.3390/molecules24132392.

Muhammad T, Foziah JA, Fazal R, Hassane A, Nizam U *et.al.* Synthesis, characterization, biological evaluation, and kinetic study of indole base sulfonamide derivatives as acetylcholinesterase inhibitors in search of potent anti-Alzheimer agent. Journal of King Saud University – Science 2021; 33 (2021): 101401-8.

Michael CVZ, Brian D, Diane RS, Janet S, Alberto DP. Discovery of [2-(4, 5, 7-trifluoro-benzothiazol-2-ylmethyl)- pyrrolo[2,3-b]pyridine-1-yl]acetic acid as highly potent and selective inhibitors of aldose reductase for chronic diabetic complications. Bioorg Med Chem Lett 2009; 19: 2006–08.

Song EY, Navneet K, Park MY, Yinglan J, Kyeong L, Kim Guncheol et al. Synthesis of amide and urea derivatives of benzothiazole as Raf-1 inhibitor. Eur J Med Chem 2008; 43: 1519-24.

Rana A, Siddiqui N, Khan SA. , Ehtaishamul Haque Syed, Mashooq AB. N-{[(6-Substituted-1, 3-benzothiazole-2-yl) amino]carbonothioyl}-2/4- substituted benzamides: Synthesis and pharmacological evaluation. Eur J Med Chem 2008; 43: 1114-22.

Hipparagi SM, Majumder UK, Vyavahare M, Panpaliya P, Soni K, Nargund LVG. et al. Synthesis and antimicrobial activity of 8-substituted-7-fluoro-benzo[4,5]thiazolo[2,3-C][1,2,4]triazole. Orient J Chem 2007; 23(1): 325-28.

Serdons K, Verduyckt T, Vanderghinste D, Cleynhens J, Borghgraef P, Vermaelen P. Synthesis of ^{18}F-labelled 2-(4'-fluorophenyl)-1,3-benzothiazole and evaluation as amyloid imaging agent in comparison with [^{11}C]PIB. Bioorg Med Chem Lett 2009; 19: 602–05.

Xiangji C, Pingrong Y, Lianfeng Z, Boli L. Synthesis, and biological evaluation of ^{99m}Tc, Re-monoamine-monoamide conjugated to 2-(4-aminophenyl) benzothiazole as potential probes for β-amyloid plaques in the brain. Bioorg Med Chem Lett 2008; 18: 1442-45.

Sze MC, Fang DS, Wrigley S, Bradshaw TD, Hutchinson, Nicholas PIS et al. Antitumor benzothiazoles. Synthesis of 2-(4-acylaminophenyl) benzothiazoles and investigations into the role of acetylation in the antitumor activities of the potent amines. J Med Chem 1999; 42: 381-92.

Choi SJ, Park HJ, Sang KL, Sang WK, Gyoonhee H, Young HPC. Solid phase combinatorial synthesis of benzothiazoles and evaluation of topoisomerase II inhibitory activity. Bioorg Med Chem Lett 2006; 14: 1229-35.

Wang M, Gao M, Mock BH, Miller KD, Sledge GW, Hutchins GD. Synthesis of carbon-11 labeled fluorinated 2-arylbenzothiazoles as novel potential PET cancer imaging agents. Bioorg Med Chem 2006; 14: 8599–07.

Lee A, Lee K, Kim D. Using reverse docking for target identification and its applications for drug discovery. Expert Opin. Drug Dis. 2016; *11*: 707–715.

Peter W. Rose, Bojan Beran, Chunxiao Bi *et al*. The RCSB Protein Data Bank: redesigned web site and web services. Nucleic Acids Res. 2011; 39: 392-401.

Adeniji SE, Shallangwa GA, Arthur DE, Abdullahi M, Mahmoud AY, Haruna A. Quantum modelling and molecular docking evaluation of some selected quinoline derivatives as antitubercular agents. Heliyon.2020; 1(2): 1010-1020.

Trott O, Olson AJ. Autodock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010; 22:455–461.

Azzam RA, Osman RR, Elgemeie GH. Efficient Synthesis and Docking Studies of Novel Benzothiazole-Based Pyrimidinesulfonamide Scaffolds as New Antiviral Agents and Hsp90α Inhibitors. ACS Omega. 2020; 5(3): 1640–55.

Gul H, Demirtas A, Ucar G, Taslimi P, Gulcin I. Synthesis of Mannich bases by two different methods and evaluation of their acetylcholine esterase and carbonic anhydrase inhibitory activities. Lett Drug Des Discov.2017; 14: 573–580.

Vijay KMMJ, Jayadevaiah KV, Nagaraja TS, Bharathi DR, Shameer H, Jayachandran E. et al. Synthesis, characterization, and antimicrobial activity of new *N*-substituted-3-chloro-2-azetidinones. Arch Pharm Sci & Res 2009; 1(1): 31-39.

Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR. Vogel's textbook of practical organic chemistry, ELBS; 5th ed. 1991; 5: 1272-73.

Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR. Vogel's textbook of practical organic chemistry, ELBS; 5th ed. 1991; 5: 919.

Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR. Vogel's textbook of practical organic chemistry, ELBS; 5th ed. 1991; 5: 920.

Kumaresh G, Indrajit S. Anthracene-based ortho-phenylenediamine clefts for sensing carboxylates. Tetrahedron Lett 2008; 49 : 4591–95

Wishar N, Davidsen SK, Michaelides MR. Thienopyrimidine ureas as novel and potent multitargeted receptor tyrosine kinase inhibitors. J Med Chem. 2005; 48: 6066-83.

Jayachandran E, Sreenivasa GM, Nargund LVG, Shanmukha I, Gaikwad PM, Inhibition of albumin denaturation (anti-inflammatory) and anthelmintic activity of fluoro substituted benzothiazoles comprising azetidinones. Ultra Chem 2006; 2(2): 147-56.

Ellman GL, Courtney D, Andres V, Featherstone RM. A new rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 961; 7: 88-95.

Saglık BN, Ilgın S, Özkay Y. Synthesis of new donepezil analogues and investigation of their effects on cholinesterase enzymes. Eur. J. Med. Chem.2016; 124: 1026–1040.

Hussein W, Saglık BN, Levent S, Korkut B, Ilgın S, Özkay Y, Kaplancıklı ZA. Synthesis and biological evaluation of new cholinesterase inhibitors for alzheimer's disease. Molecules.2018; 23: 2033-43.